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Original Article

Synthesis and cytotoxic activity of some 2-amino-4-aryl-3-cyano-7-(dimethylamino)-4*H*-chromenes

M. Vosooghi[1], S. Rajabalian[2], M. Sorkhi[1], M. Badinloo[3], M. Nakhjiri[4], A.S. Negahbani[4], A. Asadipour[2], M. Mahdavi[5], A. Shafiee[1] and A. Foroumadi[1,2,*]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, I.R.Iran.*
[2]*Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, I.R.Iran.*
[3]*Kerman Physiology Research Center, Kerman University of Medical Sciences, Kerman, I.R.Iran.*
[4]*Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran, I.R.Iran.*
[5]*Institute of Biochemistry & Biophysics, University of Tehran, Tehran, I.R.Iran.*

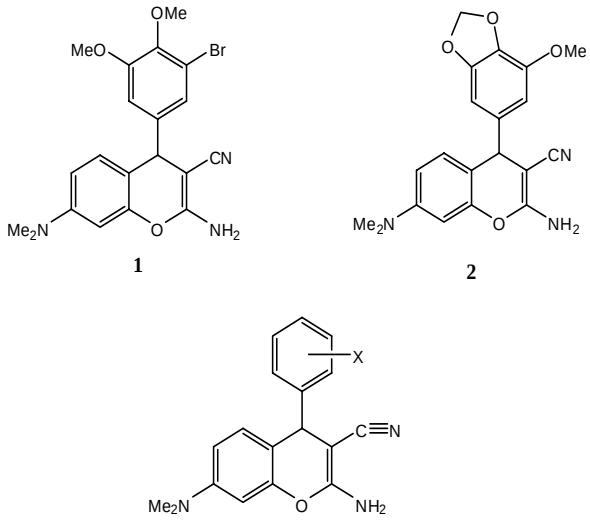
Abstract

A series of 2-amino-4-aryl-3-cyano-7-(dimethylamino)-4*H*-chromenes was synthesized by condensation of 3-(dimethylamino)phenol, an aromatic aldehyde and malonitrile in ethanol containing piperidine. The assignments of the structure of all synthesized compounds were based on spectral data (IR, Mass and ^{1}H NMR). The cytotoxic activities of the synthesized compounds against six human tumor cell lines were determined by MTT assay. Several compounds showed significant cytotoxic activity.

Keywords: Synthesis; Cytotoxic Activity; 4*H*-chromenes

INTRODUCTION

Cancer is a disease characterized by the uncontrolled growth of abnormal cells. It is now well documented that most cytotoxic anticancer agents induce apoptosis and several novel anticancer agents have been identified using apoptosis inducing activity in cancer cell lines. Apoptosis is one of the main types of programmed cell death. This involves a series of biochemical events that lead to a variety of morphological changes, including membrane blebbing, cell shrinkage, DNA fragmentation, chromatin condensation and nuclear fragmentation (1). One of the important factors in preserving tissue homeostasis and organ morphogenesis is the correct balance between apoptosis induction and inhibition (2). Abnormal inhibition of apoptosis is one of the hallmarks of tumorogenesis (3). Since many cancerous cells exhibit abnormal inhibition of apoptosis, researchers are interested in the discovery and development of apoptotic inducers which serve as potential anti-cancer agents (3). The discovery of compounds 1 and 2 (Fig. 1) which belong to the 4-aryl-4*H*-chromenes family has been recently reported and is shown to possess anti-cancer activity (4). These compounds, which are potent



3a, X = 2-F; **3b**, X = 3-F; **3c**, X = 4-F; **3d**, X = 2-Cl; **3e**, X = 3-Cl; **3f**, X = 4-Cl; **3g**, X = 2-Br; **3**h, X = 3-Br; **3i**, X = 4-Br

Fig. 1. Chemical structures of compounds 1, 2 and 3a-3i.

*****Corresponding author: Alireza Foroumadi
Tel. 0098 21 66954708, Fax. 0098 21 66461178
Email: aforoumadi @yahoo.com

apoptosis inducers, were found to be highly active in the growth inhibition MTT (3- (4,5-Dimethylthiazol- 2-yl)- 2,5- diphenyltetrazolium bromide) assay, with the concentration causing 50% cell growth inhibition (IC50) values in the low nanomolar range. The MTT test has been widely used as a rapid and sensitive method for screening anticancer drugs as well as for the assessment of cytotoxicity of compounds (5,6).

Herein, we would like to report the synthesis of some 4-aryl-4*H*-chromenes having substitution of fluoro, chloro and bromo atoms in 2-, 3- and 4-positions of the phenyl ring (compounds 3a-3i, Fig. 1) and their cytotoxic activities were evaluated against cancer cell lines using MTT assay.

MATERIALS AND METHODS

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H NMR spectra were recorded on a Bruker FT-80 NMR spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. The purity of the compounds was monitored by thin layer chromatography using several solvents with different polarities.

Chemistry

2-amino- 7-(dimethylamino)- 4-(substituted phenyl)- 4*H*-chromene- 3-carbonitriles (compound 3a-i) were synthesized by condensation of 3-dimethylaminophenol (compound 4), a substituted benzaldehyde (compound 5a-i) and malonitrile in ethanol in the presence of piperidine (Scheme 1) (7). The synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral data.

2- amino- 7-(dimethylamino)- 4-(2-fluorophenyl) -4H-chromene- 3-carbonitriles (compounds 3a-i): General procedure

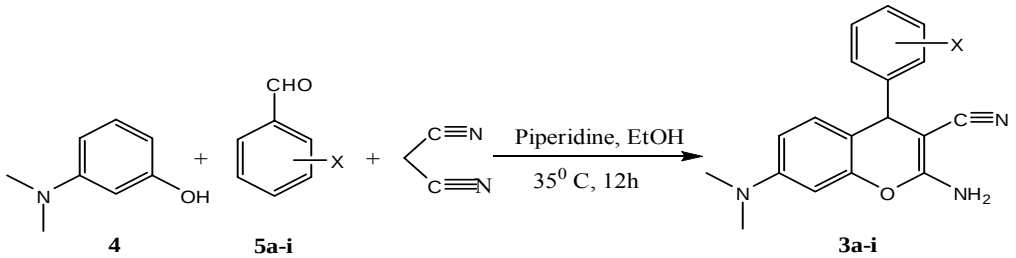
Piperidine (0.85 g, 10 mmol) was added to a mixture of 3-dimethylaminophenol (compound 4, 0.68 g, 5 mmol), substituted benzaldehyde (compound 5a-i, 5 mmol) and malonitrile (0.03 g, 5 mmol) in ethanol (20 ml). The reaction mixture was stirred at 35 °C for 12 h. After cooling, the precipitated solid was filtered, washed with cold ethanol and crystallized from the same solvent.

2- amino- 7-(dimethylamino)- 4-(2-fluorophenyl)- 4H-chromene- 3-carbonitrile (compound 3a)

Yield 49%; m.p. 202-204 °C; white powder; IR (KBr, cm^{-1}): 3371, 3180 (NH_2), 2193 (CN); ^{1}HNMR (DMSO-d_6, 80 MHz) δ:7.26-6.24 (m, 4H, phenyl), 6.83 (d, 1H, J = 8.4 Hz, H_5 chromene), 6.38 (d, 1H, , J = 2.4 Hz, H_8 chromene), 6.46-6.22 (m, 1H, H_6 chromene), 5.88 (brs, 2H, NH_2), 4.97 (s, 1H, H_4 chromene), 2.91 (s, 6H, NMe_2); Ms (m/z, %): 309(M^+, 25), 214(100), 198(11), 149(4), 97(4).

2- amino- 7-(dimethylamino)- 4-(3-fluorophenyl)-4H-chromene- 3-carbonitrile (compound 3b)

Yield 39%; m.p. 159-160 °C; white powder; IR (KBr, cm^{-1}): 3459, 3320 (NH_2), 2193 (CN); ^{1}HNMR ($CDCl_3$) δ: 7.40-6.70 (m, 4H, phenyl), 6.58-6.20 (m, 3H, $H_{5,6,8}$ chromene), 4.63 (s, 1H, H_4chromene), 4.56 (s(brs), 2H, NH_2), 2.93(s, 6H, NMe_2); Ms (m/z, %): 309(M^+, 94), 292(25), 220(18), 214(100), 197(100), 170(57), 154(66), 106(100), 98(33), 95(25), 75(18).



Scheme 1. Synthesis of 4-aryl-4*H*-chromenes 3a-i

2- amino- 7-(dimethylamino)- 4-(4-fluorophenyl)- 4H-chromene- 3-carbonitrile (compound 3c)

Yield 22%; m.p. 133-134 °C; yellow powder; IR (KBr, cm^{-1}): 3469, 3320 (NH_2), 2197 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.28-6.90 (m, 4H, phenyl), 6.80 (d, 1H, *J* = 8.4 Hz, H_5 chromene), 6.50 (d, 1H, , *J* = 2.4 Hz, H_8 chromene), 6.44-6.23 (m, 1H, H_6 chromene), 4.63 (s, 1H, H_4 chromene), 4.59 (s(brs), 2H, NH_2), 2.93(s, 6H, NMe_2); Ms (m/z, %): 309(M^+,86), 291(14), 220(14), 214(100), 197(71), 169(20), 106(15).

2- amino- 4-(2-chlorophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3d)

Yield 75%; m.p. 187-188 °C; white powder; IR (KBr, cm^{-1}): 3483, 3320 (NH_2), 2202 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.67-7.23 (m, 4H, phenyl), 6.75 (d, 1H, *J* = 8.3 Hz, H_5 chromene), 6.47 (d, 1H, , *J* = 2.3 Hz, H_8 chromene), 6.42-6.28 (m, 1H, H_6 chromene), 4.71 (s, 1H, H_4 chromene), 4.57 (s(brs), 2H, NH_2), 2.93 (s, 6H, NMe_2); Ms (m/z, %): 325(M^+, 27), 324(46), 290(11), 245(11), 214(100), 197(87), 162(19), 144(52), 106(32), 105(19).

2- amino- 4-(3-chlorophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3e)

Yield 62%; m.p. 166-167 °C; yellow powder; IR (KBr, cm^{-1}): 3426, 3324 (NH_2), 2197 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.30-7.05 (m, 4H, phenyl), 6.66 (d, 1H, , *J* = 8.8 Hz, H_5 chromene), 6.47 (m, 1H, *J* = 2.4 Hz , H_8 chromene), 6.40-6.26 (m, 1H, H_6 chromene), 4.61 (brs, 1H, H_4 chromene), 4.58 (brs, 2H, NH_2), 2.92 (s, 6H, NMe_2); Ms (m/z, %): 325(M^+, 90), 324(100), 214(100), 197(100), 169(60), 106(80), 75(75).

2- amino- 4-(4-chlorophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3f)

Yield 47%; m.p. 198-200 °C; white powder; IR (KBr, cm^{-1}): 3475, 3314 (NH_2), 2191 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.29 (d, 2H, *J* = 8.6 Hz , H_3 and H_5 phenyl), 7.19 (d, 2H, , *J* = 8.6 Hz , H_2 and H_6 phenyl), 6.65 (d, 1H, , *J* = 8.2 Hz, H_5 chromene), 6.70 (m, 1H, *J* = 2.6 Hz , H_8 chromene), 6.41-6.24 (m, 1H, H_6 chromene), 4.62 (s, 1H, H_4 chromene), 4.55 (brs), 2H, NH_2), 2.93(s, 6H, NMe_2); Ms (m/z, %): 325(M^+, 20), 269(52), 214(100), 198(52), 144(16), 106(10), 75(10).

2- amino- 4-(2-bromophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3g)

Yield 49%; m.p. 197-198 °C; yellow powder; IR (KBr, cm^{-1}): 3457, 3313 (NH_2), 2202 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.60-7.50(m, 1H, H_3 phenyl), 7.21-7.01(m, 3H, H_{4-6} phenyl), 6.85 (d, 1H, *J* = 8.5 Hz, H_5 chromene), 6.47 (d,1H, J=2.5, H_8 chromene), 6.45-6.21 (m, 1H, H_6 chromene), 5.31(s, 1H, H_4 chromene), 4.58(brs, 2H, NH_2), 2.91(s, 6H, NMe_2); Ms (m/z, %): 371(M^++2, 13), 369(M^+, 13), 214(100), 198(15).

2- amino- 4-(3-bromophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3h)

Yield 67%; m.p. 178-180 °C; yellow powder; IR (KBr, cm^{-1}):3457, 3349 (NH_2), 2192 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.32-7.05(m, 4H, phenyl), 6.75 (d, 1H, *J* = 8.5 Hz, H_5 chromene), 6.47 (d, 1H, J=2.5, H_8 chromene), 6.40-6.22 (m, 1H, H_6 chromene), 4.59 (brs, 3H, H_4 chromene and NH_2), 2.92(s, 6H, NMe_2); Ms (m/z, %): 371(M^++2, 95), 369(M^+, 98), 368 (56), 354(15), 214(100), 198(15), 170(30), 144(50).

2- amino- 4-(4-bromophenyl)- 7-(dimethylamino)- 4H-chromene- 3-carbonitrile (compound 3i)

Yield 25%; m.p. 202-204 °C; pale yellow powder; IR (KBr, cm^{-1}): 3477, 3324 (NH_2), 2182 (CN); ^{1}HNMR ($CDCl_3$, 80 MHz) δ: 7.42 (d, 2H, *J* = 8.2 Hz , H_3 and H_5 phenyl), 7.05 (d, 2H, , *J* = 8.2 Hz , H_2 and H_6 phenyl), 6.73 (d, 1H, *J* = 8.4 Hz, H_5 chromene), 6.47-6.26 (m, 2H, H_6 and H_8 chromene), 4.61 (s,2H, H_4chromene), 4.58 (s(brs), 1H, NH_2), 2.93(s, 6H, NMe_2); Ms (m/z, %): 371(M^++2, 10), 369(M^+, 11), 214(100), 198(19).

Biological activity

Cell lines and cell culture

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 (Pastor Institute, Tehran, Iran). 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-i was assessed in monolayer cultures using MTT colorimetric assay (8,9). 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 µl of the cell suspension was seeded into 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) (10) 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 using a microplate reader at 570 nm. Each set of experiments was independently performed three times. For each compound, IC50 compared with the control was calculated from concentration-response curves by regression analysis.

RESULTS

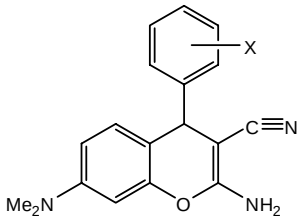
The compounds 3a-3i were tested *in vitro* against a panel of six human tumor cell lines. The percentage of growth was evaluated using MTT colorimetric assay versus controls not treated with test agents. For each compound, IC50 was determined and reported in Table 1. The data for vincristine was included for comparison. The obtained results revealed that compounds 3f, 3g and 3i possessed poor activity (IC50 >100 µM) against all cell lines.

DISCUSSION

In KB cell line, compound 3b was found to be the most active compound with IC50 less than 1 µM. Compound 3b followed by compound 3e were the most active compounds against EJ cell line (IC50 ≤3.3 µM). No cytotoxic effect was observed against breast carcinoma cell line MCF-7 except for compound 3h with moderate cytotoxic activity (IC50 = 7.3 µM). Compounds 3b followed by 3h were found to be the most potent compounds against astrocytoma cell line 1321N1 with IC50 of <1 µM. Moreover, they were strongly more potent than standard drug vincristine. These compounds were also found to be the most potent compounds against osteosarcoma cell line Saos with IC50 of <1 µM. In the ovary carcinoma cell line A 2780 CP, compounds 3b and 3i showed good activity (IC50 <0.6 µM). In general, it was observed that compounds 3b and 3h had the best cytotoxicity results against nearly all cell lines (Table 1).

The results highlight the relationship between structure and biological selectivity of the drugs. It seems that at least part of growth or proliferation inhibitory effects of the compounds could be particularly attributed to the substitution at 2, 3 or 4 positions of the phenyl ring. Among these positions, substitution at the C3 position greatly influences their potency and spectrum of cytotoxic activity.

Table 1. Cytotoxic activity (IC50, μM)[a] of compounds 3a–i against different cell lines in comparison with vincristine.



Compound	X	Cell line					
		KB[b]	EJ[c]	MCF-7[d]	1321N1[e]	Saos-2[f]	A 2780 CP[g]
3a	2-F	29.5 ± 0.70	88.5 ± 16.5	70.0 ± 14	77.0 ± 14.0	47.5 ± 3.00	27.0 ± 2.00
3b	3-F	0.25 ± 0.01	3.15 ± 0.35	175 ± 35	0.22 ± 0.05	0.31 ± 0.01	0.20 ± 0.01
3c	4-F	145 ± 21.0	62.0 ± 3.00	99.0 ± 57	175 ± 23.0	53.0 ± 15.0	125 ± 30.0
3d	2-Cl	76.5 ± 21.3	93.5 ± 2.00	140 ± 14	217 ± 30.0	137 ± 21.0	104 ± 20.5
3e	3-Cl	1.60 ± 0.40	3.30 ± 0.42	95.0 ± 7.0	31.5 ± 6.00	7.50 ± 2.00	2.50 ± 0.70
3f	4-Cl	120 ± 15.0	450 ± 212	425 ± 91	285 ± 21.0	135 ± 21.5	310 ± 28.0
3g	2-Br	170 ± 18.0	405 ± 190	375 ± 77	320 ± 42.0	175 ± 7.00	270 ± 28.0
3h	3-Br	0.44 ± 0.02	5.35 ± 0.35	7.50 ± 0.7	0.90 ± 0.10	0.75 ± 0.30	0.45 ± 0.08
3i	4-Br	170 ± 5.00	505 ± 21.9	1700 ± 141	225 ± 91.0	360 ± 127	415 ± 21.0
Vincristine		0.10 ± 0.01	0.24 ± 0.08	8.90 ± 3.0	5.00 ± 1.00	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.

CONCLUSION

In conclusion, we have explored the substitution of the 2, 3 and 4 positions of 4-aryl-4*H*-chromenes as potential anticancer agents. It was found that halogenations of the 3-position resulted in a large increase of activity; however substitution of the 3-position by F or Br afforded better results. Anti cancer effects of these compounds in tumor cells indicated that they are good candidates for further pharmacological studies to discover effective chemotherapeutic for the treatment of human cancer diseases.

ACKNOWLEDGMENT

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