Synthesis of novel chromenes as cytotoxic agents

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Novel substituted chromenes, chromenopyrimidines and chromenotriazolopyrimidines were synthesised to explore their anticancer activity. Several compounds were evaluated for their antitumour activity, most of them revealed promising cytotoxic activity against breast cancer cell line MCF-7 in comparison to colchicine as positive control.

Keywords: heterocycles, substituted chromenes, chromenopyrimidine, chromenotriazolopyrimidines, cytotoxic activity

Tubulin is one of the most important molecular targets of antitumour agents. Two subunits of this protein, α-and β-tubulins can undergo polymerisation to give microtubules. Antitumour agents can bind to different sites of tubulin and cause either its uncontrolled polymerisation or the inhibition of tubulin polymerisation. Tubulin polymerisation is inhibited by colchicine and its analogues. The action of colchicine is due to binding at a particular site of tubulin (the colchicine binding site), resulting in deformation of the α, β-dimer structure, which hinders the tubulin assembly into microtubules.[1,2] In recent years, several new structurally related classes of colchicine have been synthesised. For example, compound **I** (Fig. 1) that belonging to the 4-aryl-4*H*-chromene series.[3]

4-Aryl-4*H*-chromenes are potent apoptosis inducing agents possessing vascular targeting activity.[4–7] 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 and highly active as single agents or in combination with other anticancer agents in several tumour models, so they could be developed into new therapeutic anticancer agents.[8–12]

Hence, we aimed to synthesise a new series of heterocyclic compounds containing 4-aryl-4*H*-chromene moiety as shown in Schemes 1 and 2.

Results and discussion

2-Amino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile and its alkylated derivatives **1a** and **1b** were prepared.[13,14] Reacting **1a** and **1b** with formamide smoothly yielded 4-aminochromeno[2,3-*d*]pyrimidines **2a** and **2b** (Scheme 1) that were confirmed using microanalyses and spectral data. The IR spectra showed the disappearance of the cyano group as well as the presence of an NH_2 group. While, the ^{1}H NMR spectra showed the presence of C2H proton signal at δ 8.09–8.18 ppm and C5H proton signal at δ 4.89–5.30 ppm. The increased chemical shift of the C5H signal, compared to compounds **1a** and **1b** (C4H) can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons.

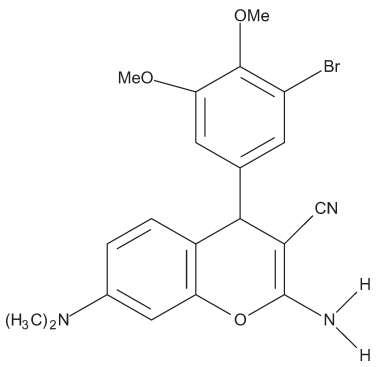

Fig. 1 Compound **1**.

Compounds **A** were intended to be prepared by reacting compounds **1a** and **1b** with acetic anhydride. Several attempts were carried out to prepare these target compounds **A** but these were unsuccessful. During monitoring the reaction using TLC it was found out that both the mono and diacetyl derivatives were formed at the exact same time. So, reaction of compounds **1a** and **1b** with acetic anhydride gave the diacetyl derivatives **3a** and **3b** (Scheme 1). The absence of NH and NH_2 absorption bands and the presence of an absorption band corresponding to C≡N group and also the appearance of a broad absorption band corresponding to (2C=O) in IR spectra confirming the formed product and proved that it was neither compounds **A** nor the starting materials. ^{1}H NMR spectra of compounds **3a** and **3b** indicated a singlet signal characteristic for six protons of diacetyl moiety (2 $COCH_3$) and the disappearance of D_2O exchangeable signals of both NH_2 or NH group.

For the preparation of chloroacylaminochromenes **B** (Scheme 1); reacting the aminocyanochromenes **1a** and **1b** with the appropriate chloro acid chloride was carried out but unexpectedly this reaction did not afford the expected acyl derivatives **B** but gave compounds that identified as 2-oxo-2*H*-chromene derivatives **4a** and **4b** (Scheme 2). The formation of the target acyl derivatives **B** was eliminated on the basis of spectral data and elemental analysis. IR spectra revealed the absence of NH group signal and the appearance of signal attributed to C=O of **4a** and **4b** respectively. ^{1}H NMR spectra showed the absence of the NH group as well as the absence of the C4H and the aliphatic CH acyl protons that exist in the target compounds **B**. Additional support for the structures of compounds **4a** was provided by ^{13}C NMR spectrum of compound **4a** which showed the disappearance of the peak of C-4 in chromene ring and the appearance of C=C instead confirming the structure of these compounds.

A suggested mechanism for the formation of compounds **4a** and **4b** is thought to be: after the formation of the chloroacylaminochromene the bond become very weak due to the inductive effect and carbon number 2 become highly positive centre and hence the attack with the conjugated carbonate base was easier with simultaneous break of the bond of the amide group (Fig. 2).

Formimidic acid ethyl esters **5a** and 5**b** were prepared by heating the corresponding *o*-aminonitrile derivatives **1a** and **1b** with triethyl orthoformate under reflux temperature. The IR spectra of compounds **5a** and **5b** showed the absence of absorption bands due to NH_2 group. Moreover, the ^{1}H NMR spectra of **5a** and **5b** revealed the appearance of a triplet and a quartet signals for the CH_2CH_3 protons and a singlet for N=CH proton.

The appearance of a singlet signal attributed to (C2H) and the downfield chemical shift for the signal of the C5H proton compared to the expected value in the ^{1}H NMR spectra confirmed the formation of tricyclic compounds.

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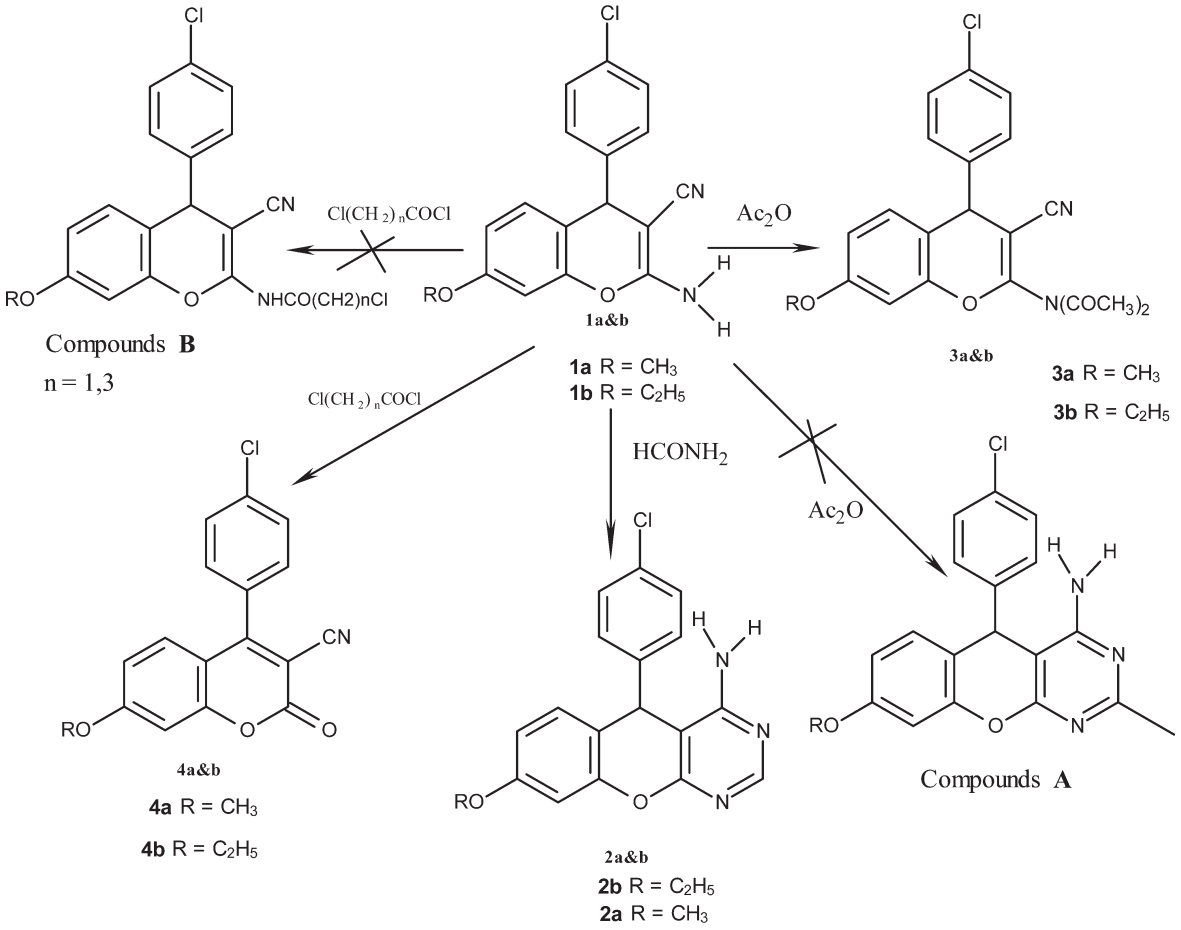
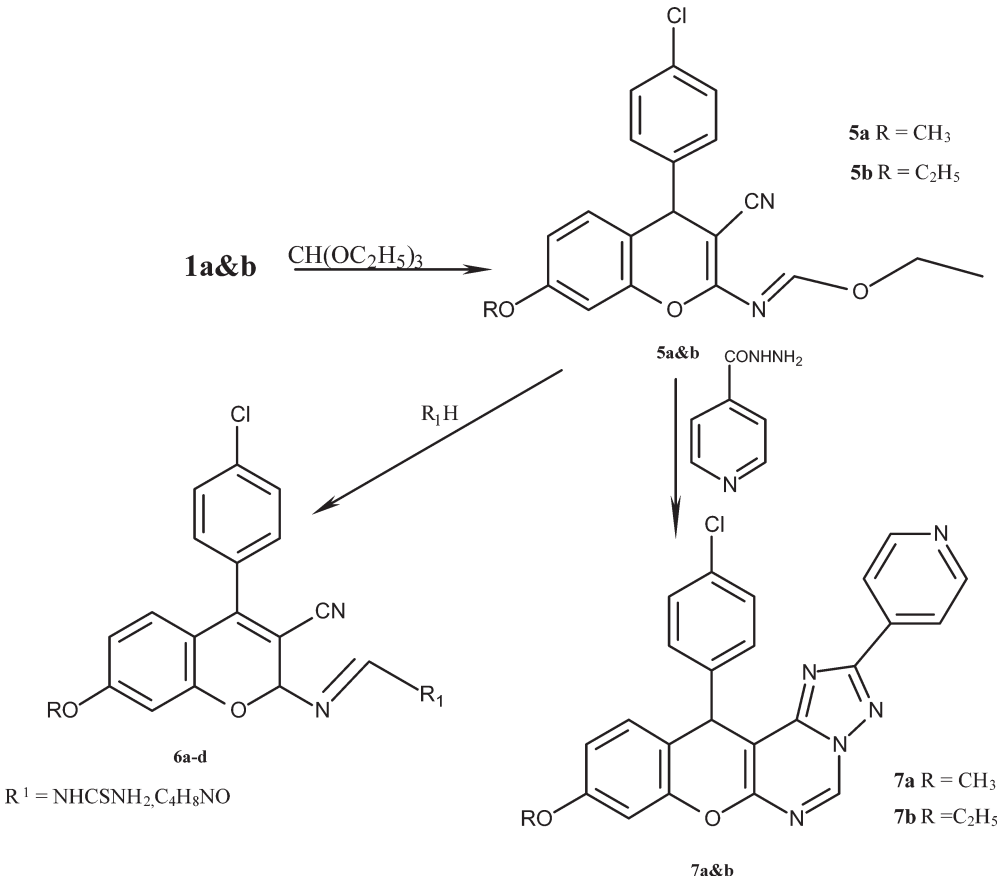


Scheme 1 Synthesis of compounds **2a** and **2b**, **3a** and **3b**, and **4a** and **4b**.



Scheme 2 Synthesis of compounds **5a** and **5b**, **6a–d** and **7a** and **7b**.

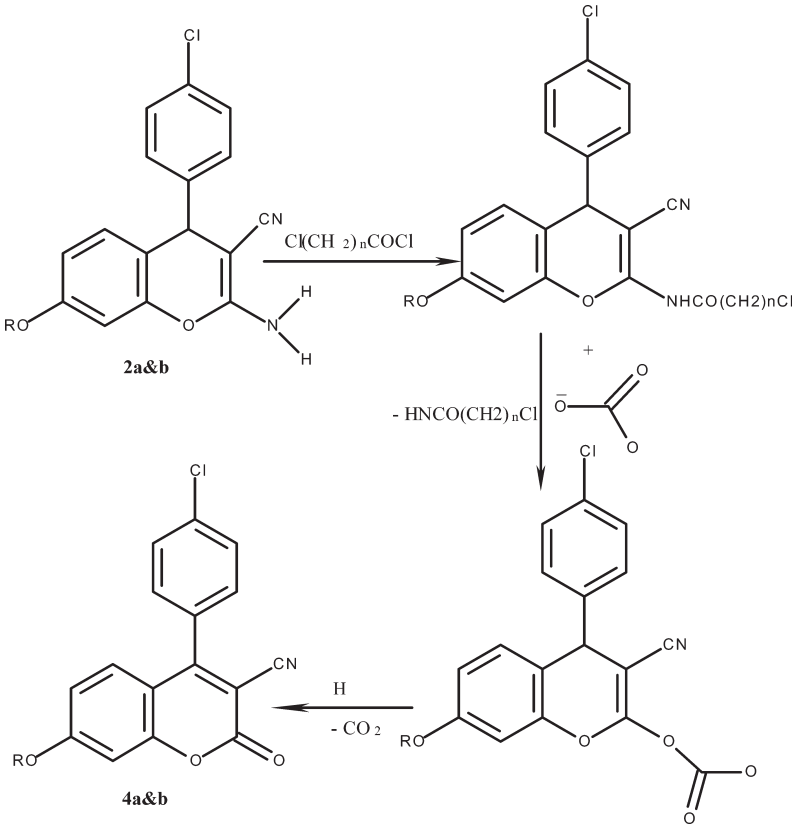


Fig. 2 The suggested mechanism of formation of compounds **4a** and **4b**.

Condensation of the key intermediate iminoethers **5a** and **5b** with different basic nitrogenous reagents such as thiourea and morpholine was adopted. Interaction of **5a** and **5b** and these reagents was carried to afford **6a** and **6b**, and **6c** and **6d** (Scheme 2). The contradiction of the reaction conditions between the two reactions may be attributed to the difference in reactivity of thiourea and morpholine, since the latter is much more basic than the former reagent. The structure of compounds **6a–d** was established on the basis of spectroscopic data and element analysis. The IR spectra of **6a** and **6b** showed characteristic absorption bands indicating the presence of amino functionality. ^{1}H NMR spectra of **6a–d** revealed the disappearance of the triplet and quartet signals attributed to the ethyl protons of the intermediates. In addition, ^{1}H NMR spectra of **6a** and **6b** showed D_2O exchangeable NH and NH_2 signals while the ^{1}H NMR spectra of **6c** and **6d** demonstrated a triplet peak attributed to two protons of the CH_2 of morpholine moiety and a multiplet peak attributed to nine protons ($3CH_2$ of morpholine moiety and OCH_3) and another multiplet one corresponding to six protons of $3CH_2$ of morpholine moiety for **6c** and **6d**.

Synthesis of chromenotriazolopyrimidines **7a** and **7b** was achieved *via* the interaction of the backbone intermediates **5a** and **b** with isonicotinic acid hydrazide in dioxane containing few drops of triethyl amine (Scheme 3). Spectral data of the obtained compounds confirmed their structures as **7a** and **7b**. Once more, ^{1}H NMR spectra showed the increased in chemical shift of C5H and C12H signals that can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons.

Antitumour activity

Colchicine was the reference drug that used in this study. The response parameter calculated was IC_{50} value (Table 1), which corresponds to the compound concentration causing 50% mortality in human breast tumour cell line (MCF-7). Six of the newly synthesised compounds were evaluated for their *in vitro* cytotoxic activity on human breast tumour cell line (MCF-7). Compounds **3a** (IC_{50} = 0.007 μM) and **6a** (IC_{50} = 0.008 μM) showed double the reactivity of colchicine (IC_{50} = 0.013 μM) while compounds **4a** (IC_{50} = 0.014 μM) and **6c** (IC_{50} = 0.011 μM) were nearly as active as colchicine. Moreover, Compounds **2b** (IC_{50} = 0.026 μM) and **7b** (IC_{50} = 0.026 μM) had half the reactivity of the positive control drug.

The objective of the present study was to synthesise and investigate the anticancer activity of the novel substituted chromenes, chromenopyrimidine derivatives and chromenotriazolo-pyrimidines. The results reveal that the corresponding chromene derivatives **3a** and **6a** showed the exquisite *in vitro* cytotoxic activity when compared to other tested compounds and colchicine as a reference drug. Additionally, compounds **4a** and **6c** revealed moderate activity while **2b** and **7b** showed the least antitumour activity. It is worth to mentioning that, compounds **3a**, **6a**, **4a** and **6c** have nearly the same structure as compound **I** (Fig. 1) and showed promising cytotoxic activity, while in case of the tricyclic compounds the activity is reduced by nearly half.

Table 1 Results of *in vitro* cytotoxic activity of test compounds

Compound no.	IC_{50} μM
Colchicine	0.013
2b	0.026
3a	0.007
4a	0.014
6a	0.008
6c	0.011
7b	0.026

IC_{50}: concentration of a drug that is required for 50% inhibition.

Experimental

Reactions were routinely monitored by TLC on silica gel sheets that precoated with UV fluorescent silica (MERCK 60 F 254) and spots were developed using I_2 vapour/UV light as visualising agents. Solvent system was chloroform: methanol (in different ratio). ^{1}H NMR spectra were determined in $CDCl_3$, or DMSO-d_6 solvent with Varian Gemini 300 MHz Spectrometer. Peak positions were given in parts per million (δ) downfield the tetramethylsilane as internal standard. ^{13}C NMR spectra were carried out on Gemini 75 MHz Spectrometer. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1}. GC Mass spectra were run on Shimadzu QP-2010 spectrometer and Mass spectra were run on Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University, Egypt. Melting points were determined on a Griffin instrument and are uncorrected. All reported products showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were performed at the Micro-analytical Center, Cairo University, Egypt. Compound **1a** and **1b** was prepared adopting a reported procedure.[13,14]

*Synthesis of compounds (RS) 8-alkoxy-5-(4-chlorophenyl)-5H-chromeno[2,3-d]pyrimidin-4-amines (***2a** *and* **2b***); general procedure*
A solution of **1a or 1b** (0.01 mol) in formamide (20 mL) was heated under reflux for 3 hnd was then cooled and poured into ice-cold water (20 mL). The precipitated solid was filtered, washed with water and crystallised from the appropriate solvent to afford **2a** or **2b** respectively.

(RS)-5-(4-Chlorophenyl)-8-methoxy-5H-chromeno[2,3-d]pyrimidin-4-amine (**2a**): Crystallised from benzene: acetone (1: 1) mixture. Yield 96%; m.p. 226–227 °C. IR (KBr): 3206 (NH_2), 1626 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3); 5.30 (s, 1H, C5H); 6.72–7.42 (m, 7H, ArH + 2H, NH_2, D_2O exchangeable); 8.18 (s, 1H, C2H) ppm. Anal. Calcd for $C_{18}H_{14}ClN_3O_2$ (339.78): C, 63.63; H, 4.15; N, 12.37. Found: C, 63.92; H, 4.29; N, 12.08%.

(RS)-5-(4-Chlorophenyl)-8-ethoxy-5H-chromeno[2,3-d]pyrimidin-4-amine (**2b**): Crystallised from benzene. Yield 92%; m.p. 209–210 °C. IR (KBr): 3385, 3335 (NH_2), 3165 (CH arom.), 2979 (CH aliph.), 1653 (C=N)cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.38 (t, J = 7.2 Hz, 3H, CH_3); 3.98 (q, J = 7.2 Hz, 2H, CH_2); 4.85 (s, 2H, NH_2, D_2O exchangeable); 4.89 (s, 1H, C5H); 6.57–7.27 (m, 7H, ArH), 8.09 (s, 1H, C2H) ppm; MS: *m/z* 355 (M+2‡, 7.93), 353 (M‡, 24.88), 242 (M-$C_6H_4Cl^{\ddagger}$,100). Anal. Calcd for $C_{19}H_{16}ClN_3O_2$ (353.81): C, 64.50; H, 4.56; N, 11.88. Found: C, 64.80; H, 4.69; N, 11.98%.

*Synthesis of compounds (RS) N-acetyl-N-[7-alkoxy-4-(4-chloro-phenyl)-3-cyano-4H-chromen-2-yl]acetamides (***3a** *and* **3b***); general procedure*
A mixture of **1a** or **1b** (0.01 mol) and acetic anhydride (20 mL) was heated under reflux for 5 h. The precipitated crystals formed after cooling were filtered and recrystallised from ethanol to give compounds **3a** or **3b** respectively.

(RS)-N-Acetyl-N-[4-(4-chlorophenyl)-3-cyano-7-methoxy-4H-chromen-2-yl]acetamide (**3a**): Yield 78%; m.p. 134–135 °C. IR (KBr): 3069 (CH arom.), 2949 (CH aliph.), 2220 (C≡N), 1744 (2 acetyl C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 6H, $2COCH_3$); 3.79 (s, 3H, OCH_3); 4.90 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.70 (d, J = 8.7 Hz, 1H, ArH); 6.87 (d, J = 8.7 Hz, 1H, ArH); 7.22 (d, J = 8.4 Hz, 2H, ArH); 7.35 (d, J = 8.4 Hz, 2H, ArH). Anal. Calcd for $C_{21}H_{17}ClN_2O_4$ (396.83): C, 63.56; H, 4.32; N, 7.06. Found: C, 63.81; H, 4.24; N, 7.05%.

(RS)-N-Acetyl-N-[4-(4-chlorophenyl)-3-cyano-7-ethoxy-4H-chromen-2-yl]acetamide (**3b**): Yield 74%; m.p. 164–165 °C. IR (KBr): 3106 (CH arom.), 2982 (CH aliph.), 2219 (C≡N), 1744 (2 acetyl C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.40 (t, J = 7.2 Hz, 3H, CH_3); 2.46 (s, 6H, $2COCH_3$); 4.01 (q, J = 7.2 Hz 2H, CH_2); 4.90 (s, 1H, C4H); 6.58 (s, 1H, ArH); 6.69 (d, J = 8.7 Hz, 1H, ArH); 6.86 (d, J = 8.7 Hz, 1H, ArH); 7.25 (d, J = 8.1 Hz, 2H, ArH); 7.34 (d, J = 8.1 Hz, 2H, ArH); GCMS: *m/z* 412 (M+2‡, 1.54), 410 (M‡, 4.71), 215 (M-$C_{10}H_8ClO_2^{\ddagger}$, 100). Anal. Calcd for $C_{22}H_{19}ClN_2O_4$ (410.86): C, 64.32; H, 4.66; N, 6.82. Found: C, 64.20; H, 4.80; N, 6.80%.

*Synthesis of compounds 7-alkoxy-4-(4-chlorophenyl)-2-oxo-2H-chromene-3-carbonitriles (***4a** *and* **4b***); general procedure*
A mixture of **1a** or **1b** (0.01mol), the appropriate acid chloride (0.01 mol) and anhydrous potassium carbonate (2.07 g, 0.015 mol) in tetrahydrofuran (30 mL) was heated under reflux for 2 h. The solid that separated after cooling was collected by filtration, washed with water, dried and crystallised from ethanol to afford compounds **4a** or **4b** respectively.

4-(4-Chlorophenyl)-7-methoxy-2-oxo-2H-chromene-3-carbonitrile (**4a**): Yield 83%; m.p. 207–208 °C. IR (KBr): 3096 (CH arom.), 2990 (CH aliph.), 2219 (C≡N), 1727 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.94 (s, 3H, OCH_3); 6.85 (s, 1H, ArH); 6.90 (d, J = 9 Hz,1H, ArH); 7.24 (d, J = 9 Hz, 1H, ArH); 7.43 (d, J = 8.4 Hz, 2H, ArH); 7.60 (d, J = 8.4 Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 56.24, 97.92, 101.27, 111.49, 113.79, 114.11, 129.53, 129.86, 130.44, 137.41, 156.43, 157.29, 162.58, 165.72; GCMS: *m/z* 313 (M+2‡, 35.63), 311 (M‡, 100), 283 (M-CO‡, 46.37). Anal. Calcd for $C_{17}H_{10}ClNO_3$ (311.73): C, 65.50; H, 3.23; N, 4.49. Found: C, 65.30; H, 3.52; N, 4.39%.

4-(4-Chlorophenyl)-7-ethoxy-2-oxo-2H-chromene-3-carbonitrile (**4b**): Yield 78%; m.p. 171–172 °C. IR (KBr): 3095 (CH arom.), 2944 (CH aliph.), 2224 (C≡N), 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.48 (t, J = 7.2 Hz, 3H, CH_3); 4.15 (q, J = 7.2 Hz, 2H, CH_2); 6.83 (s, 1H, ArH); 6.88 (d, J = 9 Hz, 1H, ArH); 7.24 (d, J = 9 Hz, 1H, ArH); 7.41 (d, J = 8.7 Hz, 2H, ArH); 7.59 (d, J = 8.7 Hz, 2H, ArH). Anal. Calcd for $C_{18}H_{12}ClNO_3$ (325.75): C, 66.37; H, 3.71; N, 4.30. Found: C, 66.14; H, 3.84; N, 3.99%.

*Synthesis of compounds (RS)-N-(EZ)-7-alkoxy-4-(4-chlorophenyl)-3-cyano-4H-chromen-2-ylformimidates (***5a** *and* **5b***); general procedure*
A mixture of compound **2a** or **2b** (0.01 mol) and triethyl orthoformate (20 mL) was heated under reflux for 4 h. The reaction mixture was evaporated under reduced pressure, and then the residue was washed with ethanol and crystallised from the appropriate solvent to give **5a** or **5b** respectively.

Ethyl (RS)-N-[(EZ)-4-(4-chorophenyl)-3-cyano-7-methoxy-4H-chromen-2-yl]formimidate (**5a**): Crystallised from absolute ethanol. Yield 88%; m.p. 130–132 °C. IR (KBr): 3068 (CH arom.), 2965 (CH aliph.), 2200 (C≡N), 1644 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.39 (t, J = 7.6 Hz, 3H, CH_2CH_3); 3.80 (s, 3H, OCH_3); 4.43 (q, J = 7.6 Hz, 2H, CH_2); 4.80 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.65 (d, J = 8.7 Hz, 1H, ArH); 6.84 (d, J = 8.7 Hz, 1H, ArH); 7.15 (d, J = 8.7 Hz, 2H, ArH); 7.30 (d, J = 8.7 Hz, 2H, ArH); 8.38 (s, 1H, N=CH). Anal. Calcd for $C_{20}H_{17}ClN_2O_3$ (368.82): C, 65.13; H, 4.65; N, 7.60. Found: C, 65.28; H, 4.66; N, 7.80%.

Ethyl (RS)-N-(EZ)-4-(4-chlorophenyl)-3-cyano-7-ethoxy-4H-chromen-2-ylformimidate (**5b**): Crystallised from methanol. Yield 61%; m.p. 134–136 °C. IR (KBr): 3067 (CH arom.), 2981 (CH aliph.), 2208 (C≡N), 1619 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.28–1.36 (m, 6H, $2CH_2CH_3$); 3.94 (q, J = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$); 4.35 (q, J = 7.6 Hz, 2H, $N{=}CHOC\underline{H}_2CH_3$); 4.71 (s, 1H, C4H); 6.49 (s, 1H, ArH); 6.54 (d, J = 8.4 Hz, 1H, ArH); 6.74 (d, J = 8.4 Hz, 1H, ArH); 7.07 (d, J = 8.4 Hz, 2H, ArH); 7.21 (d, J = 8.4 Hz, 2H, ArH); 8.30 (s, 1H, N=CH); MS: *m/z* 384 (M+2‡, 7.31), 382 (M‡, 20.40), 271 (M-$C_6H_4Cl^{\ddagger}$, 100). Anal. Calcd for $C_{21}H_{19}ClN_2O_3$ (382.85): C, 65.88; H, 5.00; N, 7.32. Found: C, 66.09; H, 4.80; N, 7.12%.

*Synthesis of compounds (RS)-N′-(EZ)-(7-alkoxy-4-(4-chloro-phenyl)-3-cyano-4H-chromen-2-yl)-N-carbamothioylformimidamides (***6a** *and* **6b***); general procedure*
Sodium ethoxide (0.01 mol) [sodium metal (0.23 g, 0.01 mol) and absolute ethanol (5 mL)] was added to a mixture of the iminoether **5a** or **5b** (0.01 mol) and thiourea (0.76 g, 0.01 mol) in absolute ethanol (30 mL),. The reaction mixture was heated under reflux for 2 h then cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried and crystallised from benzene to afford **6a** or **6b** respectively.

(RS)-N′-(EZ)-(4-(4-Chlorophenyl)-3-cyano-7-methoxy-4H-chromen-2-yl)-N-carbamothioyl formimidamide (**6a**): Yield 95%; m.p. 229–230 °C. IR (KBr): 3481, 3376, 3340 (NH, NH_2), 3186 (CH arom.), 2939 (CH aliph.), 2193 (C≡N), 1629 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.34 (br. s, 1H, NH, D_2O exchangeable); 3.78 (s, 3H, OCH_3); 4.95 (s, 1H, C4H); 5.02 (s, 2H, NH_2, D_2O exchangeable); 6.59–7.36 (m, 7H, ArH); 8.31 (s, 1H, N=CH). Anal. Calcd for $C_{19}H_{15}ClN_4O_2S$ (398.87): C, 57.21; H, 3.79; N, 14.05. Found: C, 57.30; H, 4.10; N, 13.83%.

(RS)-N′-(EZ)-(4-(4-Chlorophenyl)-3-cyano-7-ethoxy-4H-chromen-2-yl)-N-carbamothioyl formimidamide (**6b**): Yield 84%; m.p. 235–236 °C. IR (KBr): 3381, 3336 (NH, NH_2), 3180 (CH arom.), 2979 (CH aliph.), 2194 (C≡N), 1621 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.38 (t, J = 7.2 Hz, 3H, CH_3); 2.60 (br. s, 1H, NH, D_2O exchangeable); 4.00 (q, J = 7.2 Hz, 2H, CH_2); 4.96 (s, 1H, C4H); 5.26 (br. s, 2H, NH_2, D_2O exchangeable); 6.59–7.34 (m, 7H, ArH); 8.32 (s, 1H, N=CH); MS: *m/z* 414 (M+2‡, 30.86), 412 (M‡, 37.88), 411 (M-1‡, 42.01), 55 (M-$C_{18}H_{14}ClN_2O_2S^{\ddagger}$, 100). Anal. Calcd for $C_{20}H_{17}ClN_4O_2S$

(412.89): C, 58.18; H, 4.15; N, 13.57. Found: C, 58.00; H, 4.00; N, 13.57%.

*Synthesis of compounds (RS)-(EZ) -7-alkoxy-4-(4-chlorophenyl)-2-(morpholinomethyleneamino)-4H-chromene-3-carbonitriles (***6c** *and* **6d***); general procedure*

A solution of compound **5a** or **5b** (0.01mol) and morpholine (0.87 g, 0.01mol) in ethanol (30 mL) was stirred at room temperature for 1 h. The solid formed was filtered, dried and crystallised from ethanol to give **6c** or **6d** respectively.

(RS)-(EZ)-4-(4-Chlorophenyl)-7-methoxy-2-(morpholinomethyleneamino)-4H-chromene-3-carbonitrile (**6c**): Yield 66%; m.p. 191–192 °C. IR (KBr): 3100 (CH arom.), 2907(CH aliph.), 2186 (C≡N), 1595 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.51 (t, J = 6.4 Hz, 2H, CH_2 morpholine); 3.72–3.83 (m, 9H, $3CH_2$ morpholine and OCH_3); 4.76 (s, 1H, C4H); 6.58–7.28 (m, 7H, ArH); 8.23 (s, 1H, N=CH). Anal. Calcd for $C_{22}H_{20}ClN_3O_3$ (409.87): C, 64.47; H, 4.92; N, 10.25. Found: C, 64.69; H, 4.95; N, 10.05%.

(RS)-(EZ)-4-(4-Chlorophenyl)-7-ethoxy-2-(morpholinomethyleneamino)-4H-chromene-3-carbonitrile (**6d**): Yield 84%; m.p. 207–208 °C. IR (KBr): 3100 (CH arom.), 2923 (CH aliph.), 2197 (C≡N), 1603 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40 (t, J = 7.2 Hz, 3H, CH_3); 3.50 (t, J = 6.4 Hz 2H, CH_2 morpholine); 3.72–3.85 (m, 6H, $3CH_2$ morpholine); 4.00 (q, J = 7.2 Hz, 2H, CH_2-CH_3); 4.75 (s, 1H, C4H); 6.56 (s, 1H, ArH); 6.59 (d, J = 8.4 Hz, 1H, ArH); 6.83 (d, J = 8.4 Hz, 1H, ArH); 7.14 (d, J = 9 Hz, 2H, ArH); 7.26 (d, J = 9 Hz, 2H, ArH); 8.22 (s, 1H, N=CH); MS: *m/z* 425 ($M+2^{\overline{\ }|^{\ddagger}}$, 14.17), 423 ($M^{\overline{\ }|^{\ddagger}}$, 37.64), 312 ($M\text{-}C_6H_4Cl^{\overline{\ }|^{\ddagger}}$, 100). Anal. Calcd for $C_{23}H_{22}ClN_3O_3$ (423.89): C, 65.17; H, 5.23; N, 9.91. Found: C, 65.46; H, 5.10; N, 9.74%.

*Synthesis of compounds (RS)-9-alkoxy-12-(4-chlorophenyl)-2-(pyridin-4-yl)-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidines (***7a** *and* **7b***); general procedure*

A few drops of triethylamine were added to a mixture of **5a or 5b** (0.01mol) and the isonicotinic acid hydrazide (1.37 g, 0.01mol) in dioxane (30 mL). The reaction mixture was heated under reflux for 12 h then cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried and crystallised from a mixture of methanol: chloroform (3:1) to afford **7a or 7b** respectively.

(RS)-12-(4-Chlorophenyl)-9-methoxy-2-(pyridin-4-yl)-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7a**): Yield 66%; m.p. 261–262 °C. IR (KBr): 3047 (CH arom.), 2964 (CH aliph.), 1627 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.81 (s, 3H, OCH_3); 5.73 (s, 1H, C12H); 6.74–7.36 (m, 7H, ArH); 8.17 (d, J = 6 Hz, 2H, pyridinyl 3,5-H); 8.79 (d, J = 6 Hz, 2H, pyridinyl 2,6-H); 9.14 (s, 1H, C5H). Anal. Calcd for $C_{24}H_{16}ClN_5O_2$ (441.87): C, 65.24; H, 3.65; N, 15.85. Found: C, 65.50; H, 3.79; N, 15.71%.

(RS)-12-(4-Chlorophenyl)-9-ethoxy-2-(pyridin-4-yl)-12H-chromeno[3,2e][1,2,4] triazolo[1,5-c]pyrimidine (**7b**): Yield 94%; m.p. 231–233 °C. IR (KBr): 3052 (CH arom.), 2981 (CH aliph.), 1625 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.2 Hz, 3H, CH_3); 4.07 (q, J = 7.2 Hz, 2H, CH_2); 5.72 (s, 1H, C12H); 6.74 (d, J = 8.1 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.07 (d, J = 8.1 Hz, 1H, ArH); 7.23 (d, J = 8.4 Hz, 2H, ArH); 7.35 (d, J = 8.4 Hz, 2H, ArH); 8.16 (d, J = 6 Hz, 2H, pyridinyl 3,5-H); 8.80 (d, J = 6 Hz, 2H, pyridinyl 2,6-H); 9.13 (s, 1H, C5H); MS: *m/z* 457 ($M+2^{\overline{\ }|^{\ddagger}}$, 15.87), 455 ($M^{\overline{\ }|^{\ddagger}}$, 38.86), 344 ($M\text{-}C_6H_4Cl^{\overline{\ }|^{\ddagger}}$, 100), 316 ($M\text{-}C_8H_8Cl^{\overline{\ }|^{\ddagger}}$, 51.71). Anal. Calcd for $C_{25}H_{18}ClN_5O_2$ (455.90): C, 65.86; H, 3.98; N, 15.36. Found: C, 65.56; H, 3.99; N, 15.49%.

Antitumour activity

The breast tumour cell line was obtained frozen in liquid nitrogen (–180 °C) from the American Type Culture Collection (ATCC) and was maintained at the National Cancer Institute, Cairo, Egypt, by serial sub culturing. Colchicine was used in this experiment as a positive control. The tested compounds were dissolved in 20% DMSO in concentration 1mg mL^{-1} Serial dilutions were made reaching final concentration of the compounds to 0, 5, 12.5, 25 and 50 μg mL^{-1}. Previous experiments have shown that DMSO at this concentration does not modify the cellular activities that we are analysing. All chemicals used in this study are of high analytical grade. They either obtained from (Sigma-Alderich or Biorad)

Measurement of potential cytotoxic activity

The cytotoxic activity was measured *in vitro* on the human breast tumour cell line (MCF-7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan, *et al*.[15]

Cells were plated in 96 multiwell plates (104 cell/ well) for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the compound under test (0, 5, 12.5, 25, and 50 μg mL^{-1}) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37 °C and in an atmosphere of 5% CO_2. After 48 hours the cell was fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound.

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