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Synthesis and *In Vitro* Antiproliferative Effects of New Dihydropyrano[3,2-b]Chromene Derivatives

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ABSTRACT

Three-component reaction of 3-hydroxy-4H-chromen-4-one, aromatic aldehydes and malononitrile or ethyl cyanoacetate in EtOH, at reflux and in the presence of catalytic amounts of nanosized MgO has been used for the synthesis of dihydropyrano[3,2-b]chromene derivatives. A number of advantages are associated with this method such as, easy monitoring of reaction progress, convenient workup procedure and high yields. The antiproliferative effects of these compounds were tested by MTT assay on six cell lines; five cancerous (Caco-2, A549, MCF-7, HT1080, and U87) and one normal (3T3). The results revealed that some of these compounds had cytotoxicity against tested cell lines especially against Caco-2 (colorectal) cell line.

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Cytotoxic activity; dihydropyrano[3,2-b]chromene derivatives; 3-hydroxy-4H-chromen-4-one; nanosized MgO; three-component reactions

Introduction

Nowadays, cancer is an important health problem globally. It is the first to fourth leading cause of death; depending on the country.¹ Chemotherapy is usually the choice method of treatment alone or in combination with surgery and radiotherapy.² Today, by unfolding several molecular characteristics and metabolic pathways of cancer cells, many research studies are aimed at specific cancer therapies.³ Therefore, the development and synthesis of antineoplastic agents have attracted a lot of attention.

Chromene derivatives have not only attracted considerable interest due to their biological activity, but also found wide application in medicinal chemistry as well.^{4,5} Along with displaying activities that are antihyperglycemic and antidiyslipidemic,⁶ cytotoxic,⁷ molluscicidal,⁸ anti-inflammatory,⁹ and antifungal,¹⁰ they can also be used as pigments, photoactive materials, and biodegradable agrochemicals.^{11–13} In addition, chromenes have been found in plants¹⁴ and used as valuable leads for the design and synthesis of new pharmacophores for medicinal chemistry and drug development. Therefore, we can go ahead with synthesizing dihydropyrano[3,2-b]chromene derivatives using the method mentioned below.

Multi-component reactions (MCRs) represent a special attractive synthetic strategy for producing molecular complexity in a single synthetic procedure. This is because they are performed without the need to separate any intermediate step during the processes. This decreases the synthesis time and saves both energy and raw materials.^{15–17}

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Values are reported from three independent experiments.

 Supplemental data for this article can be accessed [here](#).

In fact, development of MCRs can lead to a new and diverse synthetic approach to afford many small organic molecules in the field of new organic, bioorganic, and medicinal chemistry.^{18–22} Hence, nowadays MCRs are considered as a vital technique in the synthesis of various important heterocyclic compounds such as dihydropyrano[3,2-*b*]chromene derivatives.

Conventional organic syntheses are commonly based on homogeneous or heterogeneous catalysts. Magnesium oxide (MgO) is known as a heterogeneous catalyst and requires no special handling. MgO obtained using a novel but simple procedure, was methodically investigated as a heterogeneous base catalyst for reactions taking place in the liquid phase, specifically the Michael addition and the Knoevenagel condensation.^{23–26} Considering the fact that MgO has been widely used in many chemical reactions, we investigated its use in MCRs as catalyst and then scrutinized the three-component reaction of 3-hydroxy-4H-chromen-4-one, aromatic aldehydes and malononitrile or ethyl cyanoacetate in EtOH, at reflux and in the presence of catalytic amounts of nanosized MgO for synthesis of dihydropyrano[3,2-*b*]chromene derivatives.

Material and methods

General methods

Solvents and reagents were purchased from commercial sources and were used without further purification. Melting points were measured on an Electrothermal-9100 apparatus and are uncorrected. IR spectra were recorded on a Bruker FTIR Tensor 27 infrared spectrophotometer. ¹H NMR spectra were recorded on a Bruker AVANCE III 300 MHz NMR spectrometer. ¹³C NMR spectra were recorded on the same instruments at 75 MHz, using TMS as an internal standard. Elemental analyses were performed using a Heraeus CHN-O-Rapid, Fully Automatic Elemental Analyzer. 3-Hydroxy-4H-chromen-4-one (**1**) was prepared according to literature procedures.²⁷

Preparation of nanosized MgO

The MgO nanoparticles were synthesized by precipitation of the magnesium hydroxide gels in aqueous solution using Mg(NO₃)₂ as salt and liquid ammonia as the precipitating agent. Initially, the pH of 200 mL of distilled water was adjusted to 10.5 by addition of liquid ammonia. To this solution, 0.1 M magnesium nitrate solution (0.0148 g/mL) was added dropwise with continuous stirring. The rate of addition of the salt solution was kept at 20 mL/h. During the addition, the pH of the mixture decreased due to hydrolysis of the salt. The pH was maintained at 10.5 by controlled addition of liquid ammonia solution. After completion of the precipitation procedure, the mixture was stirred at room temperature for 12 h, filtered, repeatedly washed with distilled water, dried at 120 °C, and calcined at 500 °C for 2 h.²⁸

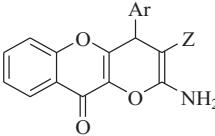
General procedure for the preparation of dihydropyrano[3,2-*b*]chromene derivatives (**4a–j**)

A mixture of 3-hydroxy-4H-chromen-4-one (**1**) (324 mg, 2 mmol), aromatic aldehydes (**2a–h**) (2 mmol), malononitrile (**3a**) (132 mg, 2 mmol) or ethyl cyanoacetate (**3b**) (0.21 mL, 2 mmol), and nanosized MgO (44 mg, 55 mol%, Table 1) in ethanol (10 mL) was refluxed for in the reported time in Table 2 (the progress of the reaction was monitored by TLC and hexane/ethyl acetate was used as an eluent). After completion of the reaction, the mixture was cooled, nanosized MgO was removed by filtration and the obtained crude product was recrystallized from ethanol to give the pure solid sample for analysis.

Table 1. Optimizing the quantity of catalyst and solvent effects on the three-component reaction of 3-hydroxy-4H-chromen-4-one (1), benzaldehyde (2a) and malononitrile (3a), for the synthesis of dihydropyrano[3,2-b]chromene derivatives.

Entry	Catalyst (mol%)	Solvent	Time (min)	Yield (%)
1	Nano MgO (25%)	Ethanol	38	80
2	Nano MgO (35%)	Ethanol	33	84
3	Nano MgO (45%)	Ethanol	27	87
4	Nano MgO (55%)	Ethanol	20	91
5	Nano MgO (65%)	Ethanol	20	91
6	Nano MgO (55%)	Methanol	22	91
7	Nano MgO (55%)	Toluene	73	70
8	Nano MgO (55%)	THF	45	78
9	Nano MgO (55%)	EtOAc	40	84
10	Nano MgO (55%)	CH ₃ CN	38	87
11	Nano MgO (55%)	H ₂ O	120	48

Table 2. Three-component reaction of 3-hydroxy-4H-chromen-4-one (1), aromatic aldehydes (2a–h) and malononitrile (3a) or ethyl cyanoacetate (3b).

				
Yield (%)	Time (min)	Z	Ar	Compd. No.
91	20	CN	C ₆ H ₅	4a
92	20	CN	4-ClC ₆ H ₄	4b
94	20	CN	2,4-diClC ₆ H ₃	4c
91	20	CN	4-BrC ₆ H ₄	4d
89	23	CN	4-CH ₃ C ₆ H ₄	4e
88	28	CN	4-CH ₃ OC ₆ H ₄	4f
89	25	COOEt	C ₆ H ₅	4g
90	23	COOEt	4-FC ₆ H ₄	4h
88	28	COOEt	4-CH ₃ C ₆ H ₄	4i
86	30	COOEt	4-CH ₃ OC ₆ H ₄	4j

2-Amino-10-oxo-4-phenyl-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile (4a)

Cream powders; yield: 91%; m.p. 268–269 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3419, 3292 (NH₂), 2193 (CN), 1657 (C=O), 1616, 1597 (C=C); ¹H NMR (300 MHz, DMSO-d₆) δ_{ppm} : 8.10 (dd, 1H, $J = 9$ Hz, $J = 3$ Hz, CH-Ar), 7.76–7.70 (m, 1H, CH-Ar), 7.50–7.30 (m, 9H, CH-Ar, NH₂), 4.95 (s, 1H, CH); ¹³C NMR (75 MHz, DMSO-d₆) δ_{ppm} : 169.02 (C=O), 159.79 (C2), 154.98 (C5a), 150.43, 141.45, 134.90, 133.75, 129.45, 128.39, 125.86, 125.74, 123.58, 119.84 (CN), 118.65, 56.10 (C3), 41.47 (C4); Anal. calcd. for C₁₉H₁₂N₂O₃: C, 72.15; H, 3.82; N, 8.86%. Found: C, 71.95; H, 3.65; N, 8.68%.

2-Amino-4-(4-chlorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile (4b)

White powders; yield: 92%; m.p. 261–262 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3379, 3318 (NH₂), 2198 (CN), 1662 (C=O), 1637, 1611 (C=C); ¹H NMR (300 MHz, DMSO-d₆) δ_{ppm} : 8.10 (dd, 1H, $J = 9$ Hz, $J = 3$ Hz, CH-Ar), 7.78–7.72 (m, 1H, CH-Ar), 7.52–7.34 (m, 8H, CH-Ar, NH₂), 5.03 (s, 1H, CH); ¹³C NMR (75 MHz, DMSO-d₆) δ_{ppm} : 169.04 (C=O), 159.78 (C2), 155.00 (C5a), 149.83, 140.37, 134.95, 133.83, 133.06, 130.39, 129.42, 125.90, 125.76, 123.60, 119.71 (CN), 118.66, 55.70 (C3), 40.81 (C4); Anal. calcd. for C₁₉H₁₁ClN₂O₃: C, 65.06; H, 3.16; N, 7.99%. Found: C, 64.88; H, 2.99; N, 7.79%.

2-Amino-4-(2,4-dichlorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile(4c)

White powders; yield: 94%; m.p. 257–258 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3393, 3331 (NH_2), 2189 (CN), 1651 ($\text{C}=\text{O}$), 1629, 1592 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, DMSO-d_6) δ_{ppm} : 8.10 (d, 1H, $J=9$ Hz, CH-Ar), 7.77–7.68 (m, 2H, CH-Ar), 7.54–7.39 (m, 6H, CH-Ar, NH_2), 5.45 (s, 1H, CH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm} : 168.93 ($\text{C}=\text{O}$), 160.03 (C2), 154.94, 148.87 (C5a), 137.19, 135.00, 134.35, 134.15, 133.98, 133.01, 129.89, 128.81, 125.94, 125.75, 123.61, 119.37 (CN), 118.68, 54.54 (C3), 38.80 (C4); Anal. calcd. for $\text{C}_{19}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_3$: C, 59.24; H, 2.62; N, 7.27%. Found: C, 59.06; H, 2.45; N, 7.08%.

2-Amino-4-(4-bromophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile (4d)

White powders; yield: 91%; m.p. 261–262 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3378, 3318 (NH_2), 2198 (CN), 1663 ($\text{C}=\text{O}$), 1637, 1610 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, DMSO-d_6) δ_{ppm} : 8.10 (d, 1H, $J=9$ Hz, CH-Ar), 7.76 (t, 1H, $J=9$ Hz, CH-Ar), 7.62–7.34 (m, 8H, CH-Ar, NH_2), 5.02 (s, 1H, CH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm} : 169.04 ($\text{C}=\text{O}$), 159.77 (C2), 155.00 (C5a), 149.77, 140.79, 134.96, 133.84, 132.34, 130.74, 125.91, 125.77, 123.60, 121.63, 119.71 (CN), 118.67, 55.63 (C3), 39.16 (C4); Anal. calcd. for $\text{C}_{19}\text{H}_{11}\text{BrN}_2\text{O}_3$: C, 57.74; H, 2.81; N, 7.09%. Found: C, 57.55; H, 2.64; N, 6.92%.

2-Amino-10-oxo-4-(p-tolyl)-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile (4e)

White powders; yield: 89%; m.p. 265–266 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3387, 3308 (NH_2), 2198 (CN), 1652 ($\text{C}=\text{O}$), 1637, 1598 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, DMSO-d_6) δ_{ppm} : 8.10 (dd, 1H, $J=6$ Hz, $J=3$ Hz, CH-Ar), 7.76–7.70 (m, 1H, CH-Ar), 7.49–7.19 (m, 8H, CH-Ar, NH_2), 4.89 (s, 1H, CH), 2.28 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm} : 169.01 ($\text{C}=\text{O}$), 159.72 (C2), 154.97 (C5a), 150.62, 138.52, 137.65, 134.88, 133.64, 129.99, 128.28, 125.84, 125.73, 123.56, 119.86 (CN), 118.64, 56.21 (C3), 41.12 (C4), 21.12 (CH_3); Anal. calcd. for $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_3$: C, 72.72; H, 4.27; N, 8.48%. Found: C, 72.53; H, 4.09; N, 8.30%.

2-Amino-4-(4-methoxyphenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carbonitrile (4f)

Cream powders; yield: 88%; m.p. 219–221 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3367, 3307 (NH_2), 2202 (CN), 1649 ($\text{C}=\text{O}$), 1639, 1600 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, DMSO-d_6) δ_{ppm} : 8.10 (d, 1H, $J=6$ Hz, CH-Ar), 7.75 (t, 1H, $J=9$ Hz, CH-Ar), 7.51–7.25 (m, 6H, CH-Ar, NH_2), 6.95 (d, 1H, $J=3$ Hz, CH-Ar), 4.89 (s, 1H, CH), 3.75 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm} : 169.03 ($\text{C}=\text{O}$), 159.67 (C2), 159.34 (ArC-OMe), 154.99 (C5a), 150.74, 134.89, 133.54, 133.45, 129.53, 125.84, 125.75, 123.56, 119.90 (CN), 118.65, 114.78, 56.34 (C3), 55.57 (OCH_3), 40.82 (C4); Anal. calcd. for $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_4$: C, 69.36; H, 4.07; N, 8.09%. Found: C, 69.18; H, 3.90; N, 7.91%.

Ethyl 2-amino-10-oxo-4-phenyl-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (4g)

Brown powders; yield: 89%; m.p. 175–176 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3453, 3402 (NH_2), 1685 (COOEt), 1666 ($\text{C}=\text{O}$), 1614, 1575 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, DMSO-d_6) δ_{ppm} : 8.10 (d, 1H, $J=9$ Hz, CH-Ar), 7.89 (s, 1H, CH-Ar), 7.75 (t, 1H, $J=6$ Hz, CH-Ar), 7.56–7.23 (m, 8H, CH-Ar, NH_2), 4.95 (s, 1H, CH), 3.97 (q, 2H, $J=6$ Hz, CH_2), 1.04 (t, 3H, $J=6$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm} : 168.96 ($\text{C}=\text{O}$), 168.08 (COOEt), 160.18 (C2), 155.04 (C5a), 153.20, 143.61, 134.79, 133.27, 129.01, 128.20, 127.65, 125.79, 125.72, 123.67, 118.71, 75.49 (OCH_2), 59.39, (C3), 41.04 (C4), 14.58 (CH_3); Anal. calcd. for $\text{C}_{21}\text{H}_{17}\text{NO}_5$: C, 69.41; H, 4.72; N, 3.85%. Found: C, 69.24; H, 4.56; N, 3.68%.

Ethyl 2-amino-4-(4-fluorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (4h)

Yellow powders; yield: 90%; m.p. 197–198 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3451, 3398 (NH₂), 1685 (COOEt), 1667 (C=O), 1615, 1576 (C=C); ¹H NMR (300 MHz, DMSO-d₆) δ_{ppm} : 8.105 (d, 1H, $J=9$ Hz, CH-Ar), 7.90 (s, 1H, CH-Ar), 7.77 (t, 1H, $J=9$ Hz, CH-Ar), 7.56–7.12 (m, 7H, CH-Ar, NH₂), 4.98 (s, 1H, CH), 3.97 (q, 2H, $J=6$ Hz, CH₂), 1.04 (t, 3H, $J=6$ Hz, CH₃); ¹³C NMR (75 MHz, DMSO-d₆) δ_{ppm} : 168.98 (C=O), 168.02 (COOEt), 163.31 (ArC-F), 160.11 (C2), 155.05 (C5a), 152.80, 139.81, 134.83, 133.24, 130.19, 125.82, 125.73, 123.66, 118.71, 115.90, 115.61, 75.36 (OCH₂), 59.41 (C3), 40.82 (C4), 14.59 (CH₃); Anal. calcd. for C₂₁H₁₆FNO₅: C, 66.14; H, 4.23; N, 3.67%. Found: C, 65.97; H, 3.06; N, 3.50%.

Ethyl 2-amino-10-oxo-4-(p-tolyl)-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (4i)

Yellow powders; yield: 88%; m.p. 194–196 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3394, 3290 (NH₂), 1678 (COOEt), 1663 (C=O), 1615, 1576 (C=C); ¹H NMR (300 MHz, DMSO-d₆) δ_{ppm} : 8.10 (d, 1H, $J=9$ Hz, CH-Ar), 7.86 (s, 1H, CH-Ar), 7.76 (t, 1H, $J=9$ Hz, CH-Ar), 7.56–7.11 (m, 7H, CH-Ar, NH₂), 4.90 (s, 1H, CH), 3.97 (q, 2H, $J=6$ Hz, CH₂), 2.24 (s, 3H, CH₃), 1.07 (t, 3H, $J=6$ Hz, CH₃); ¹³C NMR (75 MHz, DMSO-d₆) δ_{ppm} : 168.95 (C=O), 168.10 (COOEt), 160.14 (C2), 155.04 (C5a), 153.43, 140.66, 136.82, 134.77, 133.20, 129.58, 128.04, 125.78, 125.71, 123.66, 118.71, 75.57 (OCH₂), 59.41 (C3), 40.82 (C4), 21.07 (Ar-CH₃), 14.62 (CH₃); Anal. calcd. for C₂₂H₁₉NO₅: C, 70.02; H, 5.07; N, 3.71%. Found: C, 69.85; H, 4.89; N, 3.54%.

Ethyl 2-amino-4-(4-methoxyphenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (4j)

Brown powders; yield: 86%; m.p. 167–168 °C (dec.); IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3397, 3293 (NH₂), 1681 (COOEt), 1664 (C=O), 1614, 1509 (C=C); ¹H NMR (300 MHz, DMSO-d₆) δ_{ppm} : 8.10 (d, 1H, $J=6$ Hz, CH-Ar), 7.86 (s, 1H, CH-Ar), 7.75 (t, 1H, $J=6$ Hz, CH-Ar), 7.55–7.44 (m, 3H, CH-Ar, NH₂), 7.22 (d, 2H, $J=9$ Hz, CH-Ar), 6.88 (d, 2H, $J=9$ Hz, CH-Ar), 4.88 (s, 1H, CH), 3.98 (q, 2H, $J=6$ Hz, CH₂), 3.70 (s, 3H, OCH₃), 1.07 (t, 3H, $J=6$ Hz, CH₃); ¹³C NMR (75 MHz, DMSO-d₆) δ_{ppm} : 168.94 (C=O), 168.13 (COOEt), 160.11 (C2), 158.81 (ArC-OMe), 155.04 (C5a), 153.50, 135.63, 134.74, 133.13, 129.19, 125.75, 125.71, 123.66, 118.69, 114.37, 75.72 (OCH₂), 59.40 (C3), 55.47 (OCH₃), 40.82 (C4), 14.64 (CH₃); Anal. calcd. for C₂₂H₁₉NO₆: C, 67.17; H, 4.87; N, 3.56%. Found: C, 66.99; H, 4.69; N, 3.38%.

In vitro anti-cancer activity

The *in vitro* cytotoxic activities of the newly synthesized compounds were evaluated on colorectal (Caco-2), lung (A549), breast (MCF-7), fibrosarcom (HT1080), and glioblastoma (U87-MG) cancer cell lines, and a normal fibroblast cell line (3T3). The cell lines were purchased from the Iranian Biological Resource Center (IBRC, Tehran, Iran) and grown in DMEM culture media (Biosera, France) supplemented with 10% FBS (Gibco) and antibiotics (penicillin 100 U/mL and streptomycin 100 µg/mL) at 37 °C in a CO₂ incubator (5% CO₂ and 95% relative humidity). In order to evaluate the cytotoxicity of synthesized compounds, 1×10^4 cells were seeded in each well of a flat-bottom microplate and incubated for 24 h. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and the desired concentrations of each compound (0.001–1000 µg/mL) were prepared by dilution in DMEM (without FBS) and added to each well. Triplicate wells were considered for each concentration. Doxorubicin, the most potent anticancer drug, was used as the reference drug. The cells were further incubated for 24 h. MTT powder

(Sigma, St. Louis, MO) was dissolved in PBS (pH 7.2) to a concentration of 5 mg/mL. A 10 μ L of the MTT solution was then added to each well and the plates were incubated for 3–4 h at 37 °C in dark. Cell viability was determined by measuring the absorbance at 570 nm after solubilization of formazane crystals by adding 100 μ L DMSO to each well. The test was done three times for each compound and the inhibitory concentration 50 (IC₅₀) was calculated by probit analysis (SPSS ver. 18; SPSS Inc., Chicago, IL).

Results and discussion

Following our previous works on environmentally friendly MCRs,^{29–33} we have synthesized a series of dihydropyrano[3,2-b]chromene derivatives (**4a–j**) in two steps: (i) synthesis of 3-hydroxy-4H-chromen-4-one (**1**)²⁷ (Figure 1) and (ii) three-component reaction of 3-hydroxy-4H-chromen-4-one (**1**), aromatic aldehydes (**2a–h**) and malononitrile (**3a**) or ethyl cyanoacetate (**3b**) in EtOH, at reflux and in the presence of catalytic amounts of nanosized MgO (Figure 2).

Moreover, in order to optimize the conditions of reaction, the amount of used catalyst was optimized in the reaction of 3-hydroxy-4H-chromen-4-one (**1**), benzaldehyde (**2a**) and malononitrile (**3a**) for preparing compound **4a** as the model reactions. An increase in the amount of nano MgO from 25 to 55 mol% increased the yield of the compound **4a**. So the best results were obtained when the reactions were carried out in the presence of 55 mol% nanosized MgO. The results are shown in Table 1.

In addition, several polar and non-polar solvents were used to study the solvent effects on the preparation of compound **4a**. In each case, the substrates were mixed together with 55 mol% nanosized MgO agitated with 10 mL solvent at reflux. The results are summarized in Table 1. Methanol and ethanol as solvents resulted in the same reaction time and yield, but ethanol has advantages such as being eco-friendly, green, and less toxic over methanol (entry 6). The three-component reactions in water needed relatively longer reaction time and gave moderate yields of the product, probably because of the poor solubility of the reactants in comparison to ethanol

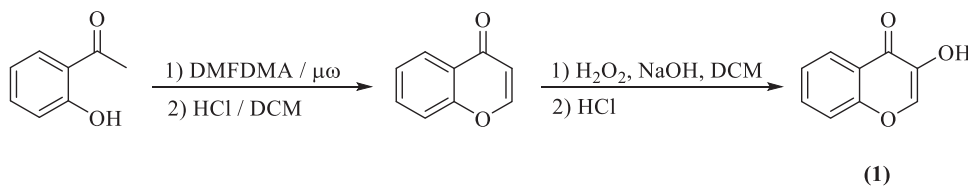
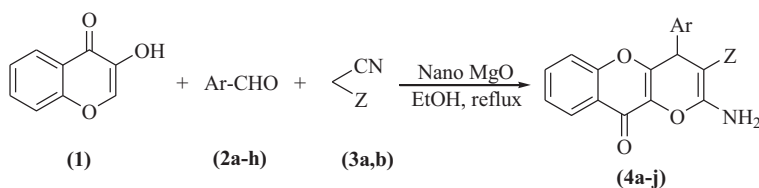


Figure 1. Synthesis of 3-hydroxy-4H-chromen-4-one.



4a: Ar= C₆H₅, Z= CN

4c: Ar= 2,4-diClC₆H₃, Z= CN

4e: Ar= 4-CH₃C₆H₄, Z= CN

4g: Ar= C₆H₅, Z= COOEt

4i: Ar= 4-CH₃C₆H₄, Z= COOEt

4b: Ar= 4-ClC₆H₄, Z= CN

4d: Ar= 4-BrC₆H₄, Z= CN

4f: Ar= 4-CH₃OC₆H₄, Z= CN

4h: Ar= 4-FC₆H₄, Z= COOEt

4j: Ar= 4-CH₃OC₆H₄, Z= COOEt

Figure 2. Synthesis of dihydropyrano[3,2-b]chromene derivatives over nanosized MgO solvents.

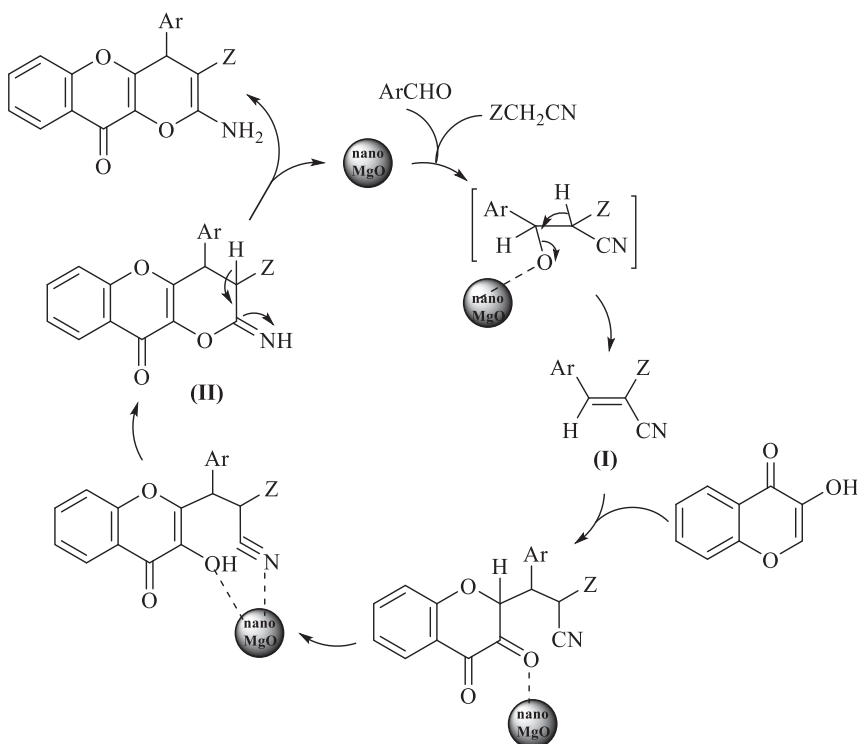


Figure 3. A plausible mechanism for the nano-MgO-catalyzed three-component reaction for the synthesis of dihydropyrano[3,2-b]chromene derivatives.

(entry 11). It is noteworthy to mention that the polar solvents such as EtOH afford better yields than non-polar. Finally, based on the above results during the optimization of catalyst and solvent, three-component reaction of 3-hydroxy-4H-chromen-4-one (**1**), aromatic aldehydes (**2a–h**), and malononitrile (**3a**) or ethyl cyanoacetate (**3b**) in EtOH, at reflux and in the presence of catalytic amounts of nanosized MgO has been used for synthesis of dihydropyrano[3,2-b]chromene derivatives (**4a–j**) (Table 2).

The mechanism of the reaction presumably proceeds in three steps: (i) a Knoevenagel condensation, (ii) a Michael addition, and (iii) an intramolecular cyclization catalyzed by nano MgO as presented in Figure 3.

The reaction firstly leads to Knoevenagel condensation between aldehydes and malononitrile or ethyl cyanoacetate in the presence of nano MgO, followed by dehydration, affords intermediate (**i**). In this step, the Knoevenagel condensation is catalyzed by amphoteric nano MgO which acts as a base during abstraction of acidic proton from active methine of malononitrile or ethyl cyanoacetate and shows acidic nature followed by loss of water molecule. The second step deals with the Michael addition reaction between 3-hydroxy-4H-chromen-4-one (**1**) and the intermediate (**i**), which nano MgO activates the nitrile group by polarizing for the intramolecular nucleophilic attack by the OH group leading to the ring closure which in turn results in the formation of intermediate (**ii**). This is followed by the shift of hydrogen for synthesis of dihydropyrano[3,2-b]chromene derivatives (**4a–j**) and again the catalyst enters into the catalytic cycle^{34,35} (Figure 3).

The structure of compounds **4a–j** was determined on the basis of their elemental analyses, ¹H and ¹³C NMR and IR spectroscopic data. The IR spectrum **4a** showed the presence of NH₂ in 3419 and 3292 cm^{−1}, CN at region 2193 cm^{−1}, and one sharp band at 1657 cm^{−1} due to vibration of the carbonyl (C=O) group. The ¹H NMR spectrum of **4a** indicated one kind of aliphatic protons at 4.95 ppm. On the other hand, the aryl protons and NH₂ appeared at 8.10–7.30 ppm (11H, m). The

Table 3. IC₅₀ (μg/mL) values of the compounds (**4a–j**) on six cell lines (mean ± standard error of mean).

Compd. No.	Caco-2	A549	MCF-7	HT1080	U87	3T3
4a	81.91 ± 2.80	88.27 ± 4.31	87.08 ± 4.69	69.77 ± 5.31	88.89 ± 5.59	70.72 ± 7.32
4b	44.20 ± 0.66	60.19 ± 4.29	85.44 ± 11.58	73.03 ± 2.10	212.22 ± 9.90	79.03 ± 2.01
4c	103.67 ± 6.34	192.47 ± 14.30	191.56 ± 22.75	159.98 ± 7.03	210.64 ± 14.51	94.71 ± 5.15
4d	153.17 ± 12.45	161.60 ± 10.32	175.15 ± 7.34	201.34 ± 13.25	442.00 ± 25.21	204.98 ± 17.29
4e	64.26 ± 2.66	189.19 ± 10.50	94.12 ± 2.59	93.52 ± 6.51	200.10 ± 17.92	87.91 ± 2.49
4f	87.26 ± 3.82	191.37 ± 14.71	184.34 ± 22.66	165.85 ± 11.86	202.57 ± 7.47	3.39 ± 0.28
4g	39.17 ± 0.60	71.59 ± 0.46	95.68 ± 9.28	77.82 ± 3.55	222.51 ± 17.73	96.74 ± 11.84
4h	100.89 ± 9.43	99.95 ± 6.18	100.64 ± 4.56	118.23 ± 8.09	288.23 ± 16.37	82.67 ± 5.24
4i	36.96 ± 0.95	80.90 ± 4.96	89.23 ± 9.81	47.22 ± 4.17	77.68 ± 4.27	86.13 ± 7.14
4j	99.45 ± 8.43	89.93 ± 5.59	94.65 ± 7.02	98.61 ± 7.89	233.71 ± 13.52	148.29 ± 9.03
Dox	5.16 ± 0.15	6.44 ± 0.15	5.20 ± 0.12	5.40 ± 0.11	7.44 ± 0.12	6.03 ± 0.16

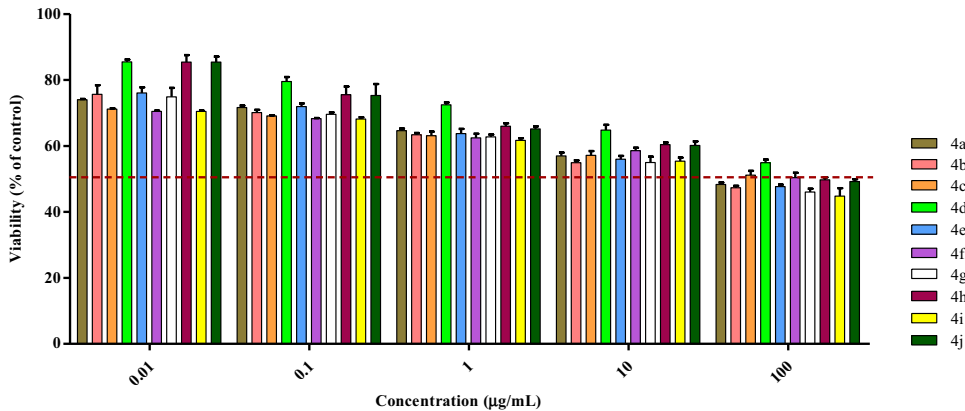


Figure 4. Effect of some concentrations of compounds **4a** to **4j** on Caco-2 cell line after 24 h incubation [mean ± standard deviation (SD)].

¹³C NMR of compound **4a** showed two signals at 169.02 and 119.84 ppm, which occur due to the carbonyl and CN carbons, respectively. The 12 aromatic carbons appeared at 159.79–118.65 ppm. Two signals at 159.79 and 154.98 ppm, which occurred due to C2 and C5a, respectively. Finally, two signals reflecting the shift of carbons at 56.10 and 41.47 ppm, which are due to the C3 and C4, respectively.

To investigate the antiproliferative effects of the compounds, MTT assay was done and the values of IC₅₀ for each compound were calculated (Table 3). The results showed that most of the synthesized compounds had inhibitory effects on growth of colorectal cancer cell line (Caco-2). The lowest IC₅₀ was 37 μg/mL for compound **4i** followed by compounds **4g** and **4b**. Generally, most of the compounds have an IC₅₀ < 100 μg/mL on this cell line (Figure 4).

The best IC₅₀ for A549 cell line was related to compound **4b** (60 μg/mL) followed by **4i**, **4g**, **4a**, **4j**, and **4h**. The other compounds had IC₅₀ values higher than 100 μg/mL.

For MCF7 cell line, the best compounds were **4a**, **4b**, **4e**, **4i**, **4g**, and **4j**. These compounds were also effective on HT1080 cell line. The most potent compound for HT1080 was compound **4i** (an IC₅₀ of 47 μg/mL).

The least effect of the compounds was on U87 glioblastoma, although compounds **4i** and **4a** had accepted cytotoxicity on this cell line (IC₅₀ values of about 77 and 88 μg/mL, respectively) (Figure 5).

In general, the best cytotoxic effects were related to compound **4i** which shows IC₅₀ values lower than 100 μg/mL on all tested cell lines; however, this compound was not as potent as the standard drug (doxorubicin).

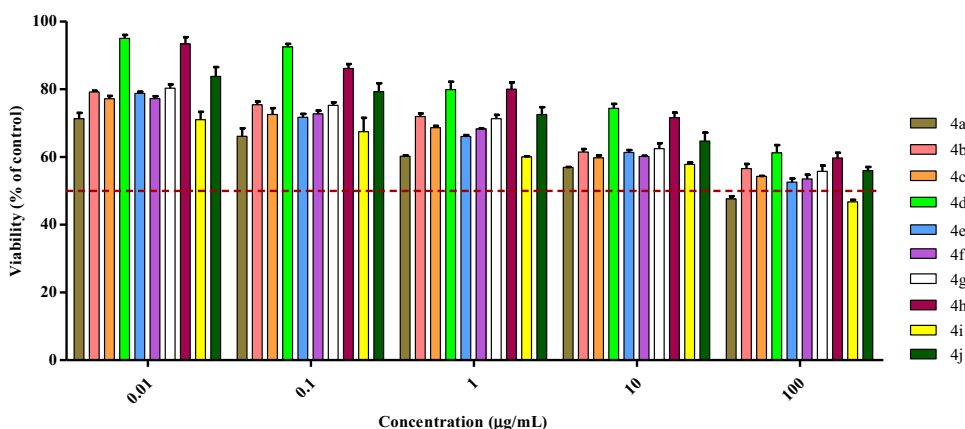


Figure 5. Effect of some concentrations of compounds **4a** to **4j** on U87 cell line after 24 h incubation [mean $\pm$ standard deviation (SD)].

All compound showed cytotoxicities close to that of other cell lines on the normal mouse fibroblast (3T3 cell line) except compound **4f**, which was extremely cytotoxic for this cell line.

Totally, the highest IC_{50} values were for compounds **4d** and **4c** then **4h** (although **4h** was better than **4c** and **4d**). Interestingly, these three compounds had halogen atoms (one Br in compound **4d**, two Cl in compound **4c**, and one F in compound **4h**) substituted on their phenyl ring (Figure 2), whereas compound **4b** with one Cl atom on its phenyl ring was one of the best compounds. Overall, the best compounds were **4i** and **4a** that had IC_{50} values <100 $\mu\text{g/mL}$ for five tested tumor cell lines. If we ignore U87 cell line, compound **4b**, **4g**, and **4j** also had acceptable cytotoxicity.

Conclusions

In summary, We have been able to report a new procedure for synthesis of dihydropyrano[3,2-*b*]chromene derivatives, *via* three-component reaction of 3-hydroxy-4*H*-chromen-4-one, aromatic aldehydes, and malononitrile or ethyl cyanoacetate in EtOH, at reflux and in the presence of catalytic amounts of nanosized MgO. The procedure offers several advantages, including high yields, reactions progress in mild conditions, and a very simple separation process, which makes it a useful practical process for the synthesis of these compounds.

The synthesized compounds showed cytotoxic effects on tested cell lines especially Caco-2 cell line (colorectal cancer). Compounds **4i** and **4a** were the best compounds effective on all tested cell lines followed by **4g** effective on all cell lines except U87. Most of the compounds were toxic for colorectal cell line while only two out of ten compounds (**4a** and **4i**) were effective on glioblastoma cell line. Here, we showed that dihydropyrano[3,2-*b*]chromene derivatives can be considered as efficient antiproliferative agents. More compounds can be synthesized by this method to find much more potent compounds.

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Disclosure statement

There are no conflicts to declare.

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