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Synthesis and *In Vitro* Cytotoxic Activity of 2-Amino-7-(dimethylamino)-4-[(trifluoromethyl)phenyl]-4*H*-chromenes

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Abstract: Three 2-amino-4-(trifluoromethylphenyl)-3-cyano-7-(dimethylamino) -4*H*-chromene derivatives were synthesized and their cytotoxic activities were determined against six human tumor cell lines using MTT assay. Condensation of 3-(dimethylamino)phenol, trifluoromethybenzaldehydes and malonitrile in ethanol containing piperidine afforded corresponding chromenes (**4a-c**). The structure of the synthesized compound was confirmed by ^{1}H NMR, IR and Mass spectral data. Among compounds tested, 3-trifluoromethyl analogue (**3b**) was the most active against all human tumor cell lines (IC_{50}=12-45 nM).

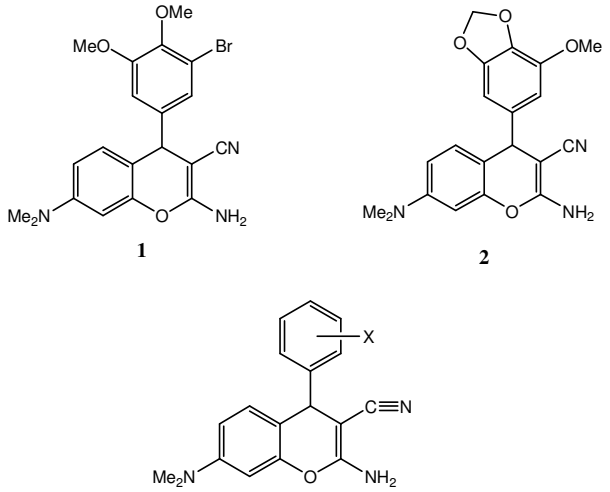
Keywords: Synthesis, 4*H*-chromenes, Cytotoxic activity.

Introduction

The correct balance between apoptosis and inhibition of apoptosis is important in preserving tissue homeostasis and organ morphogenesis[1]. Apoptosis is one of the main types of programmed cell death (PCD). This involves a series of biochemical events that lead to a variety of morphological changes, including cell shrinkage, chromatin condensation, nuclear and DNA fragmentation[2]. Since many cancer cells exhibit abnormal inhibition of apoptosis, Scientifics are interested in the discovery and development of inducers of apoptosis as potential anticancer agents[3].

The discovery of compounds **1**-**3** (Figure 1) which belong to the 4-aryl-4*H*-chromenes family has been reported and is shown to possess anti-cancer activity[4]. These compounds, which are potent apoptosis inducers, were found to be highly active in the growth inhibition MTT assay with IC_{50} values in low nanomolar ranges.

Herein, we would like to describe the synthesis of some 4-aryl-4*H*-chromenes having substitution of trifluoromethyl moiety in 2-, 3- and 4-positions of the phenyl ring (compounds **4a-c**, Figure 1) as potential drugs effecting cancer. The cytotoxic activities of synthesized compounds were determined against six cancer cell lines using growth inhibition MTT assay.



3, X =Br, Cl, F
4a, X = 2-CF_3; **4b**, X = 3-CF_3; **4c**, X =4-CF_3

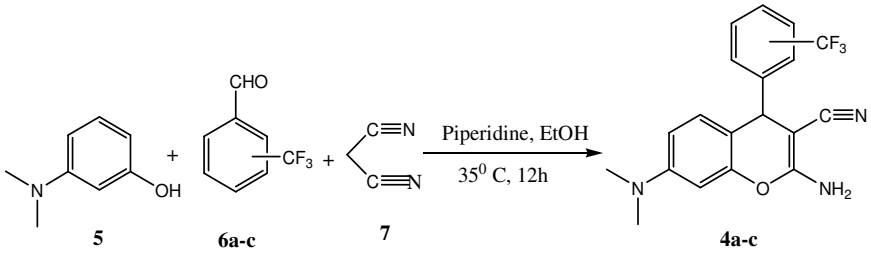
Figure 1. Chemical structures of compounds **1**-**4**

Experimental

All chemical reagents and solvents used in this study were purchased from Merck AG (Darmstadt, Germany). Melting points were determined using Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). NMR spectra were recorded using a Bruker 80 spectrometer (Bruker Bioscience, Billerica, MA, USA) and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. MS spectra were measured with a Finnigan TSQ 70 Mass spectrophotometer at 70 eV. Elemental analyses were carried out on a HERAEUS CHN-O rapid elemental analyzer (Heraeus GmbH, Hanau, Germany) for C, H and N and the results are within ± 0.4% of the theoretical values. Merck silica gel 60 F254 plates were used for analytical TLC.

Chemistry

2-Amino-7-(dimethylamino)-4-(trifluoromethylphenyl)-4*H*-chromene-3-carbonitriles (**4a-c**) were synthesized by condensation of 3-(dimethylamino)phenol (**5**), corresponding benzaldehyde (**6a-c**) and malonitrile (**7**) in ethanol in the presence of piperidine (Scheme 1)[5]. The structure of synthesized compounds was characterized by IR, ^{1}H NMR and Mass spectral data.



Scheme 1. Synthesis of 4-aryl-4*H*-chromenes **4a-c**

*2-Amino-7-(dimethylamino)-4-[(trifluoromethyl)phenyl]-4H-chromene-3-carbonitrile (**3a-c**) (General procedure)*

Piperidine (0.85 g, 10 mmol) was added to a mixture of 3-dimethylaminophenol **5** (0.68 g, 5 mmol), corresponding benzaldehyde **6a-c** (5 mmol) and malonitrile **7** (0.03 g, 5 mmol) in ethanol (20 mL). The reaction mixture was stirred at 35 °C for 12 h. After cooling, the precipitated solid was filtered off, washed with cold ethanol and crystallized from the same solvent.

*2-Amino-7-(dimethylamino)-4-[2-(trifluoromethyl)phenyl]-4H-chromene-3-carbonitrile (**3a**)*

(M.W.: 359.35) Yield 45%; m.p.205-207 ^{o}C; IR (KBr, cm^{-1}): 3318, 3191 (NH_2), 2197 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.80-7.10 (m, 4H, phenyl), 6.72 (d, 1H, *J* = 8.2 Hz, H_5 chromene), 6.47 (dd, 1H, *J* = 8.2 and 2.5 Hz, H_6 chromene), 6.30 (d, 1H, *J* = 2.5 Hz, H_8 chromene), 5.14 (s, 1H, H_4 chromene), 4.60 (brs, 2H, NH_2), 2.92 (s, 6H, NMe_2); Ms (*m/z*, %): 359(M^+,100), 341(25), 333(14), 240(87), 214(98), 197(95), 170(83), 134(87), 106(90), 77(91).

*2-Amino-7-(dimethylamino)-4-[3-(trifluoromethyl)phenyl]-4H-chromene-3-carbonitrile (**3b**)*

(M.W.: 359.35) Yield 70%; m.p.199-200 ^{o}C; IR (KBr, cm^{-1}): 3354, 3220 (NH_2), 2197 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.56 - 7.32 (m, 4H, phenyl), 6.77 (d, 1H, *J* = 8.2 Hz, H_5 chromene), 6.37 (dd, 1H, *J* = 8.2 and 2.5 Hz, H_6 chromene), 6.26 (d, 1H, *J* = 2.5 Hz, H_8 chromene), 4.72(s, 1H, H_4 chromene), 4.64 (brs, 2H, NH_2), 2.94 (s, 6H, NMe_2); Ms (*m/z*, %): 359(M^+,15), 214(100), 198(11).

*2-Amino-7-(dimethylamino)-4-[4-(trifluoromethyl)phenyl]-4H-chromene-3-carbonitrile (**3c**)*

(M.W.: 359.35) Yield 36%; m.p.209-210 ^{o}C; IR (KBr, cm^{-1}): 3329, 3196 (NH_2), 2192 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.57 (d, 2H, *J* = 7.1 Hz, H_3 and H_5 phenyl), 7.32 (d, 2H, *J* = 7.1 Hz, H_2 and H_6 Phenyl), 6.76 (d, 1H, *J* = 8.5 Hz, H_5 chromene), 6.41 (dd, 1H, *J* = 8.5 and 2.5 Hz, H_6 chromene), 6.28 (d, 1H, *J* = 2.5 Hz, H_8 chromene), 4.73 (s, 1H, H_4 chromene), 4.62 (brs, 2H, NH_2), 2.95 (s, 6H, NMe_2); Ms (*m/z*, %): 359 (M^+,10), 241(12), 214(100), 149(11), 105(11), 97(19), 84(95), 66(60).

Biological activity

Cell lines and cell cultures

The synthesized compounds were tested against six human cancer cell lines including KB (nasopharyngeal epidermoid carcinoma), EJ (bladder carcinoma), MCF-7 (breast carcinoma), 1321N1 (astrocytoma), Saos-2 (osteosarcoma) and A 2780 CP (ovary carcinoma). The cell lines were purchased from National Cell Bank of Iran (NCBI). The cells

were grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich) supplemented with 10% heat-inactivated fetal calf serum (Biochrom, Berlin, Germany), 100 µg/mL streptomycin and 100 u/mL penicillin, in a humidified air atmosphere at 37 °C with 5% CO_2.

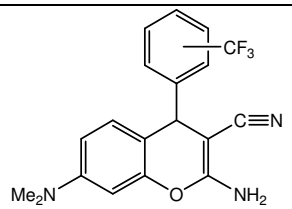
Cytotoxicity assay

The *in vitro* cytotoxic activity of each synthesized chromene derivatives **3a-c** was assessed in monolayer cultures using MTT colorimetric assay[6,7]. Briefly, each cell line in log-phase of growth was harvested by tripsinization, resuspended in complete growth medium to give a total cell count of 25×10^3 cells/ml. 100 micro liter (µL) of the cell suspension was seeded into the wells of 96-well plates (Nunc, Denmark). The plates were incubated in a humidified air atmosphere at 37 °C with 5% CO_2 overnight. Then, 50 µL of the media containing various concentrations of the compound was added per well in triplicate. The plates were incubated for further three days. The final concentration of DMSO in the highest concentration of applied compound was 0.1%. Vincristine was used as positive control for cytotoxicity while three wells containing tumor cells cultured in 150 µL of complete medium were used as controls for cell viability. After incubation, 30 µL of a 2.5 mg/mL solution of MTT (Sigma-Aldrich)[8] was added to each well and the plates were incubated for another 1 h. The culture medium was then replaced with 100 µL of DMSO and the absorbance of each well was measured by using a microplate reader at 570 nm. Each set of experiments was independently performed three times. For each compound, the concentration causing 50% cell growth inhibition (IC_{50}) compared with the control was calculated from concentration-response curves by regression analysis.

Results and Discussion

The compounds **3a-c** was tested in vitro against a panel of six human tumor cell lines. The percentage of growth was evaluated using MTT colorimetric assay versus controls not treated with test agents. For each compound, 50% inhibitory concentration (IC_{50}) was determined and reported in Table 1.

Table 1. Cytotoxic activity (IC_{50}, nM)[a] of compounds (**4a–c)** against different cell lines in comparison with vincristine



Compound	Cell line					
	KB[b]	EJ[c]	MCF-7[d]	1321N1[e]	Saos-2[f]	A 2780 CP[g]
4a (2-CF_3)	175± 7	550±95	1280±28	305±77	325±71	235±49
4b (3-CF_3)	12.5±0.7	30±4	45±5	15±1.5	12±5	12±2
4c (4-CF_3)	113±23	145±35	910±42	195±11	315±21	140±84
Vincristine	0.10±0.01	0.24±0.08	8.90±3	5±1	2.60±0.50	0.18±0.03

[a] data represented in terms of mean ±SD. [b] KB: nasopharyngeal epidermoid carcinoma. [c] EJ: bladder carcinoma. [d] MCF-7:breast carcinoma. [e]1321N1: astrocytoma. [f] Saos-2:osteosarcoma. [g] A 2780 CP: ovary carcinoma

The data for vincristine was included for comparison. The obtained results revealed that compounds **3a** and **3c** possessed poor activity (IC_{50} > 100 nM) against all cell lines in contrast with **3b**, which possess comparable activity with vincristine. These results confirmed the reported activity of **3b** as a potent inducer of apoptosis using cell-based apoptosis assay[9-11]. In general, Table 1 shows that compound **3b** was the most active compound against all tested cell lines.

The results highlight the relationship between substitutions at 2, 3 or 4 positions of the phenyl ring and cytotoxic activity. Among these positions, substitution at C3 position greatly influences their potency and spectrum of cytotoxic activity.

Conclusion

In conclusion, we have explored the substitution of the 2, 3 and 4 positions of apoptosis inducing 4-aryl-4*H*-chromenes as potential anticancer agents. It was found that substitution of the 3-position resulted in a large increase of activity. Anti cancer effects of these compounds in tumor cells indicated that **3b** is a good candidate for further pharmacological studies to discover effective chemotherapeutic for the treatment of human cancer diseases.

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