SYNTHESIS AND *IN VITRO* ANTICANCER ACTIVITY OF NEW THIADIAZOLINES AND THIAZOLINONES CONTAINING A CHROMENYL SCAFFOLD

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A series of 14 derivatives of chromenyl-dihydro-thiadiazole and chromenyl-methylene-thiazolin-4-one were synthesized and screened (using the MTT colorimetric assay) for their *in vitro* growth inhibition capacity in six human cancer cell lines. Three cell lines displayed various levels of resistance to pro-apoptotic stimuli, while two cell lines were sensitive to pro-apoptotic stimuli. Structure Activity Relationship (SAR) analyses indicate that, of the two compound series that were investigated, chromenyl-thiadiazolines displayed higher *in vitro* anticancer activity than the thiazolinone derivatives. With respect to the thiadiazoline derivatives (**3a-l** compounds), the substitution of the exocyclic amine with a substituted-phenyl moiety increased the *in vitro* growth inhibition of cancer cells, and the 3-trifluoromethyl-phenyl-derivatives (**3h**, **3l**) displayed the highest antiproliferative activity. In addition, compounds **3h**, **3k** and **3l** overcame the intrinsic resistance of cancer cells to pro-apoptotic stimuli by induction of either cytostatic (**3h**, **3l**) or cytotoxic (**3k**) effects, which was determined by quantitative videomicroscopy.

1. Introduction

Malignant tumours represent one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor [1]. In 2008, cancer accounted for approximately eight million deaths worldwide [2], and the number of cases is expected to increase by more than 45% in the next 20 years [3]. Genotoxic agents have long targeted apoptotic cell death as a primary means of treating cancer [4,5]. However, the presence of cellular defects in many cancers has contributed to an acquired resistance to apoptotic cell death [6,7], thus lowering the effectiveness of chemo- and radiotherapies [4,5]. Resistance to anoikis, a programmed cell death that occurs upon cell detachment from the extracellular matrix [8], is emerging as a hallmark of metastatic cancers that are resistant to conventional therapies [9]. Therefore, it is necessary to identify novel types of compounds that overcome the intrinsic resistance of cancer cells to pro-apoptotic stimuli by the induction of non-apoptotic cell death (such as autophagy or necrosis [4]) or by cytostatic effects [10]. For example, glioma is naturally resistant to pro-apoptotic stimuli

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[11], and therefore resistant to conventional therapies: the pro-autophagic alkylating agent temozolomide has significant therapeutic benefits for glioma patients [12,13]. Similar observations can be made for melanoma [14,15] and oesophageal cancers [16].

Molecules containing nitrogen- and sulphur-related heterocycles (thiazole, thiazolidine, thiazolinone, thiadiazoline) are considered important pharmacophores as they can possess interesting biological activities. For example, thiadiazolines have antihelmintic, antihypertensive, anticancer, anti-inflammatory, antibacterial, analgesic, and tyrosinase inhibitory activities [17].

Thiazolinones display hypoglycaemic, antibacterial, antifungal, antituberculous, anti-HIV and also antitumoural activities [18].

Chromones are a group of naturally occurring compounds that are ubiquitous, especially in plants [19,20]. In addition to forming the basic nucleus of an entire class of natural products, the flavones, the chromone moiety forms an important component of the pharmacophores found in many molecules with medicinal significance: tyrosine and protein kinase C inhibitors, antifungal, antiallergenic, antiviral, antitubulin, antihypertensive, and anticancer agents. Consequently, considerable attention is being devoted to the isolation of these compounds from natural resources and partial versus complete syntheses of chromone derivatives as well as to the evaluation of their biologic activity [19,20].

Herein we report the synthesis and *in vitro* growth inhibition characterisation of new thiadiazoline and thiazolinone derivatives substituted with a chromen-3-yl or 3-chromenyl-methylene moiety. We screened six human cancer cell lines with various levels of resistance to pro-apoptotic stimuli to investigate whether the compounds in the study are able to overcome this resistance. Three cell lines display various levels of resistance to pro-apoptotic stimuli, i.e., the A549 non-small-cell-lung cancer (NSCLC) [21,22], the SKMEL-28 melanoma [23] and the U373 glioma [24,25] cell lines. Two of the cell lines display actual sensitivity to pro-apoptotic stimuli, i.e., the Hs683 glioma [25,26] and the MCF-7 breast carcinoma [27] cell lines. The *in vitro* antiproliferative activity of each compound in the study has been determined using the MTT colorimetric assay [23-26]. The three most potent compounds were further analysed using computer-assisted phase-contrast microscopy, i.e., quantitative videomicroscopy [28,29].

2. Experimental

Chemistry

Solvents were obtained from commercial sources. Analytical thin layer chromatography was carried out on precoated Silica Gel 60F$_{254}$ sheets using UV absorption for visualisation. The melting points were taken with Electrothermal and MPM-H1 Schorpp melting point meters and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 500 MHz and were in accordance with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) as the internal standard. The samples were prepared by dissolving the powder of the synthesised compounds in DMSO-d_6 (δ_H= 2.51 ppm) and the spectra were recorded using a single excitation pulse of 12 μs (^{1}H NMR). GC-MS analyses were performed with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was measured with a Vario El CHNS instrument.

*Synthesis of chromenyl-thiosemicarbazone derivatives **2a-l** (General procedure)*

In a flask equipped with a reflux condenser, a mixture of 3-formyl-chromone **1a,b** (50 mmol) and various N^4-substituted-thiosemicarbazides (50 mmol) was reacted in 50 ml of absolute ethanol with catalytic sulphuric acid. The reaction mixture was heated under reflux for 3 h, where upon the solid product partially crystallised out. The solution was left to cool and the solid product was filtered off, washed with water, dried, and recrystallized from ethanol.

N-methyl-2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2a**)
Yield 88%. Yellow powder, mp: 250 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.46 (s, 3H, chromone-CH$_3$); 3.43 (s, 3H, *N*-CH$_3$); 7.62 (d, 1H, C$_8$-chromone-H); 7.71 (d, 1H, C$_7$-chromone-H); 7.93 (s, 1H, C$_2$-chromone-H); 8.12 (s, 1H, C$_5$-chromone-H); 8.40 (s, 1H, CH=*N*); 11.26 (s, 1H,

*N*HCS); 11.68 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 20.5 (chromone-CH$_3$); 33.3 (*N*H-CH$_3$);   113.2 (chromone-C$_3$); 116.1 (chromone-C$_8$); 121.3 (chromone-C$_{4a}$); 127.3 (chromone-C$_5$); 133.4 (CH=*N*); 136.5 (chromone-C$_6$); 138.2 (chromone-C$_7$); 151.4 (chromone-C$_{8a}$); 163.5 (chromone-C$_2$); 172.6 (C=S); 177.9 (chromone-C$_4$). Anal. Calcd. (%) for C$_{13}$H$_{13}$N$_3$O$_2$S (275.33): C, 56.71; H, 4.76; N, 15.26; S, 11.65. Found: C, 56.46; H, 4.54; N, 14.84; S, 11.34. MS (EI, 70 eV): *m/z* 276 [M$^+$].

N-phenyl-2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2b**)
Yield 90%. White powder, mp: 242 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.43 (s, 3H, chromone-CH$_3$); 7.36 (m, 5H, Ar-H); 7.64 (d, 1H, C$_8$-chromone-H); 7.73-7.74 (dd, 1H, C$_7$-chromone-H); 7.89 (s, 1H, C$_2$-chromone-H); 8.08 (s, 1H, C$_5$-chromone-H); 8.36 (s, 1H, CH=*N*); 11.06 (s, 1H, *N*HCS); 11.59 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.6 (chromone-CH$_3$); 113.4 (chromone-C$_3$); 118.6 (chromone-C$_8$); 122.1 (chromone-C$_{4a}$); 126.4 (phenyl-C$_2$, C$_6$); 127.7 (CH=*N*); 128.4 (phenyl-C$_3$, C$_5$); 128.9 (phenyl-C$_4$); 129.6 (chromone-C$_5$); 135.6 (chromone-C$_6$); 138.4 (chromone-C$_7$); 140.5 (phenyl-C$_1$); 152.7 (chromone-C$_{8a}$); 162.4 (chromone-C$_2$); 175.46 (C=S); 178.47 (chromone-C$_4$). Anal. Calcd. (%) for C$_{18}$H$_{15}$N$_3$O$_2$S (337.40): C, 64.08; H, 4.48; N, 12.45; S, 9.50. Found: C, 63.92; H, 4.70; N, 12.82; S, 9.27. MS (EI, 70 eV): *m/z* 338 [M$^+$].

N-allyl-2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2c**)
Yield 83%. Yellow powder, mp: 239 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.43 (s, 3H, chromone-CH$_3$); 4.58-4.66 (m, 2H, *N*-CH$_2$); 5.12-5.18 (m, 2H, CH=CH$_2$); 5.87-6.02 (m, 1H, CH); 7.61-7.63 (d, 1H, C$_8$-chromone-H); 7.67-7.69 (dd, 1H, C$_7$-chromone-H); 7.79-7.80 (s, 1H, C$_2$-chromone-H); 8.08 (s, 1H, C$_5$-chromone-H); 8.33 (s, 1H, CH=*N*); 11.06 (s, 1H, *N*HCS); 11.49 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 20.6 (chromone-CH$_3$); 46.1 (allyl-CH$_2$); 115.4 (chromone-C$_3$); 116.9 (CH$_2$=); 117.5 (chromone-C$_8$); 120.7 (chromone-C$_{4a}$); 129.6 (chromone-C$_5$); 134.1 (CH=*N*); 135.4 (chromone-C$_6$); 135.8 (CH=); 137.9 (chromone-C$_7$); 152.6 (chromone-C$_{8a}$); 163.8 (chromone-C$_2$); 172.9 (C=S); 179.7 (chromone-C$_4$). Anal. Calcd. (%) for C$_{15}$H$_{15}$N$_3$O$_2$S (301.36): C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.59; H, 4.92; N, 13.55; S, 10.88. MS (EI, 70 eV): *m/z* 302 [M$^+$].

N-methyl-2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2d**)
Yield 85%. White powder, mp: 260 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 3.27 (s, 3H, *N*-CH$_3$); 7.59-7.62 (d, 1H, C$_8$-chromone-H); 7.65-7.72 (d, 1H, C$_7$-chromone-H); 8.00 (s, 1H, C$_2$-chromone-H); 8.02 (s, 1H, C$_5$-chromone-H); 8.22 (s, 1H, CH=*N*); 11.31 (s, 1H, *N*HCS); 11.68 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 33.3 (CH$_3$); 113.2 (chromone-C$_3$); 121.4 (chromone-C$_{4a}$); 121.4 (chromone-C$_8$); 124.9 (chromone-C$_5$); 126.7 (chromone-C$_6$); 135.3 (CH=*N*); 137.2 (chromone-C$_7$); 154.4 (chromone-C$_{8a}$); 162.4 (chromone-C$_2$); 175.1(C=S); 181.2 (chromone-C$_4$). Anal. Calcd. (%) for C$_{12}$H$_{10}$ClN$_3$O$_2$S (295.74): C, 48.73; H, 3.41; N, 14.21; S, 10.84. Found: C, 48.93; H, 3.24; N, 14.42; S, 11.19. MS (EI, 70 eV): *m/z* 296 [M$^+$].

N-phenyl-2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2e**)
Yield 91%. White powder, mp: 238 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.54-7.58 (m, 5H, Ar-H); 7.76-7.79 (d, 1H, C$_8$-chromone-H); 7.88-7.93 (dd, 1H, C$_7$-chromone-H); 8.03-8.06 (d, 1H, C$_2$-chromone-H); 8.14 (s, 1H, C$_5$-chromone-H); 9.01 (s, 1H, CH=*N*); 11.28 (s, 1H, *N*HCS); 11.40 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 114.6 (chromone-C$_3$); 122.3 (chromone-C$_{4a}$); 123.7 (chromone-C$_8$); 125.4 (chromone-C$_5$); 128.8 (phenyl-C$_2$, C$_6$); 129.7 (chromone-C$_6$); 130.5 (CH=*N*); 131.1 (phenyl-C$_4$); 133.4 (phenyl-C$_3$, C$_5$); 137.6 (chromone-C$_7$); 138.3 (phenyl-C$_1$); 155.2 (chromone-C$_{8a}$); 163.5 (chromone-C$_2$); 173.08 (C=S); 180.6 (chromone-C$_4$). Anal. Calcd. (%) for C$_{17}$H$_{12}$ClN$_3$O$_2$S (357.81): C, 57.06; H, 3.38; N, 11.74; S, 8.96. Found: C, 56.42; H, 3.31; N, 11.39; S, 8.63. MS (EI, 70 eV): *m/z* 358 [M$^+$].

N-allyl-2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2f**)
Yield 85%. White powder, mp: 229 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 4.58-4.65 (m, 2H, *N*-CH$_2$); 5.19-5.23 (m, 2H, CH=CH$_2$); 6.02-6.06 (m, 1H, CH); 7.48-7.54 (d, 1H, C$_8$-chromone-H); 7.68-7.76 (dd, 1H, C$_7$-chromone-H); 7.89 (s, 1H, C$_2$-chromone-H); 8.05 (s, 1H, C$_5$-chromone-H); 8.46 (s, 1H, CH=*N*); 11.11 (s, 1H, *N*HCS); 11.55 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 46.3 (allyl-CH$_2$); 112.6 (chromone-C$_3$); 115.5 (CH$_2$=); 122.3 (chromone-C$_{4a}$); 123.6 (chromone-C$_8$); 126.1 (chromone-C$_5$); 127.9 (chromone-C$_6$); 133.6 (CH=*N*); 134.2 (CH=); 136.8

(chromone-C$_7$); 154.2 (chromone-C$_{8a}$); 162.3 (chromone-C$_2$); 170.6 (C=S); 182.1 (chromone-C$_4$). Anal. Calcd. (%) for C$_{14}$H$_{12}$ClN$_3$O$_2$S (321.78): C, 52.26; H, 3.76; N, 13.06; S, 9.96. Found: C, 52.13; H, 3.81; N, 12.80; S, 9.64. MS (EI, 70 eV): *m/z* 322 [M$^+$].

N-(4-methoxyphenyl)-2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2g**)

Yield 84%. White powder, mp: 230 °C. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 2.43 (s, 3H, chromone-CH$_3$); 3.77 (s, 3H, OCH$_3$); 7.22-7.25 (d, 2H, Ar-H); 7.39-7.43 (d, 2H, Ar-H); 7.46-7.50 (d, 1H, C$_8$-chromone-H); 7.62-7.64 (dd, 1H, C$_7$-chromone-H); 7.85 (d, 1H, C$_2$-chromone-H); 7.99 (s, 1H, C$_5$-chromone-H); 8.30 (s, 1H, CH=*N*); 11.07 (s, 1H, *N*HCS); 11.59 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 19.9 (chromone-CH$_3$); 55.6 (OCH$_3$); 111.5 (chromone-C$_3$); 118.8 (phenyl-C$_2$,C$_5$); 119.3 (chromone-C$_8$); 123.2 (chromone-C$_{4a}$); 127.3 (phenyl-C$_2$, C$_6$); 129.4 (CH=*N*); 130.9 (chromone-C$_5$); 131.8 (phenyl-C$_1$); 134.2 (chromone-C$_6$); 137.8 (chromone-C$_7$); 154.2 (chromone-C$_{8a}$); 156.4 (phenyl-C$_4$); 163.3 (chromone-C$_2$); 174.7 (C=S); 179.7 (chromone-C$_4$). Anal. Calcd. (%) for C$_{19}$H$_{17}$N$_3$O$_3$S (367.42): C, 62.11; H, 4.66; N, 11.44; S, 8.73. Found: C, 62.39; H, 4.79; N, 11.28; S, 8.49. MS (EI, 70 eV): *m/z* 368 [M$^+$].

N-(3-(trifluoromethyl)phenyl)-2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene) hydrazinecarbothioamide (**2h**)

Yield 80%. White powder, mp: 210 °C. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 2.45 (s, 3H, chromone-CH$_3$); 7.44-7.48 (d, 1H, C$_8$-chromone-H); 7.59-7.63 (dd, 1H, C$_7$-chromone-H); 7.72-7.78 (m, 1H, Ar-H); 7.83-7.87 (m, 3H, Ar-H); 8.03 (d, 1H, C$_2$-chromone-H); 8.07 (s, 1H, C$_5$-chromone-H); 8.34 (s, 1H, CH=*N*); 11.27 (s, 1H, *N*HCS); 11.67 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 19.0 (chromone-CH$_3$); 112.3 (chromone-C$_3$); 116.4 (chromone-C$_8$); 119.1 (phenyl-C$_2$); 121.3 (chromone-C$_{4a}$); 122.9 (CF$_3$); 124.4 (phenyl-C$_4$); 124.7 (phenyl-C$_6$); 129.1 (CH=*N*); 131.3 (phenyl-C$_5$); 132.0 (chromone-C$_5$); 132.2 (phenyl-C$_3$); 136.3 (chromone-C$_6$); 139.1 (chromone-C$_7$); 150.3 (phenyl-C$_1$); 154.8 (chromone-C$_{8a}$); 164.1 (chromone-C$_2$); 174.6 (C=S); 179.3 (chromone-C$_4$). Anal. Calcd. (%) for C$_{19}$H$_{14}$F$_3$N$_3$O$_2$S (405.39): C, 56.29; H, 3.48; N, 10.37; S, 7.91. Found: C, 56.01; H, 3.43; N, 10.09; S, 7.71. MS (EI, 70 eV): *m/z* 406 [M$^+$].

2-((6-Methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2i**)

Yield 91%. Light yellow powder, mp: 231 °C. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 2.45 (s, 3H, chromone-CH$_3$); 7.62-7.63 (d, 1H, C$_8$-chromone-H); 7.66-7.68 (dd, 1H, C$_7$-chromone-H); 7.90 (s, 1H, C$_2$-chromone-H); 8.09 and 8.25 (2 br s, 1H each, NH$_2$); 8.19 (s, 1H, C$_5$-chromone-H); 9.14 (s, 1H, CH=*N*); 11.53 (s, 1H, *N*HCS). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 20.3 (chromone-CH$_3$); 112.8 (chromone-C$_3$); 118.1 (chromone-C$_8$); 121.6 (chromone-C$_{4a}$); 131.2 (chromone-C$_5$); 135.0 (CH=*N*); 136.4 (chromone-C$_6$); 138.9 (chromone-C$_7$); 155.1 (chromone-C$_{8a}$); 163.9 (chromone-C$_2$); 174.8 (C=S); 180.4 (chromone-C$_4$). Anal. Calcd. (%) for C$_{12}$H$_{11}$N$_3$O$_2$S (261.30): C, 55.16; H, 4.24; N, 16.08; S, 12.27. Found: C, 54.88; H, 4.13; N, 15.74; S, 12.68. MS (EI, 70 eV): *m/z* 262 [M$^+$].

2-((6-Chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2j**)

Yield 83%. Light yellow powder, mp: 244 °C. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 7.69-7.72 (d, 1H, C$_8$-chromone-H); 7.74-7.80 (dd, 1H, C$_7$-chromone-H); 7.92-8.02 (d, 1H, C$_2$-chromone-H); 8.05 and 8.08 (2 br s, 1H each, *N*H$_2$); 8.11 (s, 1H, C$_5$-chromone-H); 8.36 (s, 1H, CH=*N*); 11.30 (s, 1H, *N*HCS). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 112.2 (chromone-C$_3$); 123.2 (chromone-C$_{4a}$); 123.7 (chromone-C$_8$); 127.1 (chromone-C$_5$); 128.9 (chromone-C$_6$); 135.4; (CH=*N*); 138.2 (chromone-C$_7$); 156.3 (chromone-C$_{8a}$); 164.5 (chromone-C$_2$); 175.1 (C=S); 180.1 (chromone-C$_4$). Anal. Calcd. (%) for C$_{11}$H$_8$ClN$_3$O$_2$S (281.72): C, 46.90; H, 2.86; N, 14.92; S, 11.38. Found: C, 46.69; H, 2.52; N, 14.63; S, 11.61. MS (EI, 70 eV): *m/z* 282 [M$^+$].

N-(4-methoxyphenyl)-2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2k**)

Yield 90%. White powder, mp: 227 °C. ^{1}H NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 3.81 (s, 3H, OCH$_3$); 7.04-7.09 (d, 2H, Ar-H); 7.36-7.41 (d, 2H, Ar-H); 7.66-7.69 (d, 1H, C$_8$-chromone-H); 7.85-7.89 (dd, 1H, C$_7$-chromone-H); 8.03 (s, 1H, C$_2$-chromone-H); 8.11 (s, 1H, C$_5$-chromone-H); 8.35 (s, 1H, CH=*N*); 11.11 (s, 1H, *N*HCS); 11.47 (s, 1H, CS*N*H). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz, ppm): δ 56.3 (OCH$_3$); 111.9 (chromone-C$_3$); 117.1 (phenyl-C$_3$, C$_5$); 122.9 (chromone-C$_{4a}$); 123.4 (chromone-C$_8$); 127.3 (chromone-C$_5$); 127.9 (chromone-C$_6$); 128.3 (phenyl-C$_2$, C$_6$); 131.1

(CH=N); 131.5 (phenyl-C_1); 137.4 (chromone-C_7); 156.1 (chromone-C_{8a}); 157.9 (phenyl-C_4); 164.7 (chromone-C_2); 174.9 (C=S); 179.7 (chromone-C_4). Anal. Calcd. (%) for $C_{18}H_{14}ClN_3O_3S$ (387.84): C, 55.74; H, 3.64; N, 10.83; S, 8.27. Found: C, 55.52; H, 3.75; N, 10.68; S, 7.89. MS (EI, 70 eV): m/z 388 [M^+].

N-(3-(trifluoromethyl)phenyl)-2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (**2l**)

Yield 84%. Yellow powder, mp: 235 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.73-7.80 (m, 2H, chromone-H); 7.87-7.93 (m, 3H, Ar-H); 8.02-8.03 (d, 1H, Ar-H); 8.05 (d, 1H, C_2-chromone-H), 8.12 (s, 1H, C_5-chromone-H); 8.42 (s, 1H, CH=N); 11.04 (s, 1H, NHCS); 11.45 (s, 1H, CSNH). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 112.1 (chromone-C_3); 118.1 (phenyl-C_2); 121.6 (phenyl-C_4); 122.2 (chromone-C_{4a}); 122.4 (phenyl-C_6); 122.6 (chromone-C_8); 126.2 (chromone-C_5); 127.7 (chromone-C_6); 128.3 (CF$_3$); 129.4 (CH=N); 131.3 (phenyl-C_5); 132.3 (phenyl-C_3); 136.9 (chromone-C_7); 149.2 (phenyl-C_1); 155.6 (chromone-C_{8a}); 163.3 (chromone-C_2); 174.2 (C=S); 178.9 (chromone-C_4). Anal. Calcd. (%) for $C_{18}H_{11}ClF_3N_3O_2S$ (425.81): C, 50.77; H, 2.60; N, 9.87; S, 7.53. Found: C, 50.64; H, 2.47; N, 9.63; S, 7.42. MS (EI, 70 eV): m/z 426 [M^+].

*Synthesis of chromenyl-1,3,4-thiadiazoline derivatives **3a-l** (General procedure)*

For synthesis, 5 mmol of the correspondent chromenyl-thiosemicarbazone **2a-l** was refluxed with 10 ml of acetic anhydride and 0.5 ml of pyridine for 4 h. After cooling, the mixture was poured into water and stirred for 30 minutes at room temperature. The resultant precipitate was filtered, washed with water and recrystallized from absolute ethanol.

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-methylacetamide (**3a**)

Yield 72%. White powder, mp: 285 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.27 (s, 6H, N^3-thiadiazoline-COCH$_3$, NCOCH$_3$); 2.47 (s, 3H, chromone-CH$_3$); 3.46 (s, 3H, N-CH$_3$); 6.64 (s, 1H, C_2-thiadiazoline-H); 7.58-7.60 (d, 1H, J = 8.5, C_8-chromone-H); 7.66-7.68 (d, 1H, J = 8.0, C_7-chromone-H); 7.88 (s, 1H, C_2-chromone-H); 8.11 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 20.4 (chromone-CH$_3$); 21.4 (N^3-thiadiazoline-acetyl-CH$_3$); 23.6 (N-acetyl-CH$_3$); 32.2 (N-CH$_3$); 53.1 (thiadiazoline-C_2); 116.2 (chromone-C_8); 119.2 (chromone-C_3); 123.6 (chromone-C_{4a}); 129.5 (chromone-C_5); 135.3 (chromone-C_6); 137.7 (chromone-C_7); 153.3 (chromone-C_{8a}); 155.6 (chromone-C_2); 158.9 (thiadiazoline-C_5); 168.9 (N-C=O); 170.1 (N^3-thiadiazoline-C=O); 178.5 (chromone-C_4). Anal. Calcd. (%) for $C_{17}H_{17}N_3O_4S$ (359.40): C, 56.81; H, 4.77; N, 11.69; S, 8.92. Found: C, 56.98; H, 4.56; N, 11.32; S, 8.58. MS (EI, 70 eV): m/z 360 [M^+].

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-phenylacetamide (**3b**)

Yield 66%. Light yellow powder, mp: 215 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.89 (s, 3H, N^3-thiadiazoline-COCH$_3$); 1.90 (s, 3H, NCOCH$_3$); 2.46 (s, 3H, chromone-CH$_3$); 6.69 (s, 1H, C_2-thiadiazoline-H); 7.50-7.57 (m, 5H, Ar-H); 7.60-7.61 (d, 1H, C_8-chromone-H); 7.67-7.69 (dd, 1H, C_7-chromone-H); 7.90 (s, 1H, C_2-chromone-H); 8.07 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.8 (chromone-CH$_3$); 21.5 (N^3-thiadiazoline-acetyl-CH$_3$); 23.7 (N-acetyl-CH$_3$); 54.6 (thiadiazoline-C_2); 117.1 (chromone-C_8); 119.7 (chromone-C_3); 122.5 (chromone-C_{4a}); 126.2 (phenyl-C_2, C_6); 128.3 (phenyl-C_4); 130.4 (chromone-C_5); 130.7 (phenyl-C_3, C_5); 134.8 (chromone-C_6); 137.7 (phenyl-C_1); 138.6 (chromone-C_7); 152.1 (chromone-C_{8a}); 158.9 (thiadiazoline-C_5); 159.3 (chromone-C_2); 164.3 (N-C=O); 167.9 (N^3-thiadiazoline-C=O); 177.9 (chromone-C_4). Anal. Calcd. (%) for $C_{22}H_{19}N_3O_4S$ (421.47): C, 62.69; H, 4.54; N, 9.97; S, 7.61. Found: C, 63.12; H, 4.68; N, 10.2; S, 7.43. MS (EI, 70 eV): m/z 422 [M^+].

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-allylacetamide (**3c**)

Yield 70%. White powder, mp: 217 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.23 (s, 3H, N^3-thiadiazoline-COCH$_3$); 2.26 (s, 3H, NCOCH$_3$); 2.43 (s, 3H, chromone-CH$_3$); 4.52-4.61 (m, 2H, N-CH$_2$); 5.21-5.26 (m, 2H, CH=CH$_2$); 6.00-6.05 (m, 1H, CH); 6.65 (s, 1H, C_2-thiadiazoline-H); 7.58-7.60 (d, 1H, C_8-chromone-H); 7.66-7.68 (dd, 1H, C_7-chromone-H); 7.87-7.88 (s, 1H, C_2-

chromone-H); 8.08 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.9 (chromone-CH_3); 21.5 (N^3-thiadiazoline-acetyl-CH_3); 22.9 (N-acetyl-CH_3); 48.2 (allyl-CH_2); 54.5 (thiadiazoline-C_2); 116.9 (chromone-C_8); 120.2 (chromone-C_3); 120.8 (CH_2=); 122.3 (chromone-C_{4a}); 126.8 (CH=); 129.8 (chromone-C_5); 134.2 (chromone-C_6); 136.5 (chromone-C_7); 152.4 (chromone-C_{8a}); 157.1 (chromone-C_2); 157.6 (thiadiazoline-C_5); 163.4 (N-C=O); 167.2 (N^3-thiadiazoline-C=O); 175.6 (chromone-C_4). Anal. Calcd. (%) for $C_{19}H_{19}N_3O_4S$ (385.44): C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 58.95; H, 4.83; N, 10.78; S, 8.22. MS (EI, 70 eV): *m/z* 386 [M^+].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-methylacetamide (**3d**)

Yield 68%. White powder, mp: 300 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.26 (s, 3H, N^3-thiadiazoline-$COCH_3$); 2.27 (s, 3H, $NCOCH_3$); 3.46 (s, 3H, N-CH_3); 6.63 (s, 1H, C_2-thiadiazoline-H); 7.76-7.78 (d, 1H, C_8-chromone-H); 7.89-7.91 (dd, 1H, C_7-chromone-H); 8.02-8.03 (s, 1H, C_2-chromone-H); 8.21 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 21.7 (N^3-thiadiazoline-acetyl-CH_3); 23.7 (N-acetyl-CH_3); 32.6 (N-CH_3); 54.6 (thiadiazoline-C_2); 118.6 (chromone-C_3); 119.2 (chromone-C_8); 123.4 (chromone-C_{4a}); 125. 6 (chromone-C_5); 135.3 (chromone-C_6); 136.3 (chromone-C_7); 155.1 (chromone-C_{8a}); 157.0 (chromone-C_2); 158.3 (thiadiazoline-C_5); 167.6 (N-C=O); 169.4 (N^3-thiadiazoline-C=O); 176.8 (chromone-C_4). Anal. Calcd. (%) for $C_{16}H_{14}ClN_3O_4S$ (379.82): C, 50.60; H, 3.72; N, 11.06; S, 8.44. Found: C, 50.12; H, 3.79; N, 10.59; S, 8.21. MS (EI, 70 eV): *m/z* 380 [M^+].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-phenylacetamide (**3e**)

Yield 53%. Light brown powder, mp: 251 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.90 (s, 6H, N^3-thiadiazoline-$COCH_3$, $NCOCH_3$); 6.70 (s, 1H, C_2-thiadiazoline-H); 7.50-7.56 (m, 5H, Ar-H); 7.77-7.79 (d, 1H, C_8-chromone-H); 7.90-7.92 (dd, 1H, C_7-chromone-H); 8.05-8.06 (d, 1H, C_2-chromone-H); 8.17 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 21.4 (N^3-thiadiazoline-acetyl-CH_3); 23.4 (N-acetyl-CH_3); 55.3 (thiadiazoline-C_2); 118.5 (chromone-C_3); 120.8 (chromone-C_8); 123.4 (chromone-C_{4a}); 125.6 (chromone-C_5); 126.0 (chromone-C_6); 126.5 (phenyl-C_2, C_6); 128.1 (phenyl-C_4); 130.6 (phenyl-C_3, C_5); 136.3 (chromone-C_7); 137.7 (phenyl-C_1); 153.7 (thiadiazoline-C_5); 155.1 (chromone-C_{8a}); 157.2 (chromone-C_2); 161.8 (N-C=O); 168.0 (N^3-thiadiazoline-C=O); 177.4 (chromone-C_4). Anal. Calcd. (%) for $C_{21}H_{16}ClN_3O_4S$ (441.89): C, 57.08; H, 3.65; N, 9.51; S, 7.26. Found: C, 57.21; H, 3.47; N, 9.12; S, 7.12. MS (EI, 70 eV): *m/z* 442 [M^+].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-allylacetamide (**3f**)

Yield 68%. White powder, mp: 220 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.23 (s, 3H, N^3-thiadiazoline-$COCH_3$); 2.26 (s, 3H, $NCOCH_3$); 4.52-4.62 (m, 2H, N-CH_2); 5.21-5.27 (m, 2H, $CH=CH_2$); 6.01 (m, 1H, CH); 6.65 (s, 1H, C_2-thiadiazoline-H); 7.76-7.78 (d, 1H, C_8-chromone-H): 7.89-7.91 (dd, 1H, C_7-chromone-H): 8.02-8.03 (s, 1H, C_2-chromone-H); 8.19 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 22.0 (N^3-thiadiazoline-acetyl-CH_3); 22.8 (N-acetyl-CH_3); 48.6 (allyl-CH_2); 56.3 (thiadiazoline-C_2); 118.5 (chromone-C_3); 119.7 (CH_2=); 120.8 (chromone-C_8); 123.4 (chromone-C_{4a}); 125.6 (chromone-C_5); 126.1 (chromone-C_6); 126.5 (CH=); 136.3 (chromone-C_7); 155.1 (chromone-C_{8a}); 155.0 (chromone-C_2); 155.6 (thiadiazoline-C_5); 164.3 (N-C=O); 168.4 (N^3-thiadiazoline-C=O); 178.9 (chromone-C_4). Anal. Calcd. (%) for $C_{18}H_{16}ClN_3O_4S$ (405.86): C, 53.27; H, 3.97; N, 10.35; S, 7.90. Found: C, 53.14; H, 4.03; N, 9.98; S, 7.64. MS (EI, 70 eV): *m/z* 406 [M^+].

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-(4-methoxyphenyl)acetamide (**3g**)

Yield 64%. Grey powder, mp: 171 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.89 (s, 3H, N^3-thiadiazoline-$COCH_3$); 1.93 (s, 3H, $NCOCH_3$); 2.45 (s, 3H, chromone-CH_3); 3.83 (s, 3H, OCH_3); 6.68 (s, 1H, C_2-thiadiazoline-H); 7.06-7.08 (d, 2H, J = 8.5, Ar-H); 7.43-7.45 (d, 2H, J = 8.5, Ar-H); 7.59-7.61 (d, 1H, C_8-chromone-H), 7.67-7.69 (dd, 1H, C_7-chromone-H), 7.89 (d, 1H, C_2-chromone-H), 8.05 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.9 (chromone-CH_3); 21.2 (N^3-thiadiazoline-acetyl-CH_3); 23.9 (N-acetyl-CH_3); 53.4 (thiadiazoline-

C_2); 55.8 (OCH_3); 114.3 (phenyl-C_3, C_5); 115.6 (chromone-C_8); 119.1 (chromone-C_3); 121.8 (chromone-C_{4a}); 126.7 (phenyl-C_2, C_6); 129.4 (chromone-C_5); 133.5 (phenyl-C_1); 133.7 (chromone-C_6); 137.2 (chromone-C_7); 153.4 (chromone-C_{8a}); 154.6 (thiadiazoline-C_5); 156.0 (chromone-C_2); 160.9 (phenyl-C_4); 161.2 (N-C=O); 166.9 (N^3-thiadiazoline-C=O); 176.9 (chromone-C_4). Anal. Calcd. (%) for $C_{23}H_{21}N_3O_5S$ (451.49): C, 61.18; H, 4.69; N, 9.31; S, 7.10. Found: C, 61.41; H, 4.47; N, 8.92; S, 6.83. MS (EI, 70 eV): m/z 452 [M$^+$].

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-(3-trifluoromethylphenyl)acetamide (**3h**)

Yield 70%. Light brown powder, mp: 88 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.89 (s, 3H, N^3-thiadiazoline-$COCH_3$); 1.93 (s, 3H, $NCOCH_3$); 2.45 (s, 3H, chromone-CH_3); 6.71 (s, 1H, C_2-thiadiazoline-H); 7.59-7.61 (d, 1H, C_8-chromone-H); 7.67-7.69 (dd, 1H, C_7-chromone-H); 7.78-7.81 (m, 1H, Ar-H); 7.87-7.92 (m, 3H, Ar-H); 8.08 (d, 1H, C_2-chromone-H); 8.10 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 20.9 (chromone-CH_3); 21.7 (N^3-thiadiazoline-acetyl-CH_3); 24.1 (N-acetyl-CH_3); 56.5 (thiadiazoline-C_2); 118.8 (chromone-C_8); 121.7 (phenyl-C_2); 123.2 (chromone-C_3); 124.5 (chromone-C_{4a}); 126.4 (CF_3); 126.6 (phenyl-C_4); 130.6 (phenyl-C_5); 130.8 (phenyl-C_3); 131.3 (chromone-C_5); 133.6 (phenyl-C_6);136.0 (chromone-C_6); 136.3 (chromone-C_7); 140.5 (phenyl-C_1); 151.0 (chromone-C_{8a}); 153.6 (thiadiazoline-C_5); 154.7 (chromone-C_2); 168.8 (N-C=O); 170.6 (N^3-thiadiazoline-C=O); 175.6 (chromone-C_4). Anal. Calcd. (%) for $C_{23}H_{18}F_3N_3O_4S$ (489.47): C, 56.44; H, 3.71; N, 8.58; S, 6.55. Found: C, 56.62; H, 3.83; N, 8.24; S, 6.33. MS (EI, 70 eV): m/z 490 [M$^+$].

N-(4-acetyl-5-(6-methyl-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**3i**)

Yield, 65%. Grey crystals, mp: 280 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.04 (s, 3H, N^3-thiadiazoline-$COCH_3$); 2.25 (s, 3H, $NCOCH_3$); 2.43 (s, 3H, chromone-CH_3); 6.64 (s, 1H, C_2-thiadiazoline-H); 7.55-7.57 (d, 1H, C_8-chromone-H); 7.63-7.66 (dd, 1H, C_7-chromone-H); 7.85 (s, 1H, C_2-chromone-H); 8.12 (s, 1H, C_5-chromone-H); 11.75 (s, 1H, NH).). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.6 (chromone-CH_3); 22.3 (N^3-thiadiazoline-acetyl-CH_3); 23.7 (N-acetyl-CH_3); 55.2 (thiadiazoline-C_2); 115.7 (chromone-C_8); 117.4 (chromone-C_3); 120.6 (chromone-C_{4a}); 128.6 (chromone-C_5); 134.4 (chromone-C_6); 136.2 (chromone-C_7); 155.2 (chromone-C_{8a}); 157.1 (chromone-C_2); 157.4 (thiadiazoline-C_5); 167.8 (N-C=O); 168.0 (N^3-thiadiazoline-C=O); 176.8 (chromone-C_4). Anal. Calcd. (%) for $C_{16}H_{15}N_3O_4S$ (345.37): C, 55.64; H, 4.38; N, 12.17; S, 9.28. Found: C, 56.01; H, 4.58; N, 12.51; S, 8.92. MS (EI, 70 eV): m/z 346 [M$^+$].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**3j**)

Yield 58%. Grey crystals, mp: 300 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.04 (s, 3H, N^3-thiadiazoline-$COCH_3$); 2.24 (s, 3H, $NCOCH_3$); 6.64 (s, 1H, C_2-thiadiazoline-H); 7.76-7.77 (d, 1H, C_8-chromone-H); 7.88-7.90 (dd, 1H, C_7-chromone-H); 8.02 (d, 1H, C_2-chromone-H); 8.23 (s, 1H, C_5-chromone-H); 11.77 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 21.6 (N^3-thiadiazoline-acetyl-CH_3); 24.2 (N-acetyl-CH_3); 56.4 (thiadiazoline-C_2); 118.3 (chromone-C_3); 120.1 (chromone-C_8); 122.3 (chromone-C_{4a}); 125.5 (chromone-C_5); 126.1 (chromone-C_6); 135.4 (chromone-C_7); 156.3 (chromone-C_{8a}); 156.7 (chromone-C_2); 157.0 (thiadiazoline-C_5); 168.3 (N-C=O); 169.6 (N^3-thiadiazoline-C=O); 178.5 (chromone-C_4). Anal. Calcd. (%) for $C_{15}H_{12}ClN_3O_4S$ (365.79): C, 49.25; H, 3.31; N, 11.49; S, 8.77. Found: C, 49.61; H, 3.17; N, 11.17; S, 8.91. MS (EI, 70 eV): m/z 366 [M$^+$].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-(4-methoxyphenyl)acetamide (**3k**)

Yield 69%. Grey powder, mp: 181 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.89 (s, 3H, N^3-thiadiazoline-$COCH_3$); 1.93 (s, 3H, $NCOCH_3$); 3.83 (s, 3H, OCH_3); 6.69 (s, 1H, C_2-thiadiazoline-H); 7.06-7.08 (d, 2H, J = 9.0, Ar-H); 7.43-7.45 (d, 2H, J = 9.0, Ar-H); 7.76-7.78 (d, 1H, C_8-chromone-H); 7.89-7.91 (dd, 1H, C_7-chromone-H); 8.04 (d, 1H, J = 2.5, C_2-chromone-H); 8.15 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 19.6 (N^3-thiadiazoline-acetyl-CH_3); 24.3 (N-acetyl-CH_3); 56.1 (thiadiazoline-C_2); 57.8 (OCH_3); 115.6 (phenyl-C_3, C_5); 118.8 (chromone-C_3); 121.2 (chromone-C_8); 124.0 (chromone-C_{4a}); 126.4 (chromone-C_5); 126.9 (chromone-C_6); 127.8 (phenyl-C_2, C_6); 134.3 (phenyl-C_1); 137.5 (chromone-C_7); 155.2 (thiadiazoline-C_5); 155.9 (chromone-C_{8a}) ; 156.6 (chromone-C_2); 161.8 (phenyl-C_4); 163.6 (N-C=O); 169.2 (N^3-thiadiazoline-C=O); 177.3 (chromone-C_4). Anal. Calcd. (%) for $C_{22}H_{18}ClN_3O_5S$

(471.91): C, 55.99; H, 3.84; N, 8.90; S, 6.79. Found: C, 56.26; H, 3.66; N, 8.54; S, 7.04. MS (EI, 70 eV): *m/z* 472 [M⁺].

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-(3-trifluoromethylphenyl)acetamide (**3l**)

Yield 72%. Light brown powder, mp: 117 °C. ¹H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.89 (s, 3H, N^3-thiadiazoline-COCH₃); 1.93 (s, 3H, *N*COCH₃); 6.72 (s, 1H, C₂-thiadiazoline-H); 7.77-7.81 (m, 2H, chromone-H); 7.87-7.92 (m, 3H, Ar-H); 8.04-8.05 (d, 1H, *J* = 3.0, Ar-H); 8.08 (d, 1H, C₂-chromone-H); 8.20 (s, 1H, C₅-chromone-H). ¹³C NMR (DMSO-d_6, 500 MHz, ppm): δ 21.4 (N^3-thiadiazoline-acetyl-CH₃); 23.7 (*N*-acetyl-CH₃); 55.9 (thiadiazoline-C₂); 117.1 (phenyl-C₂); 118.6 (chromone-C₃); 121.6 (chromone-C₈); 123.9 (chromone-C₄ₐ); 125.6 (chromone-C₅); 126.3 (chromone-C₆); 126.7 (CF₃); 127.5 (phenyl-C₄); 128.7 (phenyl-C₅); 129.1 (phenyl-C₃); 130.5 (phenyl-C₆); 137.2 (chromone-C₇); 139.7 (phenyl-C₁); 154.6 (thiadiazoline-C₅); 155.3 (chromone-C₈ₐ); 157.2 (chromone-C₂); 164.3 (*N*-C=O); 169.1 (N^3-thiadiazoline-C=O); 176.9 (chromone-C₄). Anal. Calcd. (%) for C₂₂H₁₅ClF₃N₃O₄S (509.89): C, 51.82; H, 2.97; N, 8.24; S, 6.29. Found: C, 51.76; H, 2.87; N, 7.91; S, 6.12. MS (EI, 70 eV): *m/z* 510 [M⁺].

Synthesis of 5-chromenyl-thiazolidine-2-thioxo-4-one **4**

For synthesis, 1 mmol (0.332 g) of 6,8-dibromo-3-formyl-chromone **1c** was refluxed for 3 h with 1 mmol (0.133 g) of 2-thioxo-4-thiazolidinone in 5 ml of acetic acid in the presence of 4 mmol (0.328 g) of anhydrous sodium acetate. The reaction mixture was cooled, and the crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallisation from ethanol.

5-((6,8-Dibromo-4-oxo-4H-chromen-3-yl)methylene)-2-thioxothiazolidin-4-one (**4**)

Yield 95%. Yellow powder, mp: 308-312 °C. ¹H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.63 (s, 1H, C₇-chromone-H); 8.17 (s, 1H, C₂-chromone-H); 8.40 (s, 1H, C₅-chromone-H); 9.05 (s, 1H, C=CH); 12.44 (br, s, *N*H). ¹³C NMR (DMSO-d_6, 500 MHz, ppm): δ 113.4 (chromone-C₃); 113.5 (chromone-C₆); 117.4 (chromone-C₈); 119.6 (CH); 121.4 (thioxothiazolidinone-C₅); 123.8 (chromone-C₄ₐ); 128.1 (chromone-C₅); 139.6 (chromone-C₇); 153.5 (chromone-C₈ₐ); 160.2 (thioxothiazolidinone-C₄); 162.6 (chromone-C₂); 177.7 (chromone-C₄); 191.2 (C=S). Anal. Calc. (%) for C₁₃H₅Br₂NO₃S₂ (447.12): C, 34.92; H, 1.13; N, 3.13; S, 14.34. Found: C, 34.90; H, 1.14; N, 3,14; S, 14,33. MS (EI, 70 eV): *m/z* 448 [M⁺].

Synthesis of 5-chromenyl-2-yl-thioacetamide-thiazolinone **5**

For synthesis, 1 mmol of 5-chromenyl-2-thioxo-thiazolidine-4-one **4** was stirred for 3 h at room temperature with 1.1 mmol (0.203 g) of iodoacetamide in the presence of 1.1 mmol (0.062 g) of anhydrous potassium hydroxide in 6 ml of DMF. The crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallisation from ethanol.

2-[5-(6,8-Dibromo-4-oxo-4H-chromen-3-yl-methylene)-4-oxo-4,5-dihydro-thiazol-2-yl-thio]-acetamide (**5**)

Yield 40%. Red powder, mp: 230-232 °C. ¹H NMR (DMSO-d_6, 500 MHz, ppm): δ 4.21 (s, 2H, CH₂); 7.41 and 7.81 (2 br s, 1H each, *N*H₂); 7.57 (s, 1H, C₇-chromone-H); 8.16 (s, 1H, C₂-chromone-H); 8.45 (s, 1H, C₅-chromone-H); 9.10 (s, 1H, C=CH). ¹³C NMR (DMSO-d_6, 500 MHz, ppm): δ 37.2 (CH₂); 113.7 (chromone-C₆); 118.6 (chromone-C₈); 119.3 (CH); 126 (chromone-C₄ₐ); 127.7 (chromone-C₃); 127.8 (chromone-C₅); 128.9 (thiazolinone-C₅); 140.3 (chromone-C₇); 151.8 (thiazolinone-C₄); 163.1 (chromone-C₈ₐ); 167.7 (chromone-C₂); 174.1 (thiazolinone-C₂); 179.1 (H₂*N*-C=O); 194.5 (chromone-C₄). Anal. Calcd. (%) for C₁₅H₈Br₂N₂O₄S₂ (504.17): C, 35.73; H, 1.60; N, 5.56; S, 12.72. Found: C, 35.85; H, 1.60; N, 9.88; S, 12.78. MS (EI, 70 eV): *m/z* 505 [M⁺].

Biological activity

Determination of In Vitro Growth Inhibition Activity in Human Cancer Cells. Seven human cancer cell lines were obtained from the American Type Culture Collection (ATCC,

Manassas, USA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ, Braunschweig, Germany). These seven cell lines included the A549 non-small-cell lung cancer (NSCLC; DSMZ code ACC107), the SKMEL-28 melanoma (ATCC code HTB-72), the Hs683 oligodendroglioma (ATCC code HTB-138), the U373 (ECACC code 08061901), the T98G (ATCC; code CRL-1690) and U251 (ECACC code 09063001) glioblastoma, and the MCF-7 breast cancer (DSMZ; code ACC115) cell lines.

The cells were cultured in RPMI (Lonza, Verviers, Belgium) culture medium supplemented with 10% heat-inactivated foetal calf serum (Lonza), 4 mM glutamine, 100 µg/ml gentamicin, and penicillin-streptomycin (200 U/ml and 200 µg/ml; Lonza).

The overall growth level of each cell line was determined using the colorimetric MTT (3-[4,5-dimethylthiazol-2yl-diphenyl tetrazolium bromide, Sigma, Belgium) assay as detailed previously [23-26]. The data are represented as the mean values from one experiment with six replicates in each experimental condition.

Computer-Assisted Phase-Contrast Microscopy (Videomicroscopy). The direct visualisation of the compound-induced effects on the cell proliferation and morphology was carried out by means of computer-assisted phase contrast microscopy, i.e., quantitative videomicroscopy, as detailed elsewhere [28,29].
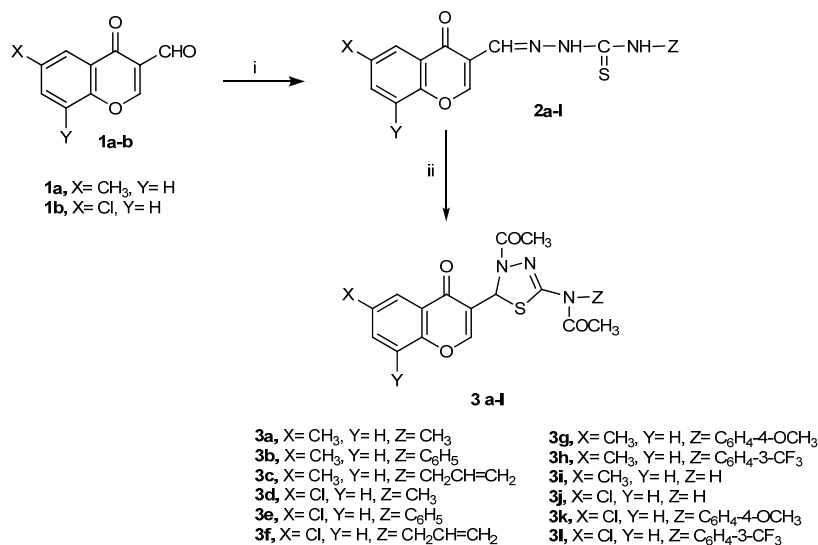
## 3. Results and discussion

Chemistry

Our synthetic routes to the target compounds **3a-l**, **5** are shown in Schemes 1-2, the synthesis of compound **6** being reported previously [30]. The structure of compound **6** is presented in Fig. 1. The derivatives of chromenyl-4,5-dihydrothiadiazoles **3a-l** were prepared by reacting the obtained chromenyl-thiosemicarbazones **2a-l** with the cyclisation agent acetic anhydride in the presence of small quantities of pyridine as a catalyst [17,31,32]. The intermediate thiosemicarbazones **2a-l** were obtained by a simple condensation between the 3-formyl-chromones **1a,b** and N^4-substituted thiosemicarbazides in absolute ethanol using concentrated sulphuric acid as catalyst [33,34]. The N^4-substituted phenyl, methyl, allyl, 3-trifluoro-methyl-phenyl, and 4-methoxy-phenyl thiosemicarbazides were obtained in good yields (>90%) by the addition of hydrazine hydrate to phenylisothiocyanate, methylisothiocyanate, allylisothiocyanate, 3-trifluoro-methylphenyl-isothiocyanate and 4-methoxyphenyl-isothiocyanate, respectively, with stirring in absolute ethanol at room temperature for 3 h [34]. These thiosemicarbazides are also commercially available.

The 5-chromenyl-methylene-2-thioxo-4-thiazolidinone **4** was obtained by the condensation of 2-thioxo-4-thiazolidinone with the 3-formyl-chromone **1c** in glacial acetic acid at reflux and in the presence of anhydrous sodium acetate. After isolation and purification, the obtained derivative **4** was alkylated with 2-iodoacetamide in the presence of anhydrous potassium hydroxide in dimethylformamide at room temperature to obtain the novel *N*-substituted compound **5**.

The purity of the compounds was confirmed by TLC, and all new compounds were characterized by m.p., elemental analysis and spectroscopic data (^{1}H NMR, ^{13}C NMR, MS).

1518

1a-b

1a, X= CH₃, Y= H
1b, X= Cl, Y= H

2a-l

3 a-l

3a, X= CH₃, Y= H, Z= CH₃
3b, X= CH₃, Y= H, Z= C₆H₅
3c, X= CH₃, Y= H, Z= CH₂CH=CH₂
3d, X= Cl, Y= H, Z= CH₃
3e, X= Cl, Y= H, Z= C₆H₅
3f, X= Cl, Y= H, Z= CH₂CH=CH₂

3g, X= CH₃, Y= H, Z= C₆H₄-4-OCH₃
3h, X= CH₃, Y= H, Z= C₆H₄-3-CF₃
3i, X= CH₃, Y= H, Z= H
3j, X= Cl, Y= H, Z= H
3k, X= Cl, Y= H, Z= C₆H₄-4-OCH₃
3l, X= Cl, Y= H, Z= C₆H₄-3-CF₃

*Scheme 1. Synthesis of chromen-yl-thiadiazoline derivatives **3a-l**: (i) H₂NNHCSNHZ/abs EtOH, reflux 3 h; (ii) Ac₂O/pyridine, reflux 4 h*

1c

4

5
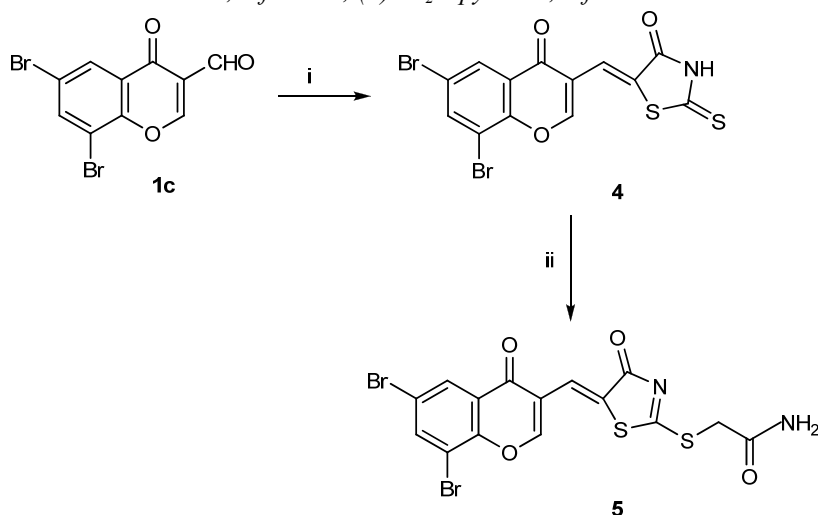
*Scheme 2. Synthesis of chromen-yl-methylene-thiazolin-2-thioacetamide **5**; (i) Thiazolidine-2-thioxo-4-one/AcOH, AcONa, reflux 3 h; (ii) ICH₂CONH₂/KOH, DMF, rt, 3 h*
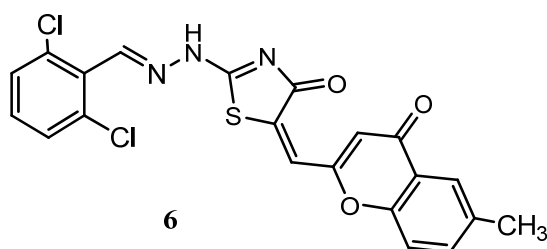
6

*Fig. 1. Chemical structure of compound **6***

Characterization of in vitro anticancer activity

The data in Table 1 show that the most potent compounds displayed an *in vitro* IC₅₀ ranging between 12 (**3h**) and 35 (**3l**, **3k**, **3j**) µM, while the remaining compounds in the study displayed weak or no (> 100 µM for **3i**) *in vitro* growth inhibition activity. In fact, nine of the 14 compounds in the study displayed an antiproliferative activity of < 100 µM against all cancer cell lines that were analysed. Compound **3h** displayed the highest *in vitro* anticancer activity (Table 1).

Table 1. Characterization of the in vitro growth inhibitory activity (using the MTT colorimetric assay) of compounds   3 a-l, 5, 6

Compounds	IC$_{50}$ concentrations (µM) after having cultured the cancer cells for 3 days with the compound of interest						
	A549	SKMEL-28	U373	U251	Hs683	MCF-7	Mean ± SEM
3a	*	*	*	79	85	84	**
3b	36	44	69	58	36	21	44 ± 7
3c	94	*	*	*	99	87	**
3d	*	43	70	21	*	22	**
3e	84	*	*	*	85	43	**
3f	75	72	48	41	42	40	53 ± 7
3g	45	39	63	77	54	34	52 ± 7
3h	19	17	12	17	27	32	21 ± 3
3i	*	*	*	*	*	*	**
3j	30	39	33	32	38	31	34 ± 2
3k	32	27	30	44	35	23	32 ± 3
3l	27	35	28	30	32	29	30 ± 1
5	77	67	82	65	73	56	70 ± 4
6	48	68	56	n.d.	n.d.	59	58 ± 4

n.d.- not determined

* IC$_{50}$> 100 µM

** The mean value could not be calculated because at least one cell line displayed an IC$_{50}$ > 100 µM

Structure Activity Relationship (SAR) analyses indicate that, of the two chemical series that were investigated, the chromenyl-thiadiazolines displayed the highest *in vitro* anticancer activity. With respect to the thiadiazoline derivatives (**3a-l** compounds), the substitution of the exocyclic amine with a substituted-phenyl moiety increased the *in vitro* growth inhibition of cancer cells (Table 1), 3-trifluoromethyl-phenyl-derivatives (**3h**, **3l**) displaying the highest growth inhibition activity (Table 1).

Thiazolinones **5** and **6** showed moderate growth inhibition activity against the cancer cell lines that were analysed in the current study.

So, it seems that the presence of fluorine atoms induces positive influences on the antiproliferative activity of these compounds against cancer cells.

The data in Table 1 further reveal that the most potent compounds, i.e., **3h**, **3j**, **3k** and **3l**, display similar growth inhibition effects in cancer cells independently of whether these cells are associated (A549 [21,22], SKMEL-28 [23], U373 [24,25]) or not associated (Hs683 [25,26], MCF-7 [27]) to various levels of resistance to pro-apoptotic stimuli.

Compounds **3h**, **3k** and **3l** were further assayed by means of quantitative videomicroscopy. Compounds **3h** and **3l** induced morphological cytostatic effects in the human MCF-7 breast cancer (Fig. 2) and T98G glioblastoma (data not shown) cell lines, while the compound **3k** induced morphological cytotoxic effects in both the human MCF-7 breast cancer (Fig. 2) and the T98G glioblastoma (data not shown) cell lines.
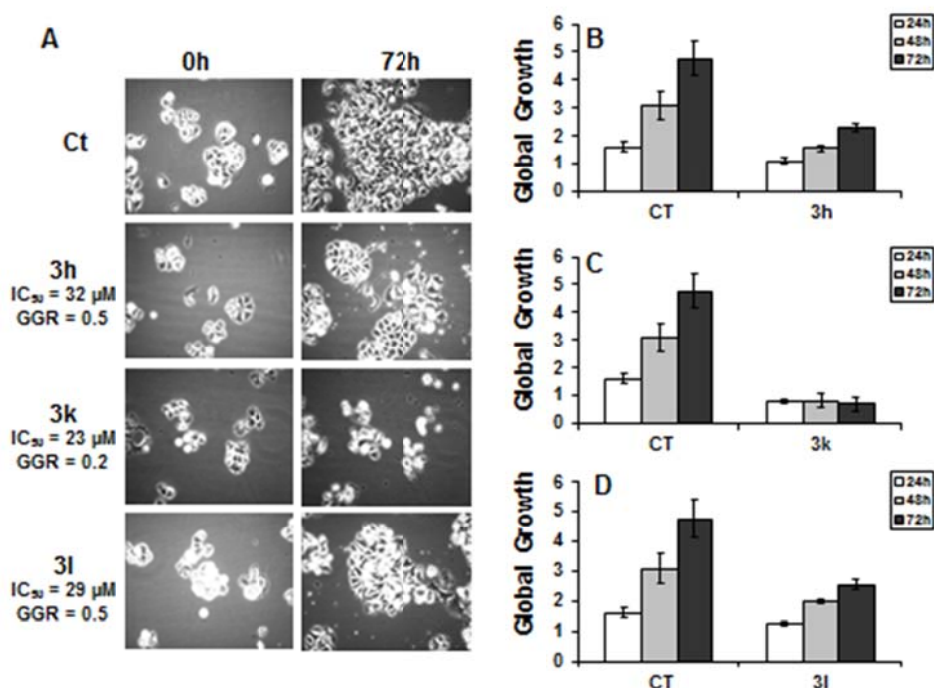
*Fig. 2. **(a)** Morphological illustrations (computer-assisted phase-contrast microscopy; Gx200) of human MCF-7 breast cancer cells cultured for 72 h with 32 µM **3h**, 23 µM **3k** or 29 µM **3l**; these concentrations refer to the MTT colorimetric test-related in vitro IC_{50} concentrations (see Table 1); **(b-d)** one image has been digitised every four minutes for a total of 1,080 digitised images during each 72 h for each experimental condition, which was performed in triplicates; all experiments were carried out in parallel, thus the controls in **Fig. 2b, 2c** and **2d** are identical. Therefore, the global growth rates correspond to the ratio of the mean number of cells present in the $1,080^{th}$ image to the number of cells present in the first image (at 0 h); we then divided this ratio obtained in the **3h**- (b), **3k**- (c) or **3l**- (d) treated experiment by the ratio obtained in the control to calculate the global growth ratio (GGR; **Fig. 2a**) index: a GGR value of 0.2 means for example that 20% of MCF-7 cancer cells grew in the treated experiment compared to the control over a 72 h observation period*

In fact, these three compounds induced cell death rates that ranged between 15 and 30% in both human MCF-7 breast cancer and T98G glioblastoma cells (Table 2). Therefore, the quantitative determination of cell death (Table 2) did not enable a clear distinction to be made between **3h**- and **3l**-related cytostatic effects versus effects from **3k**, but that information was obtained via morphological analyses (Fig. 2). Indeed, the panels on the right in Fig. 2 shows that **3h**- and **3l**-related cytostatic effects parallel a delay in MCF-7 (and also T98G, data not shown) cell growth, while **3k**-induced cytotoxic effects are paralleled by complete growth inhibition in MCF-7 breast cancer (Fig. 2) and in T98G glioblastoma (data not shown) cells.

*Table 2. The percentages of cell deaths induced by compounds **3h**, **3k** and **3l** in the human MCF-7 breast cancer and the T98G glioblastoma cell lines.*

Compounds*	Human cancer cell lines analysed	IC$_{50}$-related MTT concentration*	GGR***	% of cell death****			Global morphological effects
				24 h	48 h	72 h	
3h	MCF-7	32	0.5	7.7 ± 2.0	14.0 ± 3.8	14.7 ± 3.2	Cytostatic
	T98G**	30	0.4	9.7 ± 1.9	12.7 ± 2.3	18.7 ± 3.8	Cytostatic
3k	MCF-7	23	0.2	5.7 ± 1.2	16.3 ± 6.4	22.0 ± 8.7	Cytotoxic
	T98G	26	0.1	23.3 ± 1.9	29.0 ± 1.0	28.0 ± 2.9	Cytotoxic
3l	MCF-7	29	0.5	7.0 ± 1.5	7.7 ± 2.4	9.8 ± 2.4	Cytostatic
	T98G	62	0.3	10.7 ± 4.4	11.3 ± 1.3	22.7 ± 2.7	Cytostatic

** Compounds **3h**, **3k** and **3l** have been assayed by means of quantitative videomicroscopy (see Fig. 2) at their IC$_{50}$-related MTT colorimetric assay concentrations in the human MCF-7 apoptosis-sensitive (Dumont et al. 2007) breast cells (Table 1) and the human T98G apoptosis-resistant[**] (Branle et al. 2002) glioblastoma cells (IC$_{50}$ concentrations: **3h** = 30, **3k** = 26 and **3l** = 62 µM).*

*** Based on the phase-contrast microscopic pictures obtained by means of quantitative videomicroscopy (Fig. 2), we calculated the global growth rate (GGR), which corresponds to the ratio of the mean number of cells present in the last image captured in the experiment (conducted at 72 h) to the number of cells present in the first image (at 0 h). We divided this ratio obtained in each treated experiment by the ratio obtained in the control. Thus, for example, a GGR value of 0.4 in the current Table means that 40% of cells grew in the treated condition compared to the control condition over a 72 h observation period.

**** The % of cell deaths were determined by observation of the video films available for each experimental condition. For each experiment lasting for 72 h, one image was digitised every four minutes, yielding a whole set of 1,080 images after 72 h of observation. These 1,080 images were compressed into a one-minute video that clearly indicated whether the compound-induced effects were cytostatic or cytotoxic. Dynamic analyses of these videos allowed for easy discrimination between cytostatic (cell proliferation arrest) and cytotoxic (cell death) effects. The percentages of cell death in each control condition were < 2%.

It thus appears that compounds **3h**, **3k** and **3l** are able to overcome the intrinsic resistance of cancer cells (Table 1) to pro-apoptotic stimuli by inducing in cells either cytostatic (**3h**, **3l**) or cytotoxic (**3k**) effects (Fig. 2; Table 2).

4. Conclusions

Several types of metastatic cancers and primary cancers that have not yet metastasized by the time of initial diagnosis, including gliomas, melanomas, oesophageal cancers, pancreatic cancers, and NSCLCs, resist conventional chemotherapy and radiotherapy because these treatments induce pro-apoptotic factors in cancer cells, while the above-mentioned cancer types display various levels of resistance to pro-apoptotic stimuli.

The current study demonstrates that thiadiazoline derivatives, such as the 3-trifluoromethyl-phenyl-derivatives (**3h**, **3l**), are able to overcome cancer cell resistance to pro-apoptotic stimuli because these compounds display *in vitro* growth inhibition activity in both cancer cell lines that are sensitive to pro-apoptotic stimuli and those that are resistant to pro-apoptotic stimuli. Therefore, 3-trifluoromethyl-phenyl-derivatives deserve further investigation as potential anticancer drugs that could be assayed *in vivo* by the oral route in various mouse tumour models. We are currently pursuing this goal.

Acknowledgements

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