Synthesis and antitumour activity of a novel class of flavanones: 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyrazoles

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A new series of 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyrazoles have been synthesised. The target compounds and their analogues (2,4-diaryl-2,4-dihydrochromeno[4,3-*c*]pyrazoles) were tested for their antitumour activities *in vitro* against MCF-7 and HL-60 cells using the MTT method. Among these compounds, 1,4-bis(4-chlorophenyl)-1,4-dihydrochromeno[4,3-*c*]pyrazole was found to have potent antitumour activity *in vitro* with minimum IC_{50} of 25.31 μg mL^{-1} and 13.86 μg mL^{-1} against MCF-7 and HL-60 cells respectively.

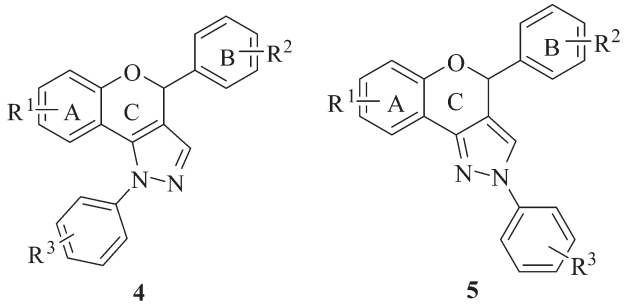
Keywords: flavonoids, pyrazoles, antitumour activity, Vilsmeier reagent

Flavanones are an important class of heterocyclic compounds found in many synthetic and natural products. They possess a wide variety of pharmacological activities, such as antiviral,[1] antitumour,[2] antibacterial,[3] anti-HIV,[4] antioxidant and anti-inflammatory[5] properties. Some of the flavanones displayed enhanced bioactivity after structural modification. Thus flavanones containing a 1,2,3-thiadiazoline ring have been found to have effective antiproliferative activity *in vivo*.[6]

Previously, we reported a new method for the preparation of 2,4-diaryl- 2,4-dihydrochromeno[4,3-*c*]pyrazoles **5** from flavanone-4- arylhydrazones utilising a Vilsmeier reagent.[7] As the extension of our previous work, we describe their analogues: 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyrazoles **4** (Scheme 1). We have evaluated their antitumour activities *in vitro* against MCF-7 and HL-60 cells.

Results and discussion

β-Chlorovinylaldehydes are important synthons in the synthesis of many natural products. An earlier report[8] showed that 4-chloro-2-aryl-2*H*- chromene-3-carbaldehydes can be easily synthesised in good yields by corresponding treatment of the flavanones treated with the Vilsmeier reagent (prepared from $POCl_3$ and DMF).[8] However, this reaction employs the toxic reagent $POCl_3$ which is harmful to human health and is an environmental pollutant. Recently, we have shown that *bis*-(trichloromethyl)carbonate (BTC, triphosgene) can be employed as a mild, highly efficient and environmentally benign reagent for the preparation of a novel Vilsmeier reagent in place of $POCl_3$ and $COCl_2$.[9–11] We provide here an improved method for the preparation of 4-chloro-2-aryl-2*H*-chromene-3-carbaldehydes **2** from the corresponding flavanones **1** using a Vilsmeier reagent derived from BTC and DMF as shown in Scheme 2. The flavanones **1** were easily prepared by the previously reported methods in good yields from commercially available arylketones and arylaldehydes.[12]



Scheme 1

In order to optimise the reaction conditions, the reaction of 2-*p*-tolylchroman-4-one **1a** and BTC/DMF was chosen as a model reaction. We first examined the influence of the ratio of **1a**/BTC/DMF using $ClCH_2CH_2Cl$ as solvent. It was found that a molar ratio 1:1:3 of **1a**/BTC/DMF was suitable. If the ratio was decreased, the yield was reduced dramatically. Then the effect of temperature on the reaction was studied. The reaction of compound **1a** with Vilsmeier reagent (BTC/DMF) was first attempted at room temperature for 5 h but it provided the product **2a** in low yield (60%), and the starting material **1a** was not completely consumed as indicated by TLC. When we treated of **1a** with BTC/DMF at 60 °C for 3 h, the reaction proceeded smoothly in 85% yield. Subsequently, solvent screening was performed by means of the reaction of **1a** with BTC/DMF under these conditions. Several conventional organic solvents such as CH_2Cl_2, CH_3CN, DMF were tested, and DMF was found to be more suitable due to its excellent dissolving power, and the yield of **2a** increased to 90%.

Our initial investigation into the synthesis of 1-phenyl-4-*p*-tolyl-1,4-dihydrochr-omeno[4,3-*c*]pyrazole **4a** used the reaction of the corresponding 4-chloro-2-*p*-tolyl-2*H*-chromene-3-carbaldehyde **2a** and commercially available phenylhydrazine hydrochloride in refluxing EtOH in the presence of triethylamine. Unfortunately, the desired product **4a** was not obtained. Instead, the intermediate 1-[(4-chloro-2-*p*-tolyl-2*H*-chromen-3-yl)methylene]-2-phenylhydrazine **3a** was obtained in good yield (92%). Numerous reagents (pyridine, AcONa, DABCO and K_2CO_3), temperature and solvents (THF, CH_3CN DMF and AcOH) were employed, but none furnished the cyclic product **4a**. To our delight, the target compound **4a** was obtained in 48% yield from corresponding **3a** by refluxing in DMF in the presence of NaH under an atmosphere of N_2.

The subsequent study was performed under the optimal conditions described above. Most of the products, 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyrazoles, were obtained in 30–40% yields (overall yields based on flavanones **1**). Their structures were confirmed by IR, ^1H NMR, ^{13}C NMR, MS and HRMS.

The compounds **4a–k** and **5a–i** were screened for their antiproliferative activity *in vitro* against two tumour cell lines including the human breast cancer cell line MCF-7 and the human leukaemia cell line HL-60. The antitumour drug, cisplatin, was used together as a positive control in the assays. The results are summarised in Table 1. Some general conclusions can be drawn from the experiment.

(1) Cytotoxicity assay demonstrated that most of compounds **4** were potentially efficient antitumour compounds against the HL-60 cell, and displayed more effective activity than **5** in this test.

(2) The compound **4e** exhibited potent antiproliferative activity against both the MCF-7 and HL-60 cells with IC_{50} values of 25.31 μg mL^{-1} and 13.86 μg mL^{-1} respectively.

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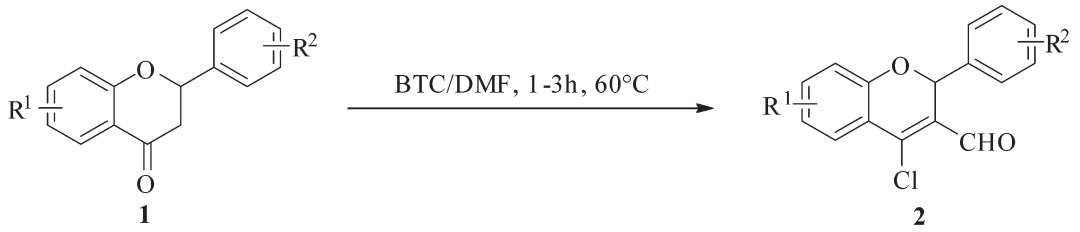
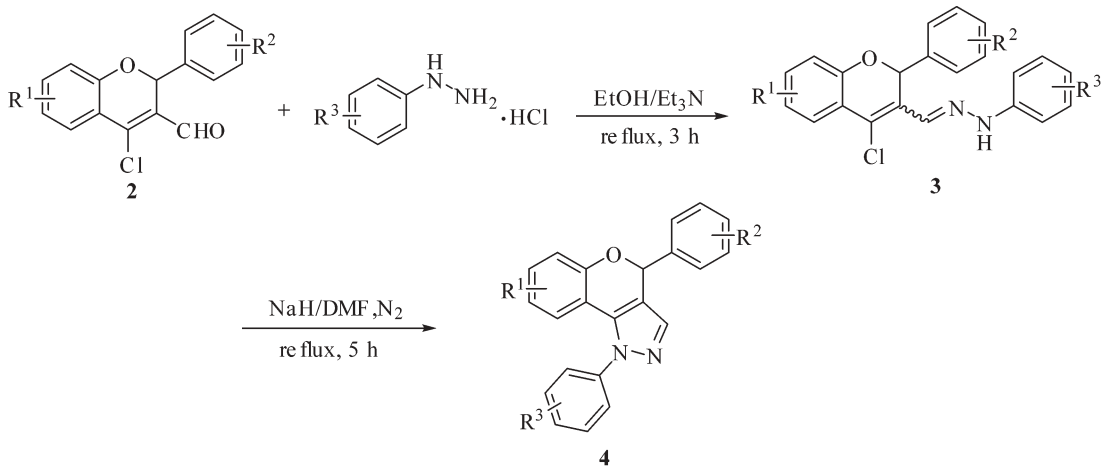


a) R^1=H, R^2=4-CH_3; b) R^1=R^2=H; c) R^1=H, R^2=4-Cl;

d) R^1 =H, R^2=4-OCH_3; e) R^1=5-CH_3, R^2=H; f) R^1=5-CH_3, R^2=4-Cl

Scheme 2



Scheme 3

(3) The compounds **4a, 4c, 4d, 4e, 4f and 4j** showed high activity against the HL-60 cell but low activity against the MCF-7 cell. Among them, **4a** and **4d** displayed more effective activity against the HL-60 cell than cisplatin.

In conclusion, a new series of 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyrazoles have been synthesised from corresponding flavanones by a novel and efficient method. Then we studied the antitumour activities *in vitro* of the target compounds and their analogues (2,4-diaryl-2,4-dihydrochromeno [4,3-*c*]pyrazoles). Most of the compound **4** displayed more effective activity than **5** in this test. In particular, **4e** showed potent activity against both the HL-60 and MCF-7 cells, whilst **4a** and **4d** displayed more effective activity against the HL-60 cell than cisplatin.

Experimental

All reagents were purchased from commercial suppliers and were used without further purification. Melting points were measured on a Büchi B-540 capillary melting point apparatus and are uncorrected. The NMR spectra were measured with a Varian 400 (400 MHz) instrument using $CDCl_3$ as the solvent with TMS as internal standard. IR spectra were recorded using KBr pellets on a Nicolet Aviatar-370 instrument. Mass spectra were measured with Thermo Finnigan LCQ-Advantage. High resolution mass spectral (HRMS) analysis was measured on an Agilent 6210 TOF LC/MS using ESI or EI (electrospray ionisation) techniques. The tumour cell lines (Bel-72 and HL-60) were obtained from Shanghai Institutes for Biological Sciences. Cisplatin was the product of Qilu Pharmaceutical Co., Ltd.

Synthesis of 4-chloro-2-p-tolyl-2H-chromene-3-carbaldehyde **(2a)***; general procedure*

BTC (0.59 g, 2 mmol) was slowly added to DMF (3 mL) at 0 ºC, the mixture was stirred at room temperature for 30 min, then 2-*p*-tolylchroman-4-one **1a** (2 mmol) was added, the reaction mixture was kept at 60 °C for 2 h. After completion of the reaction, the reaction mixture was poured into 10 mL of ice-water, neutralised with saturated $NaHCO_3$ solution and extracted with dichloromethane (15 mL× 3). The combined organic phases were washed with water, brine, dried

Table 1 *In vitro* cytotoxic activity of 1,4-diaryl-1,4-dihydrochromeno[4,3-*c*]pyr-azoles **4a–k** and 2,4-diaryl-2,4-dihydrochromeno[4,3-*c*]pyrazoles **5a–i**

Entry	R^1	R^2	R^3	Compounds	IC_{50}: μg mL^{-1}	
					MCF-7	HL-60
1	H	4-CH_3	H	**4a**	>50	13.23
2	H	4-CH_3	4-Cl	**4b**	>50	>50
3	H	H	H	**4c**	>50	19.43
4	H	4-Cl	H	**4d**	>50	12.14
5	H	4-Cl	4-Cl	**4e**	25.31	13.86
6	H	4-Cl	4-OCH_3	**4f**	>50	27.37
7	H	4-OCH_3	H	**4g**	>50	36.84
8	5-CH_3	H	H	**4h**	>50	46.81
9	5-CH_3	H	4-Cl	**4i**	>50	33.60
10	5-CH_3	4-Cl	H	**4j**	>50	29.04
11	5-CH_3	4-Cl	4-Cl	**4k**	>50	>50
12	H	4-Cl	H	**5a**	>50	>50
13	H	4-Cl	4-Cl	**5b**	>50	>50
14	H	4-Cl	4-OCH_3	**5c**	>100	>100
15	H	4-OCH_3	H	**5d**	>100	>100
16	H	4-OCH_3	4-OCH_3	**5e**	>100	>100
17	H	3,4-CH_3	H	**5f**	>100	>100
18	5-CH_3	H	H	**5g**	>100	>100
19	5-CH_3	4-OCH_3	4-OCH_3	**5h**	>100	>100
20	5-CH_3	4-Cl	H	**5i**	>100	>100
21	Cisplatin				16.52	13.37

over Na_2SO_4, evaporated. The product were recrystallised from methanol to give yellow crystals **2a** in 90% yield.

4-Chloro-2-p-tolyl-2H-chromene-3-carbaldehyde (**2a**): Yellow solid; m.p. 97–98 °C (lit.[8] 98 °C); IR (KBr): ν_{max} = 2860, 1667, 1601, 1294 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 10.27 (s, 1H), 7.71 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.32–7.28 (m, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.02–6.98 (m, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.36 (s, 1H) 2.28 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 187.8, 154.9, 143.4, 138.5, 135.0, 134.2, 129.1 (CH×2), 126.8, 126.7 (CH×2), 126.3, 121.7, 119.9, 117.3, 75.0, 21.2.; MS (EI): *m/z* (%) = 284 (25), 255 (100).

*Synthesis of 1-[(4-chloro-2-p-tolyl-2H-chromen-3-yl)-methylene]-2-phenylhydrazine (***3a***); general procedure*

A solution of **2a** (1 mmol), phenylhydrazine hydrochloride (2 mmol) and triethylamine (2 mmol) in anhydrous ethanol (10 mL) was refluxed for 5 h. After completion of the reaction, the mixture was then cooled to room temperature and evaporated under reduced pressure, poured into 20 mL of water and extracted with dichloromethane (15 mL× 3). The combined organic phases were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum, the crude product was further purified using column chromatography (silica gel, petroleum ether: EtOAc = 30:1, v/v) to give **3a** in 92% yield.

1-[(4-Chloro-2-p-tolyl-2H-chromen-3-yl)methylene]-2-phenylhydrazine (**3a**): Yellow solid; m.p. 159–161 °C; IR (KBr): ν_{max} = 3312, 1598, 1545, 1258 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.86 (s, 1H), 7.73 (s, 1H), 7.53–7.51 (m, 1H), 7.28 (s, 1H), 7.21–7.17 (m, 2H), 7.13–7.10 (m, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.93–6.78 (m, 6H), 6.56 (s, 1H), 2.23(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.8, 143.4, 138.0, 135.7, 132.7, 130.4, 129.1 (CH×2), 128.9 (CH×2), 127.1 (CH×2), 126.5, 125.8, 124.4, 121.6, 121.3, 120.6, 116.7, 112.8 (CH×2), 76.6, 21.2; MS (ESI): *m/z* (%) = 397.1 (M^++Na, 100), 399.1 (M^++Na+2, 30). HRMS-ESI: *m/z* (M^++1), Calcd for $C_{22}H_{18}ClN_2O$: 375.1264; found: 375.1273.

*Synthesis of 1-phenyl-4-p-tolyl-1,4-dihydrochromeno- [4,3-c]pyrazole (***4a***); general procedure*

A 25-mL two-necked flask was equipped with a stir bar, condenser, and placed under a nitrogen atmosphere. Into the flask was placed **3a** (3 mmol) and 3 mmol of a 60% mineral oil dispersion of NaH. Dry DMF (10 mL) was added, the mixture was stirred for 10 min at room temperature and then refluxed for 5 h. The mixture was then cooled to room temperature and poured into 20 mL of water, and extracted with dichloromethane (15 mL× 3). The combined organic phases were washed with water, brine, dried over Na_2SO_4, evaporated, and the crude product was purified by chromatography on silica gel (petroleum ether: EtOAc = 40:1, v/v) to give **4a** in 48% yield.

1-Phenyl-4-p-tolyl-1,4-dihydrochromeno[4,3-c]pyrazole (**4a**): Overall yield: 40%; yellow solid; m.p. 108–109 °C; IR (KBr): ν_{max} = 2863, 1453, 1211, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.56–7.45 (m, 5H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.30 (s, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.15–7.11 (m, 1H), 7.01–6.99 (m, 1H), 6.86–6.84 (m, 1H), 6.76–6.70 (m, 1H), 6.34 (s, 1H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.5, 140.6, 138.3, 137.0, 136.0, 133.7, 129.5, 129.4, 129.2 (CH×4), 128.7, 127.4 (CH×2), 126.2 (CH×2), 122.4, 121.1, 118.0, 115.8, 77.0, 21.2. MS (ESI): *m/z* = 339.3 (M^++1). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{19}N_2O$: 339.1497; found: 339.1498.

1-(4-Chlorophenyl)-4-p-tolyl-1,4-dihydrochromeno[4,3-c]pyrazole **(4b):** Overall yield: 31%; yellow solid; m.p. 148–150 °C; IR (KBr): ν_{max} = 2923, 1493, 1455, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48 (s, 4H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 1H), 7.22 (s, 1H), 7.20 (s, 1H), 7.18–7.11 (m, 1H), 7.02–7.00 (m, 1H), 6.88–6.86 (m, 1H), 6.79–6.75 (m, 1H), 6.31 (s, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.4, 138.9, 138.5, 136.5, 136.3, 134.5, 133.7, 129.7, 129.4 (CH×2), 129.2 (CH×2), 127.4 (CH×2), 127.2 (CH×2), 122.3, 121.2, 118.3, 118.1, 115.5, 76.9, 29.8; MS (ESI): *m/z* (%) = 373.2 (M^++1, 100), 375.2 (M^++3, 33). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{18}ClN_2O$: 373.1108; found: 373.1109.

1,4-Diphenyl-1,4-dihydrochromeno-[4,3-c]pyrazole (**4c**): Overall yield: 37%; yellow solid. m.p. 110–111 °C; IR (KBr): ν_{max} = 3066, 1511, 1496, 1212 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.55–7.48 (m, 7H), 7.43–7.37 (m, 3H), 7.30 (s, 1H), 7.17–7.10 (m, 1H), 7.03–7.01 (m, 1H), 6.86–6.84 (m, 1H), 6.76–6.72 (m, 1H), 6.38 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.4, 140.3, 139.6, 136.1, 133.7, 129.8, 129.4 (CH×2), 129.0, 128.9, 128.8 (CH×2), 127.7 (CH×2), 126.2 (CH×2), 122.6, 121.5, 118.2, 118.0, 115.9, 77.0; MS (ESI): *m/z* = 325.3 (M^++1). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{22}H_{17}N_2O$: 325.1341; found: 325.1345.

4-(4-Chlorophenyl)-1-phenyl-1,4-dihydrochromeno[4,3-c]pyrazole (**4d**): Overall yield: 39%; yellow solid; m.p. 113–115 °C; IR (KBr): ν_{max} = 2924, 1555, 1446, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51-7.50 (m, 5H), 7.45–7.36 (m, 4H), 7.29 (s, 1H), 7.16–7.12 (m, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.85–6.83 (m, 1H), 6.76–6.72 (m, 1H), 6.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.9, 140.0, 137.9, 135.6, 134.4, 133.5, 129.6, 129.2 (CH×2), 128.8 (CH×2), 128.7 (CH×2), 125.9 (CH×2), 122.4, 121.4, 117.9, 117.2, 115.5, 76.2; MS (ESI): *m/z* (%) = 359.4 (M^++1, 100), 361.3(M^++3, 35). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{22}H_{16}ClN_2O$: 359.0951; found: 359.0955.

1,4-Bis(4-chlorophenyl)-1,4-dihydrochromeno[4,3-c]pyrazole (**4e**): Overall yield: 38%; yellow solid; m.p. 161–162 °C; IR (KBr): ν_{max} = 3085, 1493, 1453, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.50–7.45 (m, 4H), 7.43–7.35 (m, 4H), 7.29 (s, 1H), 7.18–7.14 (m, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.88–6.86 (m, 1H), 6.78 (t, *J* = 7.6 Hz, 1H), 6.33 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.9, 138.5, 137.8, 136.0, 134.6, 134.5, 133.6, 129.9 (CH×2), 129.4 (CH×2), 128.8 (CH×2), 127.1 (CH×2), 122.3, 121.5, 118.1, 117.7, 115.3, 76.1; MS (ESI): *m/z* (%) = 393.4 (M^++1, 100), 395.4(M^++3, 66). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{22}H_{15}Cl_2N_2O$: 393.0561; found: 393.0564.

4-(4-chlorophenyl)-1-(4-methoxyphenyl)-1,4-dihydrochromeno[4,3-c] pyrazole (**4f**): Overall yield: 34%; yellow solid; m.p. 195–197 °C; IR (KBr): ν_{max} = 2960, 1526, 1248, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.40 (m, 4H), 7.38 (s, 1H), 7.35 (s, 1H), 7.26 (s, 1H), 7.17–7.10 (m, 1H), 7.02–6.98 (m, 3H), 6.83–6.81 (m, 1H), 6.76–6.72 (m, 1H), 6.35 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.7, 152.9, 138.0, 135.3, 134.4, 133.5, 133.0, 129.5, 128.8 (CH×2), 128.7 (CH×2), 127.3 (CH×2), 122.3, 121.4, 117.8, 116.6, 115.6, 114.4 (CH×2), 76.2, 55.6; MS (ESI): *m/z* (%) = 389.2 (M^++1, 100), 391.2 (M^++3, 29). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{18}ClN_2O_2$: 389.1057; found: 389.1063.

4-(4-Methoxyphenyl)-1-phenyl-1,4-dihydrochromeno[4,3-c]pyrazole (**4g**): Overall yield: 30%; yellow solid; m.p. 166–168 °C; IR (KBr): ν_{max} = 2958, 1514, 1454, 1247 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.57–7.41 (m, 7H), 7.29 (s, 1H), 7.16–7.09 (m, 1H), 7.00–6.98 (m, 1H), 6.95–6.90 (m, 2H), 6.86–6.83 (m, 1H), 6.75–6.71 (m, 1H), 6.32 (s, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.8, 153.2, 140.1, 135.9, 133.6, 131.4, 129.5, 129.2 (CH×2), 129.1 (CH×2), 128.7, 125.9, 122.4, 121.2, 117.9 (CH×2), 115.6, 113.9 (CH×2), 76.8, 55.3; MS (ESI): *m/z* = 355.2 (M^++1). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{19}N_2O_2$: 355.1447; found: 355.1446.

8-Methyl-1,4-diphenyl-1,4-dihydrochromeno[4,3-c]pyrazole (**4h**): Overall yield: 39%; yellow solid; m.p. 122–124 °C; IR (KBr): ν_{max} = 2923, 1468, 1221, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.55–7.50 (m, 7H), 7.40–7.36 (m, 3H), 7.29 (s, 1H), 6.96–6.90 (m, 2H), 6.64 (s, 1H), 6.32 (s, 1H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.1, 140.2, 139.5, 135.8, 133.7, 130.4, 130.1, 129.1 (CH×2), 128.7, 128.5 (CH×4), 127.4 (CH×2), 125.9, 122.9, 117.9, 117.6, 115.4, 76.8, 20.8; MS (ESI): *m/z* = 339.2 (M^++1). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{19}N_2O$: 339.1497; found: 339.1498.

1-(4-Chlorophenyl)-8-methyl-4-phenyl-1,4-dihydrochromeno[4,3-c] pyrazole (**4i**): Overall yield: 31%; yellow solid; m.p. 131–133 °C; IR (KBr): ν_{max} = 2922, 1494, 1212, 834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51–7.46 (m, 6H), 7.42–7.34 (m, 3H), 7.29 (s, 1H), 6.98–6.97 (m, 1H), 6.93–6.91 (m, 1H) 6.69 (s, 1H), 6.29 (s, 1H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.4, 139.6, 139.0, 136.6, 134.7, 134.1, 131.0, 130.7, 129.6 (CH×2), 128.9, 128.8 (CH×2), 127.7 (CH×2), 127.3 (CH×2), 123.0, 118.7, 118.2, 115.5, 77.1, 21.2; MS (ESI): *m/z* (%) = 373.1 (M^++1, 100), 375.1 (M^++3, 33). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{18}ClN_2O$: 373.1108; found: 373.1106.

4-(4-Chlorophenyl)-8-methyl-1-phenyl-1,4-dihydrochromeno[4,3-c] pyrazole (**4j**): Overall yield: 34%; yellow solid; m.p. 136–138 °C; IR (KBr): ν_{max} = 2921, 1521, 1471, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51–7.49 (m, 5H), 7.43–7.41 (m, 2H), 7.36–7.34 (m, 2H), 7.28 (s, 1H), 6.95–6.88 (m, 2H), 6.62 (s, 1H), 6.29 (s, 1H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.7, 139.9, 137.9, 135.6, 134.3, 133.6, 130.6, 130.1, 129.1 (CH×2), 128.8 (CH×2), 128.6 (CH×2), 125.8 (CH×2), 122.9, 117.6, 117.4, 115.2, 76.0, 20.9; MS

(ESI): *m/z* (%) = 373.2 (M^++1, 100), 375.2 (M^++3, 32). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{18}ClN_2O$: 373.1108; found: 373.1101.

1*,4-Bis(4-chlorophenyl)-8-methyl-1,4-dihydrochromeno[4,3-c] pyrazole* (**4k**): Overall yield: 39%; yellow solid; m.p. 176–178 °C; IR (KBr): ν_{max} = 2920, 1492, 1216, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48 (s, 4H), 7.42-7.34 (m, 4H), 7.28 (s, 1H), 6.98–6.96 (m, 1H), 6.91–6.89 (m, 1H), 6.68 (d, *J* = 1.2 Hz, 1H), 6.27 (s, 1H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.7, 138.5, 137.7, 136.0, 134.4, 133.7, 130.9, 130.4, 129.3 (CH×2), 128.7 (CH×2), 128.7 (CH×2), 126.9 (CH×2), 122.7, 117.9, 117.8, 115.1, 76.0, 21.0; MS (ESI): *m/z* (%) = 407.2 (M^++1, 100), 409.2 (M^++3, 44). HRMS-ESI: *m/z* (M^++1) Calcd for $C_{23}H_{17}Cl_2N_2O$: 407.0718; found: 407.0718.

Cytotoxic activity against human breast cancer cell line (MCF-7) and human leukaemia cell line (HL-60) in vitro

The cytotoxic activity *in vitro* was measured using the MTT method: Cells were seeded in 96-well plates at 10,000 cells per well in 100 μL of complete DMEM medium supplemented with 5% FBS, After incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. MTT solution (5 mg mL^{-1}) in normal saline was added after cells were treated with drug for 72 h, and cells were incubated for a further 3 h at 37 °C. The formazan crystals were dissolved in 150 μL DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer at 570 nm. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software, Dose-Effect Analysis with Microcomputers.

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