Synthesis and Cytotoxic Activity of Some Novel Dihyrobenzo[*h*]pyrano [3,2-*c*]chromene Derivatives

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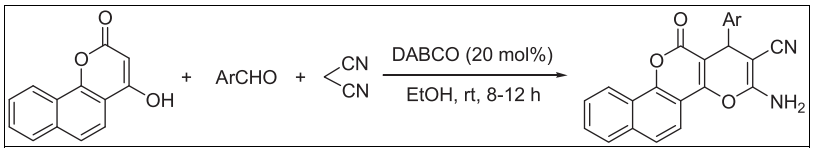
Received December 9, 2012
DOI 10.1002/jhet.1991
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).



Three-component reaction of 4-hydroxy-2*H*-benzo[*h*]chromen-2-one, aromatic aldehydes, and malononitrile in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) in ethanol at room temperature affords good yields of novel dihyrobenzo[*h*]pyrano[3,2-*c*]chromene derivatives. The synthesized compounds examined by MTT assays for cytotoxic activity in two human cancer cell lines (MOLT-4, HL-60). Most of the evaluated compounds showed low inhibitory activity against tumor cell line at micromolar concentrations.

J. Heterocyclic Chem., **00,** 00 (2014).

INTRODUCTION

Organic and medicinal significance of fused pyranochromene derivatives, one of the most important oxygen heterocycles, has been of interest mainly to chemists. Besides their widely presence in natural products [1], privileged biological properties such as antioxidant, anticancer, anti-inflammatory, antiviral, antibacterial, and anti-HIV activities have received increased attention [2]. Also, chromene moieties are of particular importance as they have represented potential diuretic, analgesic, and myorelaxant activities [3]. These facts provide continued impetus to develop a wide range of methods for the synthesis of novel fused pyran and chromene derivatives [4–8] underlying successful and important drug discoveries with a minimum number of synthetic steps to assemble compounds with interesting properties [9,10].

To meet this demand for access to a library of new pyranochromenes, multicomponent reactions (MCRs), as a powerful synthetic tool in the synthesis of heterocycles [11], can play an important role due to intrinsic benefits including atom economy, simple procedures, and straightforward reaction design [12–15].

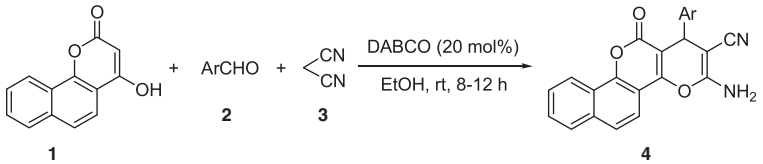
Among different reports, three-component reaction of aldehydes, malononitrile, and 4-hydroxycoumarin for the synthesis of pyrano[3,2-*c*]chromene derivatives [16] absorbed our attention. As a part of our research programs to synthesize new heterocycles [17] and also find novel cytotoxic compounds [18], we decided to investigate three-component reaction of 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **1**, aldehydes **2**, and malononitrile **3** (Scheme 1). Our results revealed that replacing 4-hydroxycoumarin with 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **1** led to the formation of novel pyrano[3,2-*c*]chromene derivatives **4** (Scheme 1) that were screened for their cytotoxic activities.

RESULTS AND DISCUSSION

Chemistry. To obtain novel pyranochromenes, we employed 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **1**, easily prepared from α-naphthol and malonic acid [9,19], as an efficient starting material containing suitable functional groups to afford the corresponding products **4** in the designed three-component reaction (Scheme 1).

To optimize the reaction conditions, reaction of 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **1** (1 mmol), benzaldehyde **2a** (1 mmol), and malononitrile **3** (1 mmol) was investigated as a model reaction in various conditions. Some results are summarized in Table 1. As can be seen in Table 1, when the model reaction was carried out in the absence of catalyst, either under solvent-free conditions or using solvent, both at room and evaluated temperatures, no product was obtained. Then, a variety of acidic and basic catalysts such as *p*-toluenesulfonic acid (*p*-TSA), Et_3N, piperidine, and DABCO were examined. The results showed that DABCO exhibited good activity, and the other mentioned catalysts were not effective and did not catalyze the model reaction efficiently. Solvent optimization results showed that ethanol is the most appropriate medium in this heterocyclization reaction, and the best results were obtained when the model reaction was achieved in the presence of DABCO in EtOH at room temperature (Table 1). It should be noted that evaluating temperature did not give any improvement

Scheme 1. Synthesis of dihyrobenzo[*h*]pyrano[3,2-*c*]chromenes **4**.



1 2 3 4

in the yield and reaction time. Also, it was found that 20 mol% of DABCO was sufficient to obtain the best yield. Under these conditions, the corresponding product **4a** was obtained in good yield (75%) after 10 h.

With optimal conditions established, we generalized our synthetic strategy for the construction of various dihyrobenzo [*h*]pyrano[3,2-*c*]chromene, we subjected a series of aromatic aldehydes having electron-donating as well as electron-withdrawing substituent's on *ortho*, *meta*, and *para* positions to obtain the corresponding compounds **4**. The results showed that the three-component reaction showed reasonable tolerance toward different aromatic aldehydes, and all products were obtained in good yields. As indicated in Table 2, the yield of the products seems to be fairly affected by the nature of substituents and their positions on benzaldehyde. It increases when electron-withdrawing *para*-substituents are present and the steric effect in the case of *ortho*-substituents benzaldehyde is evident and lower yields are observed.

Biological investigation. The cytotoxic activities of newly synthesized derivatives were assessed in two human cancer cell lines, and IC50 values were calculated for each derivative (Table 3). On the basis of IC50 values, it is obvious that most of the compounds have no effect on the two cell lines, because in most cases, the IC50 of these analogs was higher than 50 or 100 μM. In MOLT4 cell line, only six compounds, **4b**, **4d**, **4g**, **4l**, **4m**, and **4o** had an IC50 lower than 100 μM which their IC50 values were 78.2, 68.8, 99.4, 98.4, 58.5 and 61.2, respectively. Therefore, the most potent compounds in this cell line based on IC50 in a decreasing order of efficiency were **4m** > **4o** > **4d** > **4b** > **4l** > **4g**, and the rest of the compounds have no effect on HL-60 and MOLT4 cell lines. It seems that the introduction of chlorophenyl moiety on central dihyrobenzo[*h*]pyrano[3,2-*c*]chromene ring might improve cytotoxic activity of compounds.

Table 1

Investigation of various conditions for a one-pot three-component reaction of 4-hydroxy-2*H*-benzo[*h*]chromene-2-one **1**, benzaldehyde **2a**, and malononitrile **3**.

Entry	Conditions	Catalyst	Time (h)	Yield (%)
1	Solvent-free[a]	–		0
2	Ethanol/room temperature	Et_3N	24	10
3	Ethanol/room temperature	Piperidine	24	15
4	Acetonitrile/room temperature	$NaHCO_3$	24	20
5	Acetonitrile/reflux	DABCO	24	10
6	Ethanol /room temperature	DABCO	10	75
7	Toluene/reflux	DABCO	24	Trace
8	CH_2Cl_2/reflux	*p*-TSA	24	Trace

[a]At room and evaluated temperature.

CONCLUSIONS

In summary, we have developed a convenient and efficient synthesis of novel dihyrobenzo[*h*]pyrano[3,2-*c*] chromenes by the reaction of 4-hydroxy-2*H*-benzo[*h*] chromen-2-one, aromatic aldehydes, and malononitrile in the presence of DABCO in EtOH at room temperature. In addition, the results of cytotoxic activity evaluation showed that the *in vitro* anticancer effect of synthesized compounds are mainly low, it seems that this structure can be used as a novel cytotoxic scaffold for further modification and design of novel potent compounds.

EXPERIMENTAL

Chemistry. Chemistry. Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker FT-500 (Germany), using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks). The elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode (Germany).

Procedure for the synthesis of 4-hydroxy-2*H*-benzo[*h*] chromen-2-one (1). A mixture of α-naphthol (0.14 g, 1 mmol), malonic acid (0.10 g, 1 mmol), and polyphosphoric acid (0.1 g) was heated at 75°C. After completion of the reaction (checked by TLC), water was added to the reaction mixture, and the residue was extracted with ethyl acetate. The organic layer was separated, dried (Na_2SO_4), and the solvent was removed under vacuum. The resulting residue was purified using silica gel flash column chromatography (petroleum ether/ethyl acetate = 1/1).

Procedure for the synthesis of dihyrobenzo[*h*]pyrano[3,2-*c*] chromene (4). A solution of 4-hydroxy-2*H*-benzo[*h*]chromen-2-one (0.21 g, 1 mmol), aromatic aldehyde (1 mmol), and malononitrile (0.07 g, 1 mmol) in EtOH (5 mL) was stirred at room temperature. Upon completion of the reaction, monitored by TLC, the precipitated product was separated by filtration, washed with water, and recrystallized from EtOH to give compound **4** as a creamy powder.

3-Amino-12-oxo-1-phenyl-1,12-dihydrobenzo[h]pyrano[3,2-c]chromene-2-carbonitrile (4a). Yield 75%, mp 281–283°C. IR (KBr, υ, cm^{-1}): 3394, 3320 (NH_2), 2200 (CN), 1717 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.48 (s, 1H, CH, H_1), 7.23–7.34 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.43 (s, 2H, NH_2),

Table 2

Synthesis of dihyrobenzo[*h*]pyrano[3,2-*c*]chromenes derivatives **4**.

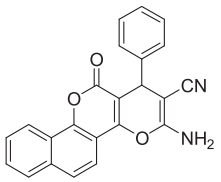
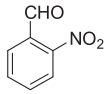
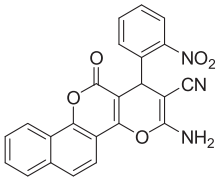
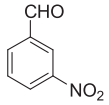
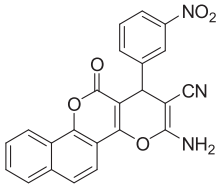
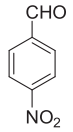
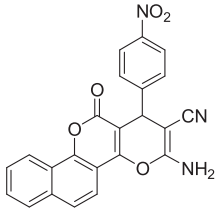
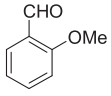
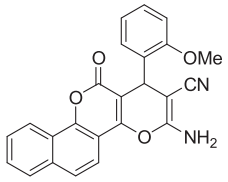
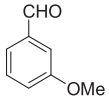
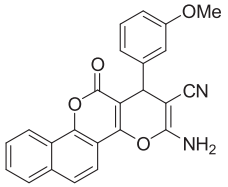
Entry	Aromatic aldehyde **2**	Product **4**	Yield (%)[a]
1		**4a** 	75
2		**4b** 	78
3		**4c** 	80
4		**4d** 	88
5		**4e** 	75
6		**4f** 	78

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Table 2
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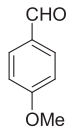
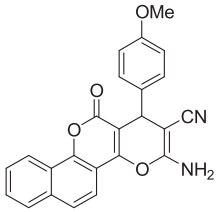
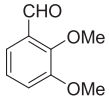
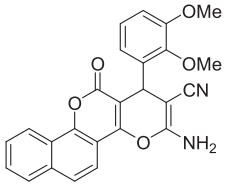
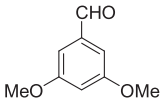
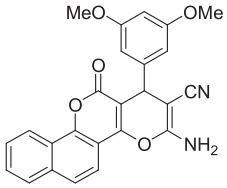
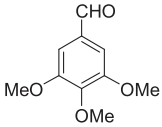
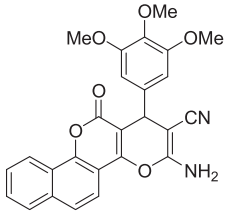
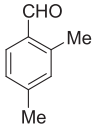
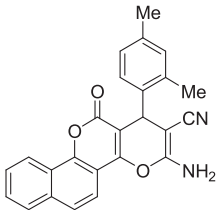
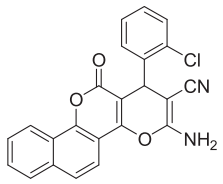
Entry	Aromatic aldehyde **2**	Product **4**	Yield (%)[a]
7		**4g** 	80
8		**4h** 	72
9		**4i** 	79
10		**4j** 	75
11		**4k** 	74
12		**4l** 	77

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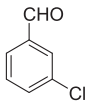
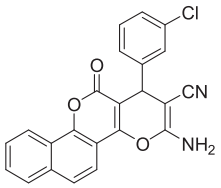
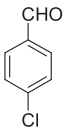
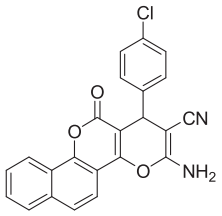
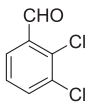
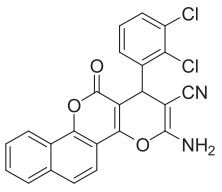
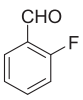
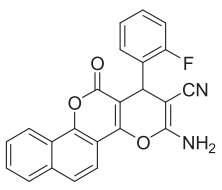
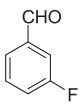
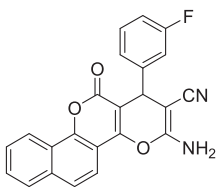
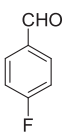
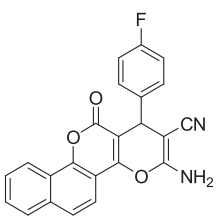
Table 2
(*Continued*)

Entry	Aromatic aldehyde **2**	Product **4**	Yield (%)[a]
13		**4m** 	73
17		**4n** 	81
15		**4o** 	70
16		**4p** 	86
17		**4q** 	79
18		**4r** 	90

[a]Isolated yields.

Table 3

Cytotoxic activity of newly synthesized compounds assessed by the MTT reduction assay.

Entry	Compounds	HL-60 cell line IC50(μM)	MOLT4 cell line IC50(μM)
1	**4a**	>50	>50
2	**4b**	>100	78.2 ± 3.7
3	**4c**	>100	>100
4	**4d**	>100	68.8 ± 3.2
5	**4e**	>100	>100
6	**4f**	>100	>100
7	**4g**	>100	99.4 ± 3.5
8	**4h**	>100	>100
9	**4i**	>100	>100
10	**4j**	>100	>100
11	**4k**	>50	>100
12	**4l**	>100	98.4 ± 1.7
13	**4m**	>100	58.5 ± 1.8
14	**4n**	>100	>100
15	**4o**	>100	61.2 ± 5.6
16	**4p**	>50	>50
17	**4q**	>50	>50
18	**4r**	>100	>100
19	Doxorubicin	ND	0.162 ± 0.3
20	Cisplatin	ND	3.6 ± 0.1

Values represent the mean ± SD of three to four different experiments. Compounds were tested at the maximum final concentration of 100 μM, except for compounds **4a**, **4p**, and **4q**, which were tested at 50 μM due to lower solubility.

7.70–7.78 (m, 2H, H_8, H_9), 7.88 (d, J = 8.8 Hz, 1H, H_5), 7.96 (d, J = 8.8 Hz, 1H, H_6), 8.07 (d, J = 8.0 Hz, 1H, H_7), 8.27 (d, J = 8.0 Hz, 1H, H_{10},); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 36.9, 57.9, 103.5, 108.4, 116.9, 117.9, 119.2, 121.4, 121.8, 124.4, 127.1, 127.6, 127.7, 128.2, 128.5, 129.1, 134.5, 143.3, 149.4, 154.2, 158.0, 159.4; *Anal*. Calcd for $C_{23}H_{14}N_2O_3$: C, 75.40; H, 3.85; N, 7.65. Found: C, 75.62; H, 4.10; N, 7.30.

3-Amino-1-(2-nitrophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4b). Yield 78%, mp 243–245°C. IR (KBr, υ, cm^{-1}): 3407, 3320 (NH_2), 2195 (CN), 1717 (CO), 1480, 1369 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 5.26 (s, 1H, CH, H_1), 7.50 (t, J = 7.5 Hz, 1H, $H_{4'}$), (d, J = 7.5 Hz, 1H, $H_{6'}$), 7.59 (s, 1H, NH_2), 7.66 (t, J = 7.5 Hz, 1H, $H_{5'}$), 7.69–7.76 (m, 2H, H_8, H_9), 7.87 (d, J = 8.7 Hz, 1H, H_5), 7.92 (d, J = 8.0 Hz, 1H, $H_{3'}$), 7.97 (d, J = 8.7 Hz, 1H, H_6), 8.07 (d, 1H, J = 8.0 Hz, H_7), 8.23 (d, 1H, J = 8.0 Hz, H_{10}); *Anal*. Calcd for $C_{23}H_{13}N_3O_5$: C, 67.15; H, 3.19; N, 10.21. Found: C, 66.90; H, 3.35; N, 10.45.

3-Amino-1-(3-nitrophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4c). Yield 80%, mp 275–278°C. IR (KBr, υ, cm^{-1}): 3383, 3314 (NH_2), 2196 (CN), 1706 (CO), 1480, 1375 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.75 (s, 1H, CH, H_1), 7.58 (s, 2H, NH_2), 7.63 (t, J = 8.0 Hz, 1H, $H_{5'}$), 7.70–7.77 (m, 2H, H_8, H_9), 7.84 (d, J = 8.0 Hz, 1H, $H_{6'}$), 7.87 (d, J = 8.7 Hz, 1H, H_5), 7.96 (d, J = 8.7 Hz, 1H, H_6), 8.07 (d, J = 7.9 Hz, 1H, H_7), 8.13 (d, J = 8.0 Hz, 1H, $H_{4'}$), 8.17 (s, 1H, $H_{2'}$), 8.26 (d, J = 7.9 Hz, 1H, H_{10}); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 30.6, 56.9, 102.3, 108.4, 117.9, 118.9, 121.4, 121.8, 122.2, 122.5, 124.4, 127.7, 128.1, 129.2, 130.0, 134.6, 134.8, 145.4, 147.8, 149.6, 154.6, 158.1, 159.5; *Anal*. Calcd for $C_{23}H_{13}N_3O_5$: C, 67.15; H, 3.19; N, 10.21. Found: C, 67.30; H, 3.05; N, 9.98.

3-Amino-1-(4-nitrophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4d). Yield 88%, mp 203–205°C. IR (KBr, υ, cm^{-1}): 3462, 34072 (NH_2), 2188 (CN), 1725 (CO), 1481, 1370 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.72 (s, 1H, CH, H_1), 7.59 (s, 2H, NH_2), 7.63 (d, J = 8.5 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.72–7.79 (m, 2H, H_8, H_9), 7.90 (d, J = 8.7 Hz, 1H, H_5), 7.99 (d, J = 8.7 Hz, 1H, H_6), 8.09 (d, J = 8.0 Hz, 1H, H_7), 8.19 (d, J = 8.5 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.30 (d, J = 8.0 Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{13}N_3O_5$: C, 67.15; H, 3.19; N, 10.21. Found: C, 67.44; H, 3.36; N, 10.40.

3-Amino-1-(2-methoxyphenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4e). Yield 75%, mp 279–281°C. IR (KBr, υ, cm^{-1}): 3374, 3325 (NH_2), 2195 (CN), 1712 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.72 (s, 3H, OCH_3), 4.73 (s, 1H, CH, H_1), 6.88 (t, J = 7.5 Hz, 1H, $H_{5'}$), 6.99 (d, J = 7.5 Hz, 1H, $H_{3'}$), 7.14 (d, J = 7.5 Hz, 1H, $H_{6'}$), 7.22 (t, J = 7.5 Hz, 1H, $H_{4'}$), 7.27 (s, 2H, NH_2), 7.68–7.75 (m, 2H, H_8, H_9), 7.89 (d, J = 8.7 Hz, 1H, H_5), 7.96 (d, J = 8.7 Hz, 1H, H_6), 8.06 (d, J = 8.0 Hz, 1H, H_7), 8.27 (d, J = 8.0 Hz, 1H, H_{10}); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 32.2, 55.7, 56.9, 102.8, 108.4, 111.8, 117.8, 119.4, 120.5, 121.3, 121.8, 124.4, 127.7, 128.1, 128.4, 129.0, 129.2, 130.7, 134.5, 149.2, 154.8, 157.2, 158.5, 159.4; *Anal*. Calcd for $C_{24}H_{16}N_2O_4$: C, 72.72; H, 4.07; N, 7.07. Found: C, 73.00; H, 4.33; N, 7.35.

3-Amino-1-(3-methoxyphenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4f). Yield 78%, mp 249–251°C. IR (KBr, υ, cm^{-1}): 3417, 3300 (NH_2), 2191 (CN), 1708 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.78 (s,3H, OCH_3), 4.48 (s, 1H, CH, H_1), 6.81–6.85 (m, 2H, $H_{2'}$, $H_{4'}$), 7.25 (d, J = 7.5 Hz, 1H, $H_{6'}$), 7.43 (s, 2H, NH_2), 7.70–7.77 (m, 2H, H_8, H_9), 7.87 (d, J = 8.7 Hz, 1H, H_5), 7.98 (d, J = 8.7 Hz, 1H, H_6), 8.08 (d, J = 8.0 Hz, 1H, H_7), 8.31 (d, J = 8.0 Hz, 1H, H_{10}); *Anal*. Calcd for $C_{24}H_{16}N_2O_4$: C, 72.72; H, 4.07; N, 7.07. Found: C, 72.44; H, 3.83; N, 6.89.

3-Amino-1-(4-methoxyphenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4g). Yield 80%, mp 275–276°C. IR (KBr, υ, cm^{-1}): 3366, 3313 (NH_2), 2195 (CN), 1708 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.72 (s, 3H, OCH_3), 4.42 (s, 1H, CH, H_1), 6.87 (d, J = 7.7 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.21 (d, J = 7.7 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.39 (s, 2H, NH_2), 7.71–7.74 (m, 2H, H_8, H_9), 7.87 (d, J = 8.3 Hz, 1H, H_5), 7.95 (d, J = 8.3 Hz, 1H, H_6), 8.06 (d, J = 7.5 Hz, 1H, H_7), 8.27 (d, J = 7.5 Hz, 1H, H_{10}); *Anal*. Calcd for $C_{24}H_{16}N_2O_4$: C, 72.72; H, 4.07; N, 7.07. Found: C, 72.50; H, 4.31; N, 6.84.

3-Amino-1-(2,3-dimethoxyphenyl)-12-oxo-1,12-dihydrobenzo [h]pyrano[3,2-c]chromene-2-carbonitrile (4h). Yield 72%, mp 282–284°C. IR (KBr, υ, cm^{-1}): 3442, 3332 (NH_2), 2192 (CN), 1721 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.72 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.73 (s, 1H, CH, H_1), 6.76 (d, J = 7.5 Hz, 1H, $H_{4'}$), 6.94 (d, J = 7.5 Hz, 1H, $H_{6'}$), 6.99 (d, J = 7.5 Hz, 1H, $H_{5'}$), 7.35 (s, 2H, NH_2), 7.71–7.77 (m, 2H, H_8, H_9), 7.91 (d, J = 8.7 Hz, 1H, H_5), 7.99 (d, J = 8.7 Hz, 1H, H_6), 8.09 (d, J = 8.0 Hz, 1H, H_7), 8.30 (d, J = 8.0 Hz, 1H, H_{10}); *Anal*. Calcd for $C_{25}H_{18}N_2O_5$: C, 70.42; H, 4.25; N, 6.57. Found: C, 70.16; H, 3.98; N, 6.28.

3-Amino-1-(3,5-dimethoxyphenyl)-12-oxo-1,12-dihydrobenzo [h]pyrano[3,2-c]chromene-2-carbonitrile (4i). Yield 79%, mp 269–271°C. IR (KBr, υ, cm^{-1}): 3442, 3356 (NH_2), 2198 (CN), 1714 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.71 (s, 6H, OCH_3), 4.44 (s, 1H, CH, H_1), 6.41 (s, 3H, $H_{2'}$, $H_{4'}$, $H_{6'}$), 7.42 (s, 2H, NH_2), 7.72–7.78 (m, 2H, H_8, H_9), 7.89 (d, J = 8.7 Hz, 1H, H_5), 7.97 (d, J = 8.7 Hz, 1H, H_6), 8.08 (d, J = 7.7 Hz, 1H, H_7),

8.31 (d, $J = 7.7$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{25}H_{18}N_2O_5$: C, 70.42; H, 4.25; N, 6.57. Found: C, 70.66; H, 4.48; N, 6.83.

3-Amino-12-oxo-1-(3,4,5-trimethoxyphenyl)-1,12-dihydrobenzo [h]pyrano[3,2-c]chromene-2-carbonitrile (4j). Yield 75%, mp 293–295°C. IR (KBr, υ, cm^{-1}): 3448, 3310 (NH_2), 2188 (CN), 1721 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.64 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 4.48 (s, 1H, CH, H_1), 6.57 (s, 2H, $H_{2'}$, $H_{6'}$), 7.41 (s, 2H, NH_2), 7.73–7.77 (m, 2H, H_8, H_9), 7.89 (d, $J = 8.7$ Hz, 1H, H_5), 7.97 (d, $J = 8.7$ Hz, 1H, H_6), 8.08 (d, $J = 8.0$ Hz, 1H, H_7), 8.31 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{26}H_{20}N_2O_6$: C, 68.42; H, 4.42; N, 6.14. Found: C, 68.22; H, 4.13; N, 5.93.

3-Amino-1-(2,4-dimethlphenyl)-12-oxo-1,12-dihydrobenzo [h]pyrano[3,2-c]chromene-2-carbonitrile (4k). Yield 74%, mp 251–253°C. IR (KBr, υ, cm^{-1}): 3411, 3321 (NH_2), 2197 (CN), 1735 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.21 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.73 (s, 1H, CH, H_1), 6.91–6.98 (m, 3H, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.35 (s, 2H, NH_2), 7.73–7.76 (m, 2H, H_8, H_9), 7.91 (d, $J = 8.7$ Hz, 1H, H_5), 7.99 (d, $J = 8.7$ Hz, 1H, H_6), 8.09 (d, $J = 8.0$ Hz, 1H, H_7), 8.29 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{25}H_{18}N_2O_3$: C, 76.13; H, 4.60; N, 7.10. Found: C, 76.30; H, 4.35; N, 7.34.

3-Amino-1-(2-chlorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4l). Yield 77%, mp 260–262°C. IR (KBr, υ, cm^{-1}): 3407, 3328 (NH_2), 2196 (CN), 1713 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 5.06 (s, 1H, CH, H_1), 7.27–7.28 (m, 2H, $H_{4'}$, $H_{6'}$), 7.34-7.35 (m, 1H, $H_{5'}$), 7.42–7.44 (m, 1H, $H_{3'}$), 7.46 (s, 2H, NH_2), 7.71–7.77 (m, 2H, H_8, H_9), 7.89 (d, $J = 8.7$ Hz, 1H, H_5), 7.98 (d, $J = 8.7$ Hz, 1H, H_6), 8.08 (d, $J = 7.8$ Hz, 1H, H_7), 8.28 (d, $J = 7.8$ Hz, 1H, H_{10}); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 34.3, 56.5, 102.4, 108.3, 117.9, 118.8, 121.4, 121.8, 124.5, 127.6, 127.8, 128.2, 128.8, 129.2, 129.5, 130.7, 132.4, 134.6, 140.2, 149.5, 154.8, 158.1, 159.2; *Anal*. Calcd for $C_{23}H_{13}ClN_2O_3$: C, 68.92; H, 3.27; N, 6.99. Found: C, 69.14; H, 2.98; N, 7.22.

3-Amino-1-(3-chlorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4m). Yield 73%, mp 259–261°C. IR (KBr, υ, cm^{-1}): 3384, 3320 (NH_2), 2207 (CN), 1699 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.54 (s, 1H, CH, H_1), 7.28–7.38 (m, 4H, $H_{2'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.50 (s, 2H, NH_2), 7.71–7.77 (m, 2H, H_8, H_9), 7.87 (d, $J = 8.7$ Hz, 1H, H_5), 7.96 (d, $J = 8.7$ Hz, 1H, H_6), 8.07 (d, $J = 8.0$ Hz, 1H, H_7), 8.28 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{13}ClN_2O_3$: C, 68.92; H, 3.27; N, 6.99. Found: C, 68.72; H, 3.05; N, 6.78.

3-Amino-1-(4-chlorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4n). Yield 81%, mp 244–246°C. IR (KBr, υ, cm^{-1}): 3385, 3305 (NH_2), 2197 (CN), 1710 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.53 (s, 1H, CH, H_1), 7.34 (d, $J = 7.5$ Hz, 2H, $H_{2'}$, $H_{6'}$), 7.37 (d, $J = 7.5$ Hz, 2H, $H_{3'}$, $H_{5'}$), 7.48 (s, 2H, NH_2), 7.72–7.76 (m, 2H, H_8, H_9), 7.89 (d, $J = 8.7$ Hz, 1H, H_5), 7.97 (d, $J = 8.7$ Hz, 1H, H_6), 8.07 (d, $J = 8.0$ Hz, 1H, H_7), 8.30 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{13}ClN_2O_3$: C, 68.92; H, 3.27; N, 6.99. Found: C, 68.64; H, 3.50; N, 7.31.

3-Amino-1-(2,3-dichlorophenyl)-12-oxo-1,12-dihydrobenzo [h]pyrano[3,2-c]chromene-2-carbonitrile (4o). Yield 70%, mp 256–259°C. IR (KBr, υ, cm^{-1}): 3432, 3311 (NH_2), 2195 (CN), 1709 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 5.11 (s, 1H, CH, H_1), 7.31–7.37 (m, 3H, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.53 (s, 2H, NH_2), 7.73–7.77 (m, 2H, H_8, H_9), 7.90 (d, $J = 8.7$ Hz, 1H, H_5), 7.99 (d, $J = 8.7$ Hz, 1H, H_6), 8.09 (d, $J = 8.0$ Hz, 1H, H_7), 8.30 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{12}Cl_2N_2O_3$: C, 63.47; H, 2.78; N, 6.44. Found: C, 63.19; H, 3.02; N, 6.71.

3-Amino-1-(2-fluorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4p). Yield 86%, mp 248–250°C. IR (KBr, υ, cm^{-1}): 3403, 3325 (NH_2), 2200 (CN), 1713 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.77 (s, 1H, CH, H_1), 7.14–7.19 (m, 2H, $H_{3'}$, $H_{5'}$), 7.30–7.40 (m, 2H, $H_{4'}$, $H_{6'}$), 7.48 (s, 2H, NH_2), 7.72–7.78 (m, 2H, H_8, H_9), 7.90 (d, $J = 8.7$ Hz, 1H, H_5), 7.99 (d, $J = 8.7$ Hz, 1H, H_6), 8.09 (d, $J = 8.0$ Hz, 1H, H_7), 8.30 (d, $J = 8.0$ Hz, 1H, H_{10}); ^{13}CNMR (125 MHz, DMSO-d_6) δ ppm: 31.3, 56.4, 102.19, 108.3, 115.4, 115.6, 117.8, 119.0, 121.4, 121.8, 124.5, 124.6, 127.8, 128.2, 129.2, 129.8, 129.9, 130.3, 134.6, 149.5, 154.7, 158.3, 159.3, 160.3 ($J_{C-F} = 231.2$ Hz); *Anal*. Calcd for $C_{23}H_{13}FN_2O_3$: C, 71.87; H, 3.41; N, 7.29. Found: C, 71.59; H, 3.18; N, 7.44.

3-Amino-1-(3-fluorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4q). Yield 79%, mp 270–272°C. IR (KBr, υ, cm^{-1}): 3415, 3326 (NH_2), 2199 (CN), 1712 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.56 (s, 1H, CH, H_1), 7.09 (dd, $J = 5.6$, 2.5 Hz, 1H, $H_{2'}$), 7.10-7.18 (m, 2H, $H_{4'}$, $H_{6'}$), 7.35–7.39 (m, 1H, $H_{5'}$), 7.48 (s, 2H, NH_2), 7.72–7.76 (m, 2H, H_8, H_9), 7.89 (d, $J = 8.7$ Hz, 1H, H_5), 7.97 (d, $J = 8.7$ Hz, 1H, H_6), 8.08 (d, $J = 8.0$ Hz, 1H, H_7), 8.29 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{13}FN_2O_3$: C, 71.87; H, 3.41; N, 7.29. Found: C, 71.54; H, 3.56; N, 6.98.

3-Amino-1-(4-fluorophenyl)-12-oxo-1,12-dihydrobenzo[h] pyrano[3,2-c]chromene-2-carbonitrile (4r). Yield 90%, mp 248–250°C. IR (KBr, υ, cm^{-1}): 3422, 3320 (NH_2), 2194 (CN), 1711 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.51 (s, 1H, CH, H_1), 7.31–7.34 (m, 2H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.45 (s, 2H, NH_2), 7.73–7.77 (m, 2H, H_8, H_9), 7.94 (d, $J = 8.7$ Hz, 1H, H_5), 7.98 (d, $J = 8.7$ Hz, 1H, H_6), 8.05 (d, $J = 8.0$ Hz, 1H, H_7), 8.26 (d, $J = 8.0$ Hz, 1H, H_{10}); *Anal*. Calcd for $C_{23}H_{13}FN_2O_3$: C, 71.87; H, 3.41; N, 7.29. Found: C, 72.08; H, 3.62; N, 7.50.

Biology. ***Reagents and chemicals.*** Roswell Park Memorial Institute (RPMI) 1640, fetal bovine serum (FBS) and phosphate-buffered saline (PBS) were purchased from Biosera (Ringmer, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) was obtained from Sigma (Saint Louis, MO, USA), and penicillin⁄streptomycin was purchased from Invitrogen (San Diego, CA, USA). Doxorubicin and dimethyl sulfoxide were obtained from EBEWE Pharma (Unterach, Austria) and Merck (Darmstadt, Germany), respectively.

Cell lines and cell culture. HL-60 (Human promyelocytic leukemia cells) and MOLT4 (Human acute lymphoblastic leukemia cell line) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were maintained in RPMI 1640 supplemented with 10% FBS, 100 units⁄mL penicillin-G, and 100 μg⁄mL streptomycin. Cells were grown in monolayer cultures, except for Raji cells, which were grown in suspension, at 37°C in humidified air containing 5% CO_2.

Cytotoxicity assay. Cell viability following exposure to synthetic compounds was estimated using the MTT reduction assay [20,21]. HL-60 and MOLT4 cells were plated in 96-well microplates at a density of 5×10^4 cells⁄mL (100 μL⁄well). Control wells contained no drugs, and blank wells contained only growth medium for background correction. After overnight incubation at 37°C, half of the growth medium was removed, and 50 μL of medium supplemented with different concentrations of synthetic compounds dissolved in DMSO was added in triplicate. Plates were centrifuged before this procedure. Maximum concentration of DMSO in the wells was 0.5%. Cells were further incubated for 72 h. At the end of the incubation time, the medium was removed, and MTT was added to each well at a final concentration of 0.5 mg⁄mL, and

plates were incubated for another 4 h at 37°C. Then, formazan crystals were solubilized in 200 μL DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). The percentage of inhibition of viability compared with control wells was calculated for each concentration of the compound, and IC50 values [21] were calculated with the software CURVEEXPERT version 1.34 for Windows (Hyams Development, OH, USA). Each experiment was repeated four times. Data are presented as mean ±SD.

Acknowledgments. This research was supported by grants from the research council of Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

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