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Original Article

Searching for new cytotoxic agents based on chromen-4-one and chromane-2,4-dione scaffolds

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Abstract

Cancer is a major cause of death worldwide and novel anticancer agents for its better management are much needed. Benzopyrone-based compounds, such as chromones, possess several distinctive chemical and biological properties, of which the cytotoxicity against cancer cells seems to be prominent. In this study, two series of compounds based on chromen-4-one (**3-10**) and chromane-2,4-dione (**11-18**) scaffolds were synthesized in moderate/high yields and evaluated for cytotoxicity against HL-60, MOLT-4, and MCF-7 cancer cells using MTT assay. In general, the compounds exhibited moderate cytotoxic effects against the cancer cell lines, among which, a superior potency could be observed against MOLT-4 cells. Chroman-2,4-dione (**11-18**) derivatives had overall higher potencies compared to their chromen-4-one (**3-10**) counterparts. Compound **13** displayed the lowest IC_{50} values against HL-60 (IC_{50}, 42.0 ± 2.7 μM) and MOLT-4 cell lines (IC_{50}, 24.4 ± 2.6 μM), while derivative **11** showed the highest activity against MCF-7 cells (IC_{50}, 68.4 ± 3.9 μM). In conclusion, this study provides important information on the cytotoxic effects of chromone derivatives. Benzochroman-2,4-dione has been identified as a promising scaffold, which its potency can be modulated by tailored synthesis with the aim of finding novel and dissimilar anticancer compounds.

Keywords: Antineoplastic agents; Cancer; Chromones; Drug screening.

INTRODUCTION

Benzopyrone-based compounds, such as chromone, coumarin, and flavonoids have come to the attention of many investigators in recent years, due to their distinctive chemical and biological properties (1-4). In this context, benzopyran and its derivatives constitute an important class of natural compounds representing a broad range of biological activities such as antibacterial and antiviral (5,6), anticancer (7,8), antioxidant (5,9), anti-inflammatory (10), monoamine oxidase B inhibition (11-13), interaction with A3 adenosine receptor (14,15), and anticoagulant effects (16). Moreover, they are important intermediates in the synthesis of several natural products and medicinal agents (1,3,17).

Cancer continues to be among one of the most important causes of death worldwide (18). Chemotherapeutic agents that are available today invariably suffer from poor toxicity profile and adverse effects that cause dose reduction and therefore lack of efficacy (19). Therefore, discovery of novel anticancer agents is one of the top priorities of drug discovery programs. In this regard, benzopyran based compounds, like chromones, may be regarded as privileged scaffolds for rational design of anti-cancer agents (20). Cytotoxic activity has been reported for several chromone derivatives such as 3-formylchromones (21), 2,3-diarylchromanones (22), 3-hydroxychromones (23), 4H-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates (24), 3-(3-oxobenzofuran-2(3H)-ylidene)methyl)-4H-chromones (25) and also for organometallic complexes of chromone derivatives (26).

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The anticancer effect of these compounds has been reported against leukemia (22,25,27) and breast cancer cells (20,28,29). Furthermore, chromones seem also to exert antiproliferative effects against lung and colon cancer cells (8). Several derivatives of chromones seem to induce apoptosis in different cancer cells (28,29).

In view of the literature evidence on the potential anticancer effect of chromone derivatives and also in continuation of our work on the development of novel heterocyclic based cytotoxic agents (30-32), herein we report on the synthesis, characterization, and *in vitro* cell based cytotoxic activities of a series of chromen-4-one and chromane-2,4-dione derivatives.

MATERIALS AND METHODS

Reagents, general procedures, and apparatus

3- (4,5-Dimethylthiazol-2-yl)- 2, 5- diphenyl-tetrazolium bromide (MTT), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluoro-phosphate (PyBOP), N,N-diisopropylethyl-amine (DIPEA), chromone-2-carboxylic (**1**), and chromone-3-carboxylic (**2**) acids as well as primary amines were obtained from Sigma-Aldrich (USA).

Fetal bovine serum (FBS), RPMI1640, phenol red free RPMI1640, phosphate buffered saline (PBS), trypsin, and trypan blue were purchased from Biosera (France). Penicillin/streptomycin was purchased from Invitrogen (USA). Dimethyl sulfoxide (DMSO) and cisplatin were from Merck (Germany) and EBEWE Pharma (Austria), respectively. All other reagents and solvents were pro analysis grade and acquired from Merck, Sigma-Aldrich (USA) and PanReac AppliChem (Germany) and used without further purification.

Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F254 (Merck, Portugal). The thickness of TLC layer was 0.2 mm. The spots were visualized under UV detection at 254 and 366 nm. Flash column chromatography was performed using silica gel 60 (0.2-0.5 or 0.040-0.063 mm; Carlo Erba, Portugal). The organic phases were dried over anhydrous Na_2SO_4 after workup and extraction. Whenever needed, the solutions were decolorized using activated charcoal. A Buchi Rotavapor® (Switzerland) was used to evaporate the solvents.

Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 NMR (^{13}C NMR) data were acquired, at room temperature, on a Brüker AMX 400 spectrometer (Spain) operating at 400.15 and 100.63 MHz, respectively. Chemical shifts were expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference; coupling constants (*J*) were reported in Hz. Electron impact mass spectra (EI-MS) were carried out on a VG AutoSpec instrument (Spain); the data were reported as *m/z* (% of relative intensity of the most important fragments).

General synthesis procedure

A solution of PyBOP (1 mmol) in dichloromethane (2.5 mL) was added to a solution of chromone carboxylic acid (1 mmol) in dimethylformamide (2.5 mL) and DIPEA (1 mmol) at 4 °C. The mixture was stirred on ice for 30 min. Afterwards the (hetero) aromatic amine was added to the reaction that was then warmed up to the ambient temperature. Then, the reaction was stirred for 4 h. The crude product was extracted (CH2Cl2) and purified by flash chromatography (CH2Cl2/MeOH or EtOAc/n-hexane). Final purification was performed by recrystallization (EtOAc/n-hexane).

N- Cyclohexyl - 4- oxo - 4 H –chromene - 2 - carboxamide (3)

Yield: 60%. ^{1}H NMR ($CDCl_3$): δ = 1.73 – 1.18 (6H, m, 2 x H(3'), 2 x H(4'), 2 x H(5')), 2.11 – 1.74 (4H, m, 2 x H(2'), 2 x H(6')), 4.06-3.91 (1H, m, H(1')), 6.70 (1H, d, *J* = 7.2, CONH), 7.17 (1H, s, H(3)), 7.45 (1H, ddd, *J* = 8.1, 7.2, 1.0, H(6)), 7.53 (1H, dd, *J* = 8.5, 0.6, H(8)), 7.74 (1H, ddd, *J* = 8.7, 7.2, 1.7, H(7)), 8.22 (1H, dd, *J* = 8.0, 1.5 Hz, H(5)). ^{13}C NMR (DMSO): 24.9 (C3', C5'), 25.4 (C4'), 32.9 (C2', C6'), 49.1 (C1'), 112.1 (C3), 118.0 (C8), 124.4 (C4a), 125.9 (C6), 126.2 (C5), 134.4 (C7), 155.0 (C8a), 155.3 (C2), 158.2 (CONH), 178.2 (C4). EI-MS *m/z*: 271 ($M^{+\bullet}$, 38), 228 (12), 191 (19), 190 (100), 173 (16), 145 (12), 89 (39).

N- Phenyl - 4 -oxo - 4H – chromene – 2 - carboxamide (4)

Yield: 81 %. ^{1}H NMR (DMSO): δ = 6.99 (1H, s, H(3)), 7.21 (1H, ddd, *J* = 7.6, 7.5, 1.1 H(4')) 7.44 (2H, ddd, *J* = 7.0, 6.9, 1.8, H(3'), H(5')), 7.58 (1H, ddd, *J* = 8.0, 6.9, 1.1, H(6)), 7.81 (2H, dd, *J* = 8.1, 1.1, H(2'), H(6')), 7.86 (1H, dd, *J* = 8.3, 0.8, H(8)), 7.95 (1H, ddd, *J* = 8.5, 6.8,1.6, H(7)), 8.10 (1H, dd, *J* = 7.9, 1.6, H(5)), 10.77 (1H, s, CONH). ^{13}C NMR (DMSO): δ = 111.1 (C3), 119.1 (C8), 121.1 (C2', C6'), 123.7 (C4a), 124.9 (C6), 125.0 (C5), 126.2 (C4'), 128.9 (C3', C5'), 135.1 (C7), 137.5 (C1'), 155.2 (C8a), 155.7 (C2), 157.8 (CONH), 177.4 (C4). EI-MS m/z: 265 ($M^{+\bullet}$, 18), 264 (83), 236 (32), 145 (18), 117 (19), 92 (1689 (100), 69 (15), 65 (16), 63 (19).

N-(4-Chlorophenyl)-4-oxo-4H- chromene- 2 - carboxamide (5)

Yield: 50%. ^{1}H NMR (DMSO): δ = 6.97 (1H, s, H(3)), 7.48 (2H, d, *J* = 8.8, H(3'), H(5')), 7.56 (1H, m, H(6)), 7.83-7.96 (4H, m, H(7), H(8), H(2'), H(6')), 8.08 (1H, dd, *J* = 7.9, 1.6, H(5)), 10.87 (1H, s, CONH). ^{13}C NMR (DMSO): δ = 111.2 (C3), 119.0 (C8), 122.7 (C2', C6'), 123.7 (C4a), 125.0 (C5), 126.2 (C6), 128.7 (C4'), 128.8 (C3', C5'), 135.2 (C7), 136.6 (C1'), 155.2 (C8a), 155.5 (CONH), 157.9 (C2), 177.3 (C4). EI-MS m/z: 301 (34), 300 (33), 299 ($M^{+\bullet}$, 100), 298 (50), 282 (15), 270 (24), 173 (14), 145 (28), 101 (18), 90 (10), 89 (89), 69 (16), 63 (14).

N-(4- (Methylthio) phenyl)-4 -oxo- 4H - chromene-2-carboxamide (6)

Yield: 60%. ^{1}H NMR ($CDCl_3$): δ = 2.51(3H, s, SCH_3), 7.28 (1H, s, H(3)), 7.31 (2H, d, *J* = 8.6, H(3'), H(5')), 7.50 (1H, ddd, *J* = 8.0, 7.2, 1.0, H(6)), 7.60 (1H, d, *J* = 8.5, H(8)), 7.66 (2H, d, *J* = 8.6 H(2'), H(6')), 7.78 (1H, ddd, *J* = 8.0, 7.1, 1.0, H(7)), 8.26 (1H, dd, *J* = 8.0, 1.5, H(5)), 8.51 (1H, s, CONH)). ^{13}C NMR ($CDCl_3$): δ = 16.2 (SCH_3), 112.7 (C3), 118.0 (C8), 121.0 (C3', C5'), 124.4 (C4a), 126.2 (C5), 126.3 (C6), 127.6 (C2', C6'), 133.7 (C1'), 134.7 (C7), 135.74 (C4'), 154.4 (C8a), 155.1 (C2), 156.8 (CONH), 178.7 (C4). EI-MS m/z: 312 (M^{+1}, 21), 311 ($M^{+\bullet}$, 100), 278 (16), 140 (11), 138 (65), 89 (23).

N-(4- (Methylsulfonyl)phenyl) -4 -oxo- 4H - chromene -2- carboxamide (7)

Yield: 54%. ^{1}H NMR (DMSO): δ = 3.23 (3H, s, CH_3), 7.03 (1H, s, H(3)), 7.59 (1H, ddd, *J* = 8.1, 7.1, 1.1, H(6)), 7.86 (1H, dd, *J* = 8.5, 0.7, H(8)), 7.98 – 7.92 (1H, m, H(7)), 8.01-7.98 (2H, m, H(3'), H(5')), 8.12-8.07 (3H, m, H(2'), H(5), H(6')), 11.10 (1H, s, NH). ^{13}C NMR (DMSO): δ = 43.7 (CH_3), 111.5 (C3), 119.1 (C8), 121.0 (C2', C6'), 123.8 (C8a), 125.0 (C6), 126.3 (C5), 128.2 (C3', C5'), 135.2 (C7), 136.4 (C4'), 142.2 (C1'), 155.1 (C8a), 155.2 (C2), 158.4 (CONH), 177.3 (C4). EI-MS m/z: 343 ($M^{+\bullet}$, 19), 343 (100), 214 (9).

N- (2-Thiazolyl) -4-oxo- 4H-chromene- 2 - carboxamide (8)

Yield: 55%. ^{1}H NMR ($CDCl_3$): δ= 7.05 (1H, s, H(3)), 7.38 (1H , d, *J* = 3.7, H(3'), 7.55 (1H, ddd, *J* = 8.1, 7.0, 1.2, H(6)), 7.64 (1H, d, *J* = 3.7, H(4')), 7.84 (1H, dd, *J* = 8.5, 0.8, H(8)), 7.90 (1H, ddd, *J* = 8.6, 7.0, 1.7, H(7)), 8.06 (1H, dd, *J* = 8.0, 1.3 Hz, H(5)), 13.32 (1H, bs, NH). ^{13}C NMR ($CDCl_3$): δ = 111.8 (C3), 114.7 (C3'), 119.1 (C8), 124.9 (C4a, C6), 126.2 (C5), 135.1 (C7, C4'), 155.3 (C1', C2, C8a, CONH), 159. 5 (C1'), 177.4 (C4). EI-MS m/z: 272.1 ($M^{+\bullet}$, 58), 244 (18), 216 (13), 173 (49), 145 (30), 89 (100).

N- (5-Methyl -2 -thiazolyl) – 4 - oxo- 4H - chromene-2-carboxamide (9)

Yield: 48%. 1H NMR ($CDCl_3$): δ = 2.39 (3H, d, J = 1.1 Hz, CH_3), 7.04 (1H, s, H(3)), 7.32 (1H, q, J = 1.1 Hz, H(4')), 7.56 (1H, ddd, J = 8.1, 7.2, 1.1 Hz, H(6)), 7.83 (1H, d, J = 7.9 Hz, H(8)), 7.91 (1H, ddd, J = 8.6, 7.1, 1.7 Hz, H(7)), 8.07 (1H, dd, J = 8.0, 1.4 Hz, H(5)).^{13}C NMR ($CDCl_3$): δ = 11.4 (CH3), 111.7 (C3), 119.1 (C8), 123.8 (C3'), 124.9 (C4a, C6), 126.1 (C5), 135.1 (C7, C4'), 155.4 (C1', C2, C8a, CONH), 177.4 (C4). EI-MS m/z: 288 ($M^{+\bullet}$, 53), 286 (86), 258 (72), 230 (62), 230 (62), 173 (95), 145 (82) 68 (100).

N- (Pyridin-2-yl)- 4-oxo - 4H-chromene – 2 - carboxamide (10)

Yield: 22%. ^{1}H NMR ($CDCl_3$): δ = 7.19 (1H, ddd, *J* = 7.4, 5.0, 0.9, H(4')), 7.30 (1H, s,

H(3)), 7.50 (1H, ddd, *J* = 8.1, 7.2, 1.0, H(6)), 7.63 (1H, dd, *J* = 8.5, 0.6, H(6')), 7.89-7.72 (2H, m, H(5'),H(8)), 8.25 (1H, dd, *J* = 8.0, 1.4, H(3')), 8.45-8.32 (2H, m, H(5), H(7)), 9.31 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ = 113.0 (C3), 114.8 (C6'), 118.2 (C8), 121.1 (C4'), 124.4 (C4a), 126.2 (C6), 126.3 (C5), 134.9 (C7), 139.0 (C5'), 148.1 (C3'), 150.1 (C1'), 154.0 (C8a), 155.2 (C2), 157.3 (CONH), 177.9 (C4). EI-MS m/z: 267 (22), 266 ($M^{+\bullet}$, 99), 238 (58), 237 (66), 210 (83), 89 (100).

(E/Z) - 3 - ((Cyclohexylamino) methylene) chromane-2,4-dione (11)

Yield: 30%. ^{1}HNMR (DMSO): δ = 1.32-1.94 (10H, m, 2 x H(2'), 2 x H(3'), 2 x H(4'), 2 x H(5'), 2 x H(6')), 3.70 (1H, m, H(1')), 7.32 (2H, m, H(6), H(8)), 7.66 (1H, dd, *J* = 8.0, 7.3, H(7)), 8.02 (0.7H, d, *J* = 7.6, H(5)), 8.11 (0.3H, d, *J* = 7.6, H(5)), 8.44 (0.7H, d, *J* = 14.8, H(C<u>H</u>NH)), 8.60 (0.3H, d, J = 14.8, H(C<u>H</u>NH)), 10.31 (0.3H, *br*s, NH), 11.85 (0.7H, *br*s, NH). ^{13}C NMR (DMSO): δ: 23.89 (C), 24.41 (C), 30.74 (C,), 32.18 (C), 35.75 (C), 58.56 (C1'), 95.6 (C3), 116.9 (C8), 120.2 (C4a), 123.9 (C6), 125.2 (C5), 134.3 (C7), 154.2 (C8a), 160.3 (CHNH), 162.4 (C2), 179.6 (C4). EI-MS m/z: 271 (M^{+1}, 100), 228 (18), 188 (23), 175 (23), 173 (18), 121 (31), 97 (22), 57 (17).

(E/Z)-3- ((Phenylamino)methylene)chromane -2,4-dione (12)

Yield 60%. ^{1}H NMR ($CDCl_3$) δ: 7.40-7.26 (5H, m, H(2'), H(3'), H(4'), H(5'), H(6')), 7.52 - 7.45 (2H, m, H(6), H(8)), 7.64-7.57 (1H, m, H(7)), 8.14 (0.7H, dd, *J* = 7.8, 1.6, H(5)), 8.08 (0.3H, dd, *J* = 7.8, 1.7, H(5)), 8.91 (0.7H, d, *J* = 13.6, H(C<u>H</u>NH), 9.05 (0.3H, d, *J* = 14.5, H(C<u>H</u>NH), 11,94 (0.3H, d, *J* = 13.8, NH), 13.70 (0.7H, d, *J* = 12.2, NH). ^{13}C NMR ($CDCl_3$) δ: 99.0/98.9 (C3), 117.6/117.5 (C8), 118.6/118.7 (C2', C6'), 120.9/120.5 (C4a), 124.6/124.4 (C6), 126.7/126.0 (C5), 127.7/127.5 (C4'), 130.4/130.3 (C3', C5'), 135.0/134.9 (C7), 137.9 (C1'), 154.9/153.6 (C8a), 155.2/155.1 (CHNH), 165.4/163.8 (C2), 182.0/178.8 (C4). EI-MS m/z: 266 (6), 265($M^{+\bullet}$, 61), 173 (100), 144 (16), 121 (35), 117 (36).

(E/Z)-3- ((4-Chlorophenyl)amino)methylene) chromane-2,4-dione (13)

Yield: 47%. ^{1}H NMR (DMSO) δ: 7.34 (1H, d, *J* = 7.8, H(8)), 7.38 (1H, dd, *J* = 7.6, 1.3, H(6)), 7.54 (2H, d, *J* = 8.8, (H3'), H(5')), 7.70-7.73 (3H, m, H(2'), H(6'), H(7)), 7.99 (1H, dd, *J* = 7.8, 1.5 Hz , H(5)), 8.85 (0.7H, d, *J* = 13.8, C<u>H</u>NH), 8.88 (0.3H, d, *J* = 14.8, C<u>H</u>NH), 11.84 (0.3H, d, *J* = 14.8, NH), 11.84 (0.3H, d, *J* = 14.8, NH), 13.39 (0.7H, d, *J* = 13.8, NH). ^{13}C NMR (DMSO) δ: 98.3 (C3), 117.2 (C8), 119.9 (C4a), 121.6/121.3 (C2', C6'), 124.3 (C5), 125.5 (C6), 129.6/129.1 (C3', C5'), 131.2 (C4'), 135.1 (C7), 137.1 (C1'), 154.5 (C8a) 155.8/154.5 (CHNH), 162.2 (C2), 180.3 (C4). EI-MS m/z: 301 (37), 300 (21), 299 ($M^{+\bullet}$, 88), 174 (16), 173(100), 151 (18), 121 (37), 92 (11), 89 (10).

(E/Z) – 3 - ((4-(Methylthio)phenyl)amino) methylene)chromane-2,4-dione (14)

Yield: 51%. ^{1}H NMR ($CDCl_3$) δ: 2.52 (3H, s, CH_3), 7.35-7.25 (5H, m, H(6), H(8), H(2'), H(3'), H(5'), H(6')), 7.64-7.57 (1H, m, H7), 8.07 (0.7H, dd, *J* = 7.8, 1.7, H(5)), 8.14 (1H, dd, *J* = 7.8, 1.7, H(5)), 8.85 (1H, d, *J* = 13.6 Hz, C<u>H</u>NH), 8.99 (0.3H, d, *J* = 14.5, C<u>H</u>NH), 11.93 (0.3H, d, *J* = 14.5, NH), 13.73 (0.7 H, d, *J* = 13.2, NH). ^{13}C NMR (DMSO) δ: 15.9/15.8 (CH_3), 98.8/98.7 (C3), 117.5/117.4 (C8), 119.0/118.9 (C2', C6'), 120.4 (C4a), 124.5/124.2 (C5), 126.6/125.8 (C6), 127.9/127.8 (C3', C5'), 134.82/134.7 (C7), 134.83 (C4'), 138.6/138.4 (C1'), 154.3/152.9 (CHNH), 155.0 (C8a), 163.6 (C2), 181.8 (C4). EI-MS m/z: 313 (27), 312 (76), 311 ($M^{+\bullet}$, 100), 296 (25), 174(29), 173(90), 148 (24), 121 (70).

(E/Z) - 3 - ((4-(Methylsulfonyl)phenyl)amino) methylene)chromane-2,4-dione (15)

Yield: 47%. ^{1}H NMR (DMSO) δ: 2.78 (3H, s, CH_3), 7.40-7.33 (2H, m, H(8), H(6)), 7.75-7.69 (1H, m, H(7)), 7.81-7.76 (2H, m, H(3'), H(5')), 7.91-7.86 (2H, m, H(2'), H(6')), 8.00 (1H, dd, *J* = 7.8, 1.5, H(5)), 8.94 (0.7H, d, *J* = 13.7, C<u>H</u>NH), 8.97 (0.3H, d, *J* = 14.7, C<u>H</u>NH), 11.91 (0.3H, d, *J* = 14.7 Hz, NH), 13.46 (0.7, d, *J* = 13.8, NH). ^{13}C NMR (DMSO) δ: 43.1 (CH_3), 98.6 (C3), 117.3/117.2 (C8), 119.9 (C4a), 120.4/120.1 (C2', C6'),

124.5/124.4 (C5), 125.3/125.2 (C3', C5'), 125.5 (C6), 135.2/135.1 (C7), 140.0 (C4'), 144.5/1144.4 (C1'), 154.4/154.2 (C8a), 155.8 (C2), 163.2/162.2 (CHNH), 180.5/177.5 (C4). EI-MS m/z: 343 ($M^{+\bullet}$, 17), 183(20), 173(21), 155 (100).

(E/Z)- 3 - ((Thiazol-2-ylamino) methylene) chromane-2,4-dione (16)

Yield 15%. ^{1}H NMR ($CDCl_3$) δ: 7.17-7.13 (1H, m, H(4')) 7.36 – 7.25 (2H, m, H(8), H(7)), 7.59-7.55 (1H, m, H(3')), 7.67-7.60 (1H, m, H(6)), 8.07 (0.7H, dd, *J* = 7.8, 1.5, H(5)), 8.14 (0.3H, dd, *J* = 7.8, 1.5, H(5)), 9.18 (0.7H, d, J = 11.9, C<u>H</u>NH), 9.25 (0.3H d, *J* = 13.1 Hz, C<u>H</u>NH), 12.20 (0.3H, d, *J* = 12.4 Hz, NH), 13.97 (0.7H, d, *J* = 9.2 Hz, NH). ^{13}C NMR ($CDCl_3$) δ: 100.7/100.6 (C3), 115.9/116.1 (C3'), 117.8/117.1 (C8), 120.6/120.1 (C4a), 124.9/124.6 (C5), 127.0/126.4 (C6), 135.8/135.5 (C7), 141.3/141.1 (C4'), 153.2 (C8a), 154.8/153.3 (CHNH), 159.8/159.7 (C2), 165.1/162.7 (C1'), 182.8/178.7 (C4). EI-MS m/z: 272 ($M^{+\bullet}$, 58), 173 (49), 145 (30), 89 (100).

(E/Z) – 3 - ((5- Methylthiazol - 2 - yl)amino)methylene)chromane-2,4-dione (17)

Yield 10%. ^{1}H NMR ($CDCl_3$) δ: 2.46 (5H, d, J = 1.3 Hz, CH_3), 7.34-7.19 (3H m, H(3'), H(8), H(7)), 7.64-7.55 (1H, m, H(6)), 8.02 (0.7H, dd, *J* = 7.8, 1.6, H(5)), 8.10 (0.3H, dd, *J* = 7.8, 1.6, H(5)), 9.04 (0.7H,), 9.12 (0.3H, s, C<u>H</u>NH), 12.08 (0.3H, *br*s, NH), 13.82 (0.7H, *br*s, NH). ^{13}C NMR ($CDCl_3$) δ: 12.2 (CH_3), 100.2/100.1 (C3), 117.6/117.5 (C8), 120.5/120.0 (C4a), 124.7/124.4 (C5), 126.8/126.2 (C6), 131.5/131.5 (C7), 135.5/135.2 (C3'), 138.3/138.2 (C4'), 154.3/152.7 (CHNH), 155.1/154.7 (C8a), 157.5/157.3 (C2), 164.9/162.7 (C1'), 182.4/178.5 (C4). EI-MS m/z: 286 ($M^{+\bullet}$, 100), 258 (27), 188 (39), 173 (50), 121 (56), 90 (74).

(E/Z)-3-((Pyridin-2-ylamino)methylene)chromane-2,4-dione (18)

Yield 61%. ^{1}H NMR (DMSO) δ: 7.42 – 7.28 (3H, m, H(4'), H(5'), H(6')), 7.77-7.64 (2H, m, H(6), H(8)), 8.05-7.90 (2H, m, H(3'), H(7')), 8.50 (1H, d, *J* = 4.7, C<u>H</u>NH), 9.39 (0.7H, d, *J* = 13.2, C<u>H</u>NH), 9.58 (0.3H, d, *J* = 14.1, C<u>H</u>NH), 11.84 (0.3H, d, *J* = 14.6 Hz, NH), 13.15 (0.7H, d, *J* = 13.1 Hz, NH), ^{13}C NMR (DMSO) δ: 99.0/98.9 (C3), 114.9/114.6 (C6'), 117.3/117.2 (C8), 120.0/120.2 (C4a), 122.4/122.2 (C4'), 124.5/124.4 (C6), 126.1/125.6 (C5), 135.4/135.3 (C7), 139.6/139.5 (C5'), 148.8/148.7 (C3'), 149.5/149.5 (C1'), 153.0/151.7 (C8a), 154.5/154.3 (CHNH), 162.9/162.6 (C2), 180.5/178 (C4). EI-MS m/z: 266 ($M^{+\bullet}$, 19), 265 (55), 210 (30), 209 (100), 79 (35).

Biological activity

Cell lines and culture

HL-60 (human promyelocytic leukemia), MOLT-4 cells (human acute lymphoblastic leukemia), and MCF-7 (human breast adenocarcinoma) were provided by Pasteur Institute of Iran (Tehran, I.R. Iran). The growth medium was prepared adding 10% FBS and antibiotics (penicillin-G and streptomycin) to RPMI1640 medium.

MTT assay

In order to evaluate the cytotoxic effect of synthesized compounds, MTT reduction assay was performed as described previously (33,34). Stock solutions of synthesized derivatives were prepared by their dissolving in DMSO. The stock solutions were diluted several times in growth medium in order to keep the final DMSO concentration below 0.25%. Three cell lines including HL-60, MCF-7, and MOLT-4 at densities of 4×10^4, 3×10^4 and 5×10^4 cells/mL, respectively, were plated in 96-well microplates (100 µL per well). The cells were cultured at 37 °C in humidified air (containing 5% CO2). Six wells were used as controls, which did not contain any synthetic compound. Blank wells were also used which contained only growth medium for background correction. After 24 h of incubation at 37 °C, 50 µL of the growth medium was removed and 50 µL media containing 3-4 different concentrations of synthesized derivative in the range 10-100 µM, depending on the potency of the compound, were added in duplicate. In experiments with HL-60 and MOLT-4 cells the plates were centrifuged before media removal. After 72 h, the medium was removed

and replaced with 80 µL of MTT solution dissolved in RPMI without phenol red at a final concentration of 0.5 mg/mL and the plates were incubated for additional 4 h at 37 °C. In order to solubilize the formazan crystals, 200 µL DMSO was added to each well. The optical density was measured at 570 nm by a microplate reader. For each concentration of the test compounds, the percent viability was calculated compared to untreated cells and IC_{50} values were calculated with Curve Expert version 1.34 for Windows. Each test was repeated between 3-5 times.

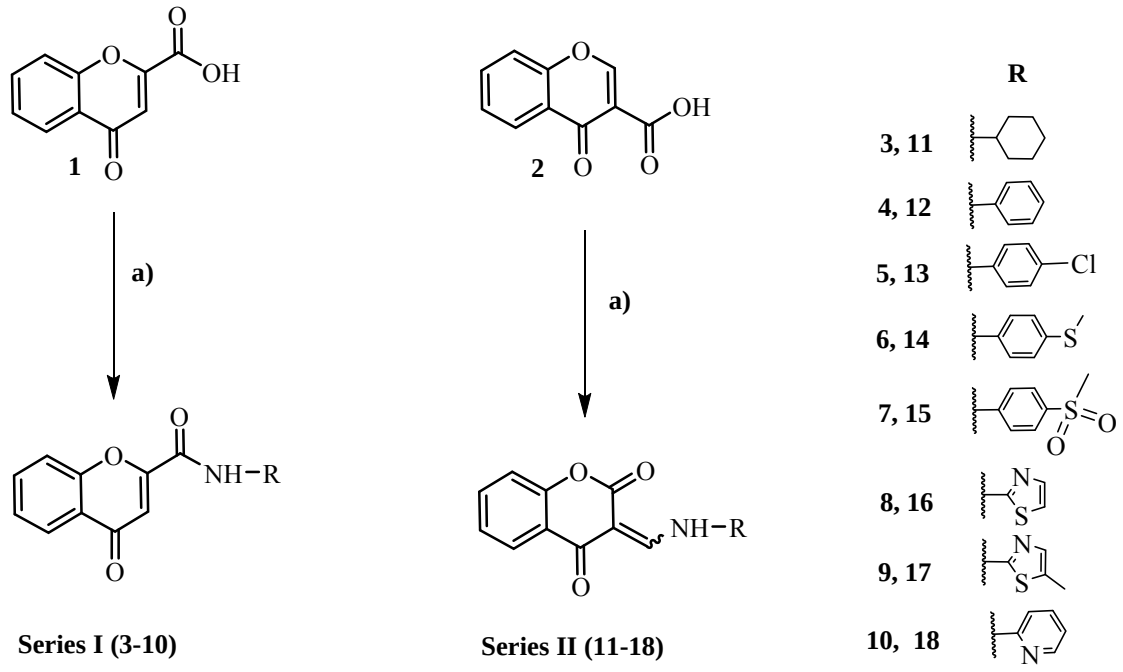
RESULTS

Chemistry

Two series of compounds based on chromen-4-one and chromane-2,4-dione scaffolds were synthesized by a straightforward one-pot synthetic strategy in moderate/high yields (Scheme 1). The chromone carboxamide derivatives from series I (3-10, Table 1) were obtained by the condensation of chromone-2-carboxylic acid (1) with the appropriate amine (Scheme 1) using the coupling reagent PyBOP in the presence of DIPEA (35-37). Chromane-2,4-dione derivatives from series II (11-18, Table 1) were obtained with similar reaction conditions by using chromone-3-carboxylic acid (2) as starting material (Scheme 1). As it was previously reported (13), the high reactivity at C-2 position of the ester intermediate formed *in situ* between the carboxylic acid and PyBOP, changed the course of the reaction resulting in a nucleophilic attack by the (hetero) aromatic amine at C-2 position of the benzopyran ring. As stated before (13) after the ring-opening and the ring closing assisted-processes the formation of the chromane-2,4-dione derivatives took place in the reaction.

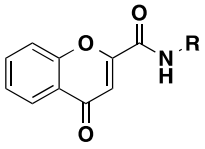
Cytotoxic activity

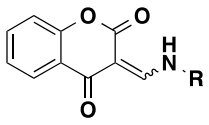
Chromone-3-carboxamide (**3-10**) and chromane-2,4-dione (**11-18**) derivatives were assayed for their cytotoxic activity on HL-60, MOLT-4, and MCF-7 cancer cells and IC_{50} values are reported in Table **2** as mean ± SEM.



Scheme 1. Strategy followed to synthetize chromen-4-one and chromane-2,4-dione derivatives. a, Reagents: PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; DIPEA, N,N-diisopropylethylamine; RNH_2.

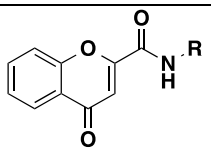
Table 1. Structure and reaction yields of synthesized chromone-2-carboxamide (series I) and chromane-2,4-dione (series II) derivatives.

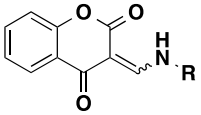




	Series I		Series II	
R	**Compounds**	**Yield (%)**	**Compounds**	**Yield (%)**
Cyclohexyl	**3**	60	**11**	30
Phenyl	**4**	81	**12**	60
4-Chlorophenyl	**5**	50	**13**	47
4-(Methylthio)phenyl)	**6**	60	**14**	51
4-(Methylsulfonyl)phenyl	**7**	54	**15**	47
2-Thiazolyl	**8**	55	**16**	15
5-Methyl-2-thiazolyl	**9**	48	**17**	10
Pyridin-2-yl	**10**	22	**18**	61

Table 2. Cell growth inhibitory activities of chromone-2-carboxamide (series I) and chromane-2,4-dione (series II) derivatives were assessed by the MTT reduction assay (IC_{50} values, μM; mean ± SEM).





	Series I				Series II			
R	**Compound**	**HL-60**	**MCF-7**	**MOLT-4**	**Compound**	**HL-60**	**MCF-7**	**MOLT-4**
Cyclohexyl	**3**	> 100	> 100	90.8 ± 1.2	**11**	64.6 ± 7.1	68.4 ± 3.9	33.2 ± 2.1
Phenyl	**4**	> 100	> 100	> 100	**12**	> 50	> 50	47.1 ± 3.6
4-Chlorophenyl	**5**	> 50	> 50	> 50	**13**	42.0 ± 2.7	> 50	24.4 ± 2.6
4-(Methylthio)phenyl)	**6**	> 100	> 100	> 100	**14**	> 50	> 50	41.6 ± 4.1
4(Methylsulfonyl)phenyl	**7**	> 50	> 50	> 50	**15**	71.8 ± 6.1	86.1 ± 19.0	48.6 ± 8.2
2-Thiazolyl	**8**	> 100	> 100	> 100	**16**	95.2 ± 7.0	85.8 ± 9.7	48.5 ± 8.3
5-Methyl-2-thiazolyl	**9**	> 100	> 100	> 100	**17**	91.5 ± 3.7	> 100	55.6 ± 3.6
Pyridin-2-yl	**10**	> 100	> 100	> 100	**18**	> 50	> 50	> 50
Cisplatin		2.7 ± 0.2	9.3 ± 2.4	2.9 ± 0.1				

DISCUSSION

In this study, two series of compounds with chromen-4-one and chromane-2,4-dione scaffolds were synthesized and their cytotoxicity was assessed against 3 human cancer cell lines using MTT assay. Chroman-2,4-dione (**11-18**) derivatives possessed higher cytotoxic capacities compared to their chromen-4-one (**3-10**) counterparts.

Several benzopyran derivatives have been reported to hold considerable cytotoxic effects against cancer cells. A series of synthesized chromone derivatives based on lavendustin structure showed anticancer effects against A-549 (lung adenocarcinoma) and HCT-15 (colon adenocarcinoma) cells (8). Also 2,3-diarylchromanones have shown potential cytotoxic effect against HL-60 promyelocytic leukemia cells (22). In another report, a series of 3-benzylidene-4-chromanones were synthesized and tested against MDA-MB-231 (triple negative breast adenocarcinoma), SK-N-MC (neuroblastoma), and KB (nasopharyngeal carcinoma) cells. Some of the synthesized derivatives were more effective than etoposide, a standard chemotherapeutic agent, against these cancer cells (20).

Different mechanisms of action have been proposed for the anticancer effect of chromone derivatives. They have been suggested to exert their effect via inhibition of topoisomerase enzymes (7,8,20). An *in silico* study has shown that

4H-chromone-1,2,3,4-tetrahydropyrimidine -5-carboxylates derivatives could be inhibitors of Bcr-Abl tyrosine kinase, which has important oncogenic functions in certain types of leukemia (27). Another investigation on breast and lung cancer cells has demonstrated that sulfonamide containing chromone derivatives possess inhibitory activity against carbonic anhydrase IX and XII, two important targets in certain types of cancer (29).

Our findings showed that chroman-2,4-dione derivatives possessed cytotoxic activity specially against leukemia cell lines and to a less extent against breast cancer cells. Although, the synthesized set of compounds in this study is not a numerous, in comparison with their anticancer activities (Table 2), the following structure activity relationships could be proposed: a) Chromane-2,4-dione derivatives (**11-18**) displayed a superior cytotoxicity profile when compared to the chromene-2-carboxamide compounds (**3-10**); b) Chromane derivatives (**11-18**) exhibited higher cytotoxic effects against MOLT-4 cell line compared to HL-60 or MCF-7 cell lines; 3) The observed order of cytotoxic activity in MOLT-4 cell line was as follows: **13** > **11** > **14** > **12** > **16** > **15** > **17** > **18**; 4) In our synthesized series of compounds, it seems that the chromane-2,4-dione derivative bearing halogen in the exocyclic phenyl ring (**13**) shows the highest potency against MOLT-4 and HL-60 cell lines; 5) Aromatization of the *N*-substituted ring reduced the cytotoxic activity against MOLT-4 cell line (**12**, IC_{50} = 47.1 ± 3.6 and **11**, IC_{50} = 33.2 ± 2.1 µM).

CONCLUSION

Our *in vitro* cell based cytotoxic evaluation showed that chromane-2,4-dione derivatives were better cytotoxic agents compared to chromone-2-carboxamide compounds against HL-60, MCF-7, and MOLT-4 human cancer lines. In particular, superior potencies have been detected in the case of acute lymphoblastic leukemia (MOLT-4) cells. Chromone-2-carboxamide compounds exhibited no cytotoxic activity against tested cancer cells; this activity loss may be related to the orientation of 2-carboxamide substituents in the site of action. The outcomes of this study may provide some information for rational discovery of chromane-2,4-dione based derivatives with the aim of finding more potent cytotoxic agents.

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REFERENCES

1. Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr Med Chem. 2005;12(8):887-916.
2. Lee T, Gong YD. Solid-phase parallel synthesis of drug-like artificial 2H-benzopyran libraries. Molecules. 2012;17(5):5467-5496.
3. Gaspar A, Matos MJ, Garrido J, Uriarte E, Borges F. Chromone: a valid scaffold in medicinal chemistry. Chem Rev. 2014;114(9):4960-4992.
4. Gomes LR, Low JN, Cagide F, Gaspar A, Reis J, Borges F. Structural characterization of some N-phenyl-4-oxo-4H-2-chromone carboxamides. Acta Crystallogr B Struct Sci Cryst Eng Mater. 2013;69(Pt 3):294-309.
5. Kim MK, Yoon H, Barnard DL, Chong Y. Design, synthesis and antiviral activity of 2-(3-amino-4-piperazinylphenyl) chromone derivatives. Chem Pharm Bull. 2013;61(4):486-488.
6. Raj T, Bhatia RK, Sharma RK, Gupta V, Sharma D, Ishar MP. Mechanism of unusual formation of 3-(5-phenyl-3H-[1,2,4]dithiazol-3-yl)chromen-4-ones and 4-oxo-4Hchromene-3-carbothioic acid N-phenylamides and their antimicrobial evaluation. Eur J Med Chem. 2009;44(8):3209-3216.
7. Ishar MP, Singh G, Singh S, Sreenivasan KK, Singh G. Design, synthesis, and evaluation of novel 6-chloro-/fluorochromone derivatives as potential topoisomerase inhibitor anticancer agents. Bioorg Med Chem Lett. 2006;16(5):1366-1370.

8. Nam DH, Lee KY, Moon CS, Lee YS. Synthesis and anticancer activity of chromone-based analogs of lavendustin A. Eur J Med Chem. 2010;45(9): 4288-4292.
9. Kim SH, Lee YH, Jung SY, Kim HJ, Jin C, Lee YS. Synthesis of chromone carboxamide derivatives with antioxidative and calpain inhibitory properties. Eur J Med Chem. 2011;46(5):1721-1728.
10. Shaveta, Singh A, Kaur M, Sharma S, Bhatti R, Singh P. Rational design, synthesis and evaluation of chromone-indole and chromone-pyrazole based conjugates: Identification of a lead for antiinflammatory drug. Eur J Med Chem. 2014;77:185-192.
11. Alcaro S, Gaspar A, Ortuso F, Milhazes N, Orallo F, Uriarte E, *et al.* Chromone-2- and -3-carboxylic acids inhibit differently monoamine oxidases A and B. Bioorg Med Chem Lett. 2010;20(9): 2709-2712.
12. Gaspar A, Reis J, Fonseca A, Milhazes N, Viña D, Uriarte E, *et al.* Chromone 3-phenylcarboxamides as potent and selective MAO-B inhibitors. Bioorg Med Chem Lett. 2011;21(2):707-709.
13. Cagide F, Silva T, Reis J, Gaspar A, Borges F, Gomes LR, *et al.* Discovery of two new classes of potent monoamine oxidase-B inhibitors by tricky chemistry. Chem. Commun. 2015;51(14): 2832-2835.
14. Cagide F, Gaspar A, Reis J, Chavarria D, Vilar S, Hripcsak G, *et al.* Navigating in chromone chemical space: discovery of novel and distinct A_3 adenosine receptor ligands. RSC Advances. 2015;5:78572-78585.
15. Gaspar A, Reis J, Kachler S, Paoletta S, Uriarte E, Klotz KN, *et al.* Discovery of novel A3 adenosine receptor ligands based on chromone scaffold. Biochem Pharmacol. 2012;84(1):21-29.
16. Stankovic´ N, Mladenovic´ M, Matic´ S, Stanic´ S, Stankovic´ V, Mihailovic´ M, *et al.* Serum albumin binding analysis and toxicological screening of novel chroman-2,4-diones as oral anticoagulants. Chem Biol Interact. 2015;227:18-31.
17. Ravichandran S, SubramanI K, Arunkumar R. Synthesis of some chromene derivatives. Int J ChemTech Res. 2009;1(2):329-331.
18. Keri RS, Budagumpi S, Pai RK, Balakrishna RG. Chromones as a privileged scaffold in drug discovery: a review. Eur J Med Chem. 2014; 78:340-374.
19. Narang AS, Desai D. Anticancer Drug Development. In: Lu Y, Mahato RI, editors. Pharmaceutical Perspectives of Cancer Therapeutics. New York: Springer; 2009. pp. 49-50.
20. Noushini S, Alipour E, Emami S, Safavi M, Kabudanian Ardestani S, Gohari AR, *et al.* Synthesis and cytotoxic properties of novel (E)-3-benzylidene-7-methoxychroman-4-one derivatives. DARU J Pharm Sci. 2013;21:31-40.
21. Kawase M, Tanaka T, Kan H, Tani S, Nakashima H, Sakagami H. Biological activity of 3-formylchromones and related compounds. In Vivo. 2007;21(5):829-834.
22. Kanagalakshmi K, Premanathan M, Priyanka R, Hemalatha B, Vanangamudi A. Synthesis, anticancer and antioxidant activities of 7-methoxyisoflavanone and 2,3-diarylchromanones. Eur J Med Chem. 2010;45(6):2447-2452.
23. Lee J, Park T, Jeong S, Kimb KH, Hong C. 3-Hydroxychromones as cyclindependent kinase inhibitors: synthesis and biological evaluation. Bioorg Med Chem Lett. 2007;17(5):1284-1287.
24. Raju BC, Rao RN, Suman P, Yogeeswari P, Sriram D, Shaik TB, *et al.* Synthesis, structure-activity relationship of novel substituted 4H-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates as potential anti-mycobacterial and anticancer agents. Bioorg Med Chem Lett. 2011;21(10):2855-2859.
25. Zwergel C, Valente S, Salvato A, Xu Z, Talhi O, Mai A, *et al.* Novel benzofuran-chromone and -coumarin derivatives: synthesis and biological activity in K562 human leukemia cells. Medchemcommun. 2013;4(12):1571-1579.
26. Ili DR, Jevti VV, Radi GP, Arsikin K, Risti B, Harhaji-Trajkovi L, *et al.* Synthesis, characterization and cytotoxicity of a new palladium(II) complex with a coumarine-derived ligand. Eur J Med Chem. 2014;74:502-508.
27. Dolatkhah Z, Javanshir S, Sadr AS, Hosseini J, Sardari S. Synthesis, molecular docking, molecular dynamics studies, and biological evaluation of 4H-chromone-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives as potential antileukemic agents. J Chem Inf Model. 2017;57(6):1246-1257.
28. Amin KM, Syam YM, Anwar MM, Ali HI, Abdel-Ghani TM, Serry AM. Synthesis and molecular docking study of new benzofuran and furo[3,2-g]chromone-based cytotoxic agents against breast cancer and p38alpha MAP kinase inhibitors. Bioorg Chem. 2018;76:487-500.
29. Awadallah FM, El-Waei TA, Hanna MM, Abbas SE, Ceruso M, Oz BE, *et al.* Synthesis, carbonic anhydrase inhibition and cytotoxic activity of novel chromone-based sulfonamide derivatives. Eur J Med Chem. 2015;96:425-435.
30. Miri R, Firuzi O, Peymani P, Nazarian Z, Shafiee A. Synthesis and cytotoxicity study of new cyclopenta [b]quinoline-1,8-dione derivatives. Iran J Pharm Res. 2011;10(3):489-496.
31. Sarkarzadeh H, Miri R, Firuzi O, Amini M, Razzaghi-Asl N, Edraki N, *et al.* Synthesis and antiproliferative activity evaluation of imidazole-based indeno[1,2-b]quinoline-9,11-dione derivatives. Arch Pharm Res. 2013;36(4):436-447.
32. Mohammadi MK, Firuzi O, Khoshneviszadeh M, Razzaghi-Asl N, Sepehri S, Miri R. Novel 9-(alkylthio)-Acenaphtho[1,2-e]-1,2,4-triazine derivatives: synthesis, cytotoxic activity and molecular docking studies on B-cell lymphoma 2 (Bcl-2). DARU. 2014;22(1):2-11.
33. Ranjbar S, Edraki N, Khoshneviszadeh M, Foroumadi A, Miri R, Khoshneviszadeh M. Design, synthesis, cytotoxicity evaluation and docking studies of 1,2,4-triazine derivatives bearing different arylidene-hydrazinyl moieties as

potential mTOR inhibitors. Res Pharm Sci. 2018;13(1):1-11.
34. Haghighijoo Z, Rezaei Z, Jaberipoor M, Taheri S, Jani M, Khabnadideh S. Structure based design and anti-breast cancer evaluation of some novel 4-anilinoquinazoline derivatives as potential epidermal growth factor receptor inhibitors. Res Pharm Sci. 2018;13(4):360-367.
35. Rusiecki V, Warne S. Synthesis of Nα-Fmoc-Nε-Nvoc-Lysine and use in the preparation of selectively functionalized peptides. Bioorganic Med Chem Lett. 1993;3(4):707-710.
36. Gaspar A, Silva T, Yáñez M, Vina D, Orallo F, Ortuso F, *et al.* Chromone, a privileged scaffold for the development of monoamine oxidase inhibitors. J Med Chem. 2011;54(14): 5165-5173.
37. Cagide F, Borges F, Gomes L, Low J. Synthesis and characterisation of new 4-oxo-N-(substituted-thiazol-2-yl)-4H-chromene-2-carboxamides as potential adenosine receptor ligands. J Mol Struct. 2015;1089:206-215.