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Synthesis And Anticancer Screening Of Novel Spiro[Chroman-2,4′-Piperidin]-4-One Derivatives With Apoptosis-Inducing Activity

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ABSTRACT

A novel series of spiro[chroman-2,4′-piperidin]-4-one derivatives was synthesized and evaluated as cytotoxic agents against three human cancer cell lines; MCF-7 (human breast carcinoma), A2780 (human ovarian cancer) and HT-29 (human colorectal adenocarcinoma) using MTT assay. Compound **16** with a sulfonyl spacer exhibited the most potent activity with IC_{50} values between 0.31 and 5.62 µM. However, the trimethoxyphenyl derivative **15** was the least potent with IC_{50} values between 18.77 and 47.05 µM. The most active compound **16** was selected for further mechanistic studies, which revealed that it induced more than three folds early apoptosis in MCF-7 cells treated for 24 h. Additionally, it increased MCF-7 cells in the sub-G1 and G2-M cell cycle phases, following the same treatment duration. Together, these compounds could be promising cytotoxic candidates, thus further structural optimization, *in vitro* and *in vivo* studies are recommended to be developed into potential cytotoxic agents.

INTRODUCTION

Cancer is a life-threating disease that is manifested by out-of-control abnormal cell growth with the possibility of invading other tissues. Currently, there are several approaches for cancer treatment including chemotherapy, hormonal therapy, immunotherapy, surgery and radiation. However, chemotherapy remains one of the most prevailing options used for treating cancer (Gerber, 2008). Despite all the treatment approaches mentioned above and the remarkable breakthrough in cancer chemotherapy, the existence of a perfect and outright cure is still a challenging goal (Raguz and Yague, 2008). Accordingly, there is an urgent need to develop more potential anticancer agents. The discovery of apoptosis-inducing agents has become one of the most promising strategies of cancer treatment (Sun *et al.*, 2004). Apoptosis is considered a highly regulated and critically biological programmed cell death mechanism used to get rid of the defective and imperfect cells (McDonald and El-Deiry, 2005). It has an essential role in maintenance of tissue growth and homeostasis of multicellular organisms (Fulda and Debatin, 2006). Consequently, it was believed that the deregulation or impairment of this pivotal process is one of the main causes of incidence of cancer, autoimmune and neurodegeneration diseases (Lowe and Lin, 2000; Ponder, 2001). On the contrary, the induction of apoptosis machinery is feasibly one of the most potent defense strategies against cancer (Zhang *et al.*, 2007). Therefore, the discovery and design of novel apoptotic-inducing agents have received widespread attention as a potential, less toxic and promising approach in cancer chemotherapy (Sharma *et al.*, 2016; Jung *et al.*, 2017).

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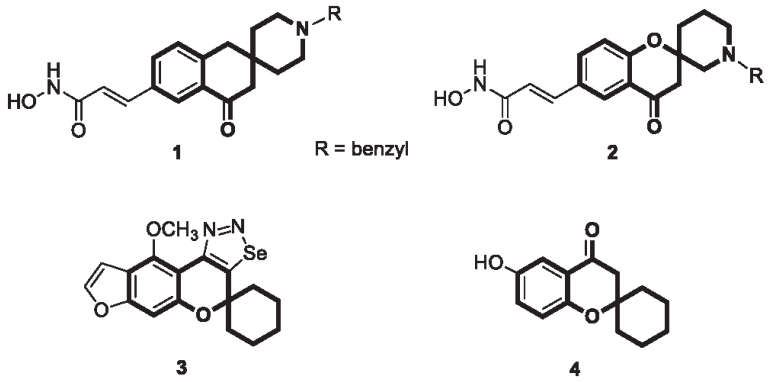


Fig. 1: Some reported spirochromane derivatives with anticancer activity.

On the other hand, spiro heterocycles have currently attracted a great attention due to their existence as a main skeleton in a wide variety of biologically active natural as well as synthetic compounds in addition to their interesting conformational features. Indeed, these molecules have become an elegant target for medicinal chemists to develop a large number of derivatives and evaluate their pharmacological potentials with emphasis on their medicinal applications. Among this class of compounds, spirochromanone is a well-recognized privileged structure that has been commonly found in a plenty of chemical compounds with diverse biological activities. These compounds exhibit significant biological activities such as anticancer (Atta *et al.*, 2010; El-Desoky *et al.*, 2013), antitubercular (Mujahid *et al.*, 2013; Mujahid *et al.*, 2015), antimicrobials (Feng *et al.*, 2014), histamine-3 antagonists (Becknell *et al.*, 2012), antiarrhythmic (Elliott *et al.*, 1992), acetyl-CoA carboxylase (ACC) inhibitors (Shinde *et al.*, 2009; Huang *et al.*, 2015), stearoyl-CoA desaturase-1 (SCD)-1 inhibitors (Uto *et al.*, 2010), histone deacetylase (HDAC) inhibitors (Varasi *et al.*, 2011; Thaler *et al.*, 2016), antimalarial (Roberts *et al.*, 2016), growth hormone secretagogues (Yang *et al.*, 1998) and δ opioid receptor agonists (Le Bourdonnec *et al.*, 2008). Thaler et al. have reported a series of spirochromanes and evaluated their activity against HDAC as a well-established anticancer target (Thaler *et al.*, 2016). These studies demonstrated that the prepared spirochromane derivatives possessed potent HDAC inhibitory activity that could be successfully developed into potential anticancer agents. Compounds **1** and **2** were the most potent HDAC inhibitors with good oral bioavailability and tumor growth inhibition. Moreover, a series of spiro furochromones was synthesized and screened for their antitumor activity where the selenium-containing compound **3** recorded the most promising activity against breast carcinoma (MCF-7) (Atta *et al.*, 2010), Figure 1. Another set of benzopyranones was reported and tested *in vitro* and *in vivo* for their anticancer potential. Compound **4** was highly potent against a panel of cancerous cell lines with IC_{50} values between 0.09 and 0.21 μM compared to 5-fluorouracil as a reference drug (El-Desoky *et al.*, 2013).

Furthermore, trimethoxyphenyl and adamantyl moieties are frequently used in design of several anticancer agents. Trimethoxyphenyl has an important role in mitosis, microtubules which represent a crucial target in development of new anticancer agents (Jordan and Wilson, 2004). In this regard, colchicine **5** (Lin *et al.*, 2016), the diarylthiazole derivative **6** (Wang *et al.*, 2015) and combretastatin A4 7 (Pettit *et al.*, 1989), are examples of this class where their anticancer activity is mediated by binding to tubulin. Meanwhile, adamantyl moiety was found to enhance the various activities of several compounds positively such as anticancer agents. One of such compounds, the diaminophenyladamantane derivative 8 exhibited strong anticancer activity *in vitro* and *in vitro* with G_0/G_1 cell cycle arrest mechanism (Wang *et al.*, 2004). Moreover, the adamantyl-1,2,4-triazole-3(4*H*)-thione derivative **9** was found to exert moderate *in vitro* anticancer activity against breast cancer with IC_{50} value of 8.15 μM with a suggested inhibition to BRCA2 protein (Genc *et al.*, 2015). The anti-prostate cancer activity was reported for compound **10** with potent inhibitory activity against 17_{α}-hydroxylase and $C_{17,20}$-lyase activities of human testicular cytochrome $P450_{17\alpha}$ (Chan *et al.*, 1996). The adamantine-gold complex **11** displayed potent cytotoxic activity in comparison to cisplatin and auranofin and could inhibit the thioredoxin reductase enzyme (Garcia *et al.*, 2016), Figure 2.

As part of our ongoing research work to develop novel anticancer agents, we describe herein the design and synthesis of some new chemical entities based on a spirochromanone framework tethered to known small moieties such as trimethoxyphenyl and adamantyl via various linkers using fragment-based drug design approach. The evaluation of their anticancer activity against a panel of cancer cell lines in addition to their ability to induce apoptosis and cell cycle analysis were also performed. It was conceptualized that these novel spiro hybrids could serve as promising leads for developing potent and safer anticancer agents. Figure 3 shows the suggested pharmacophore of the newly synthesized compounds.

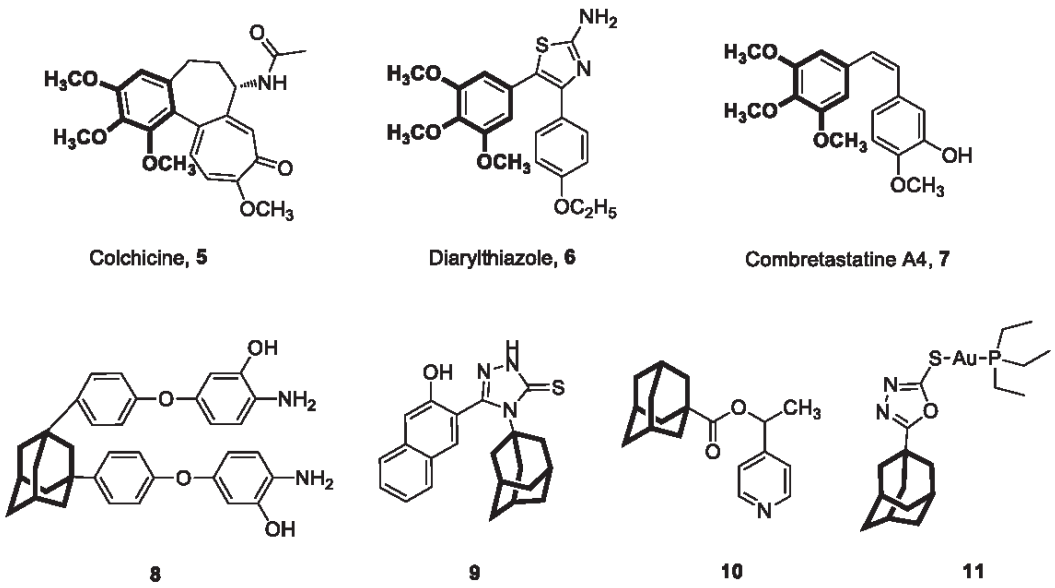


Fig. 2: Some reported trimethoxyphenyl- and adamantyl-containing compounds with anticancer activity.

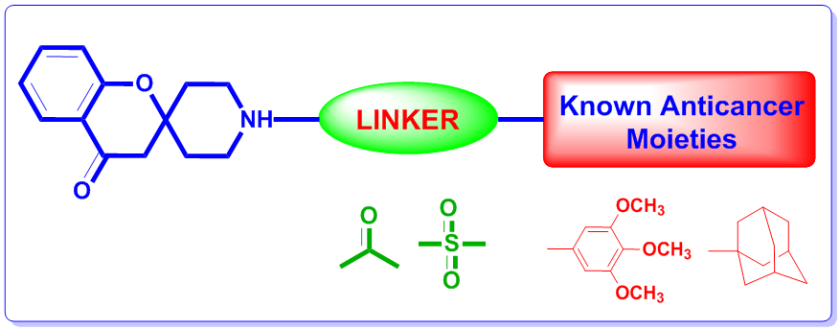


Fig. 3: Our newly designed scaffold.

MATERIALS AND METHODS

Chemistry

Chemical reagents and solvents were obtained from commercial sources, Solvents were dried by standard methods when necessary. Elemental analyses were carried out at the micro-analytical center in the Faculty of Science, Cairo University. ^{1}H NMR spectra were recorded with Bruker APX400 spectrometer at 400 MHz in DMSO-d_6. Chemical shifts were reported on the δ scale. The high-resolution mass spectra (HRMS) were recorded on Agilent 6230 Series Accurate-Mass Time-Of-Flight (TOF) LC/MS. Thin layer chromatography (TLC) was done by silica gel plates 60 GF254, cellulose plates (20 × 20 cm) from Sigma-Aldrich company for chemicals and ethyl acetate/hexane were used as the eluting system.

Tert-butyl 4-oxospiro[chroman-2,4'-piperidine]-1'-carboxylate (**12**) (Kabbe, 1978). Pyrrolidine (4.2 mL, 0.05 mol) was added under stirring to a mixture of N-Boc piperidone (5 g, 0.0249 mol) and 2-hydroxyacetophenone (3.4 g, 0.0249 mol) in anhydrous methanol (40 mL) at rt. The reaction mixture was refluxed overnight, then concentrated under reduced pressure and ethyl acetate (40 mL) was added. The combined organic layers were washed with 1 N HCl, 1 N NaOH, brine, and dried over Na_2SO_4. The organic solvent was removed in vacuo and hexane (30 mL) was added. The resulting off-white solid was filtered off, washed with hexane, Yield: 79%. 1H NMR ($CDCl_3$) δ 7.90 (d, J = 7.5, 1H), 7.63 (t, J = 7.3, 1H), 7.11 (m, 2H), 3.89 (m, 2H), 3.25 (m, 2H), 2.75 (s, 2H), 2.12 (d, J = 7.1, 2H), 1.63 (m, 2H), 1.48 (s, 9H). HRMS Calcd. for $C_{14}H_{16}O_2$ $[M + H]^+$ 317.1627, Found 318.1631.

Spiro[chroman-2,4'-piperidin]-4-one (**13**). Trifluoroacetic acid (5.16 mL, 67.05 mmol) was added slowly to a solution of tert-butyl 4-oxospiro[chroman-2,4'-piperidine]-1'-carboxylate **12** (3.86 g, 12.19 mmol) in methylene chloride (30 mL). The mixture was stirred at rt for 5 h. The mixture was then concentrated in vacuo and a solution of $NaHCO_3$ (30 mL) was added to the residue and extracted with methylene chloride (3 x 30 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and evaporated to afford compound **13** (85%) which was used directly

for the next step without any further purification. MS (EI) *m/z* 218 (M^++1).

General procedure A for the synthesis of compounds 14 and 15. The appropriate acid chloride (1.2 mmol) was added slowly to a solution of spiro[chroman-2,4'-piperidin]-4-one **13** (0.22 g, 1 mmol) in dry THF (15 mL) in presence of catalytic amount of triethyl amine. The reaction mixture was heated at 60°C for 5 h. The volatiles were removed under reduced pressure and water (30 mL) was added. The resulted precipitate was filtered off, washed with water and then recrystallized from ethanol to afford the final targeted compounds as off-white solid in good yields.

1'-Adamantane-1-carbonyl)spiro[chroman-2,4'-piperidin]-4-one (**14**). General Procedure A, off-white solid (67%). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.86 (d, *J* = 7.5, 1H), 7.49 (t, *J* = 7.3, 1H), 7.30 – 7.21 (m, 2H), 3.76 (t, *J* = 7.1, 2H), 3.13 (t, *J* = 7.1, 2H), 2.65 (s, 2H), 2.09 (t, *J* = 7, 2H), 1.63 (t, *J* = 7, 2H), 1.55 – 1.46 (m, 6H), 1.39 – 1.29 (m, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.54 (C=O), 176.91 (N-C=O), 160.49, 133.72, 127.30, 121.29, 120.94, 114.68, 68.73, 44.61, 40.09, 39.12, 36.10, 35.41, 34.25, 26.80. HRMS Calcd. for $C_{24}H_{29}NO_3$ [M + H]$^+$ 379.2147, Found 380.2321. Anal. Calcd. for: $C_{24}H_{29}NO_3$: C, 75.96; H, 7.70; N, 3.69. Found: C, 75.73; H, 7.95; N, 3.87.

1'-(3,4,5-Trimethoxybenzoyl)spiro[chroman-2,4'-piperidin]-4-one (***15***). General Procedure A, off-white solid (69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, *J* = 7.5, 1H), 7.71 (t, *J* = 7.3, 1H), 7.65 (s, *J* = 7.1, 2H), 7.30 – 7.21 (m, 2H), 3.89 (s, 9H), 3.53 (t, *J* = 7.1, 2H), 3.21 (t, *J* = 7.1, 2H), 2.67 (s, 2H), 2.11 (t, *J* = 7, 2H), 1.59 (t, *J* = 7, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.85 (C=O), 173.67 (N-C=O), 160.54, 152.42, 140.84, 133.24, 129.65, 127.74, 121.33, 120.71, 114.99, 105.91, 68.26, 60.64, 56.53, 44.33, 38.92, 34.21. HRMS Calcd. for $C_{23}H_{25}NO_6$ [M + H]$^+$ 411.1682, Found 412.1237. Anal. Calcd. for: $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.37; H, 6.42; N, 3.81.

General procedure B for the synthesis of compounds 16-18. An appropriate sulfonyl chloride derivative (1.3 mmol) was added to a stirred solution spiro[benzo[h]chromene-2,1'-cyclohexan]-4(3H)-ylidenehydrazine **13** (0.22 g, 1 mmol) in DCM (15 mL) in presence of catalytic amount of triethyl amine. The reaction mixture was allowed to stir for 6 h at room temperature. The resulting mixture was concentrated under reduced pressure and then treated with water (30 mL). The obtained precipitate was collected, washed with water and recrystallized from ethanol to afford the targeted final compounds **16-18** in good yields.

1'-(Phenylsulfonyl)spiro[chroman-2,4'-piperidin]-4-one (***16***). General Procedure B, off-white solid (71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 – 7.83 (m, 2H), 7.77 – 7.71 (m, 4H), 7.61 – 7.39 (m, 3H), 3.38 (t, *J* = 7.1, 2H), 3.31 (t, *J* = 7.1, 2H), 2.66 (s, 2H), 2.24 (t, *J* = 7, 2H), 1.61 (t, *J* = 7, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.87 (C=O), 160.54, 137.61, 133.42, 131.63, 129.72, 127.69, 121.26, 120.27, 114.99, 68.26, 44.30, 40.83, 33.51. HRMS Calcd. for $C_{19}H_{19}NO_4S$ [M + H]$^+$ 357.1035, Found 358.1364. Anal. Calcd. for: $C_{19}H_{19}NO_4S$: C, 63.85; H, 5.36; N, 3.92. Found: C, 64.02; H, 5.81; N, 4.23.

1'-Tosylspiro[chroman-2,4'-piperidin]-4-one (***17***). General Procedure B, off-white solid (72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 – 7.86 (m, 3H), 7.80 – 7.73 (m, 3H), 7.43 – 7.21 (m, 2H), 3.33 (t, *J* = 7.1, 2H), 3.29 (t, *J* = 7.1, 2H), 2.73 (s, 2H), 2.43 (s, 3H), 2.23 (t, *J* = 7, 2H), 1.57 (t, *J* = 7, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.14 (C=O), 160.13, 137.54, 133.33, 131.46, 129.37, 127.68, 121.65, 120.08, 114.34, 68.75, 44.51, 40.79, 33.47, 21.39. HRMS Calcd. for $C_{20}H_{21}NO_4S$ [M + H]$^+$ 371.1191, Found 372.1208. Anal. Calcd. for: $C_{20}H_{21}NO_4S$: C, 64.67; H, 5.70; N, 3.77. Found: C, 64.89; H, 5.63; N, 4.02.

1'-(Naphthalen-1-ylsulfonyl)spiro[chroman-2,4'-piperidin]-4-one (***18***). General Procedure B, off-white solid (77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 – 8.15 (m, 3H), 7.93 – 7.86 (m, 3H), 7.79 – 7.68 (m, 3H), 7.24 – 7.09 (m, 2H), 3.52 (t, *J* = 7.1, 2H), 3.37 (t, *J* = 7.1, 2H), 2.68 (s, 2H), 2.36 (s, 3H), 2.17 (t, *J* = 7, 2H), 1.61 (t, *J* = 7, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.98 (C=O), 160.27, 142.61, 134.91, 133.73, 131.07, 130.90, 128.39, 127.01, 126.90, 126.31, 124.62, 121.83, 120.11, 114.40, 68.75, 44.60, 40.81, 33.43. HRMS Calcd. for $C_{23}H_{21}NO_4S$ [M + H]$^+$ 407.1191, Found 408.1203. Anal. Calcd. for: $C_{23}H_{21}NO_4S$: C, 67.79; H, 5.19; N, 3.44. Found: C, 67.91; H, 5.37; N, 3.75.

Biological screening

Cell culture

MCF-7 (human breast adenocarcinoma), A2780 (human ovary adenocarcinoma) and HT29 (human colon adenocarcinoma) were all purchased from the ATCC, USA. Cells were sub-cultured using RPMI-1640 media (10% FBS).

Cytotoxicity assay

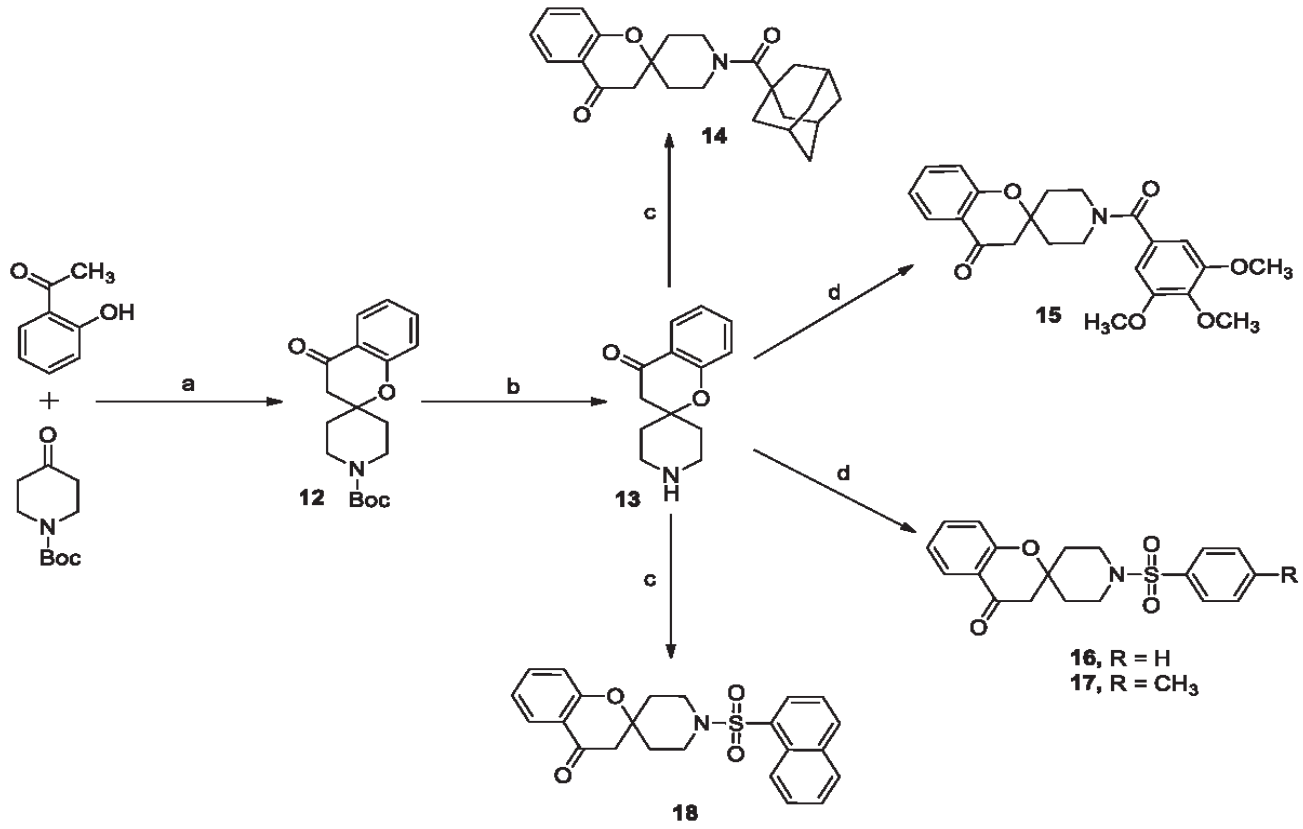
The cytotoxicity of the five compounds were evaluated by MTT assay, as previously described (Abdelazeem *et al.*, 2014; Gouda *et al.*, 2014). The three cell lines were separately cultured in 96-well (3 ×10^3/well), and incubated at 37°C overnight. Final compound concentrations: 0, 0.05, 0.5, 5, 25, 50 µM (DMSO 0.1%; n = 3). Plates were incubated for 72 h, then MTT was added to each well. Plates were incubated for 3 h, supernatant was aspirated, and DMSO was added to each well. Absorbance was read on multi-plate reader. Optical density of the purple formazan A_{550} is proportional to the number of viable cells. Compound concentration causing 50% inhibition (IC_{50}) compared to control cell growth (100%) was determined. GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA was used for analysis.

Annexin V FITC/PI apoptosis assay

Annexin V FITC/PI assay was used to evaluate possible ability to induce apoptosis. MCF-7 cells were cultured in 6 well plates (1 × 10^5 cells/well) overnight at 37°C. Compound **16** was used to treat cells (0, 5, 10 and 20 µM). After 24 h, supernatant of treated cells was collected in tubes and kept on ice, then cells were trypsinized and incubated at 37°C before being added to the tubes. MCF-7 cells were centrifuged (2000 rpm), washed with PBS (x1), centrifuged again, pellets re-suspended in binding buffer (100 µL, x1) and annexin V FITC (10 µL). Tubes were incubated at room temperature in dark for 20 minutes before adding the binding buffer (400 µL, x1) and 10 µL propidium iodide (PI). Analysis was performed by flow cytometry (NovoCyte Flow Cytometer, Acea Biosciences Inc., California, USA). Different cell populations (early apoptotic, late apoptotic, and necrotic cells) were identified by annexin V and PI staining (Vermes *et al.*, 1995).

Cell cycle analysis

Perturbation of cell cycle was done for MCF-7 cells cultured in 6 well plates (1×10^5 cells) overnight at 37°C. Treated cells with compound 3Z (0, 5, 10 and 20 µM) were washed with cold PBS x1 and trypsinized. Collected cells were spinned at 2000 rpm. The resulting pellets were washed in cold PBS x1 again, spinned and fixed overnight in 70% ice cold ethanol. Cells were re-suspended in cold PBS x1 with addition of ribonuclease A (15 min), followed by PI (2 µL/mL). Samples were held on ice and analysed by flow cytometry. Data analysis of DNA contents (PI bound to DNA) of 20000 events was carried out (Bkhaitan *et al.*, 2017).



Scheme 1: *Reagents and Reaction Conditions*: (a) Pyrrolidine, MeOH, reflux, overnight; (b) TFA, DCM, rt, 3 h; (c) appropriate sulfonyl chloride, TEA, DCM, rt, 6 h; (d) appropriate acid chloride, TEA, THF, 60°C, 5 h.

RESULTS AND DISCUSSION

Chemistry

The general synthetic pathway used for the preparation of the novel targeted spiro derivatives is shown in Scheme 1. The starting spiro material, tert-butyl 4-oxospiro[chroman-2,4'-piperidine]-1'-carboxylate **12**, was prepared using the reported multi-component reaction developed by Kabbe where a thermal condensation reaction of 2-hydroxyacetophenone and N-Boc piperidone in methanol was carried out in presence of pyrrolidine (Kabbe, 1978). The furnished spiro protected compound **12** was subjected to a deprotecting process using TFA in DCM to give the free-boc intermediate **13** in a quantitative yield which in turn was coupled with the appropriate acid chloride or sulfonyl chloride reagents in presence of TEA affording the final targeted compounds (**14**-**18**) in good yields. All the final compounds were fully characterized using NMR, mass, CHN spectral analysis.

Biological screening

Cytotoxic activity

The cytotoxic activity of the newly synthesized compounds was assessed *in vitro* against three cancer cell lines; MCF-7 (human breast carcinoma), A2780 (human ovarian cancer) and HT-29 (human colorectal adenocarcinoma) using MTT assay (Abdelazeem *et al.*, 2014; Gouda *et al.*, 2014). The results were summarized in Table 1 and expressed in terms of IC_{50} values where doxorubicin was used as a positive control. Generally, it was noticed that the compounds containing a sulfonyl moiety (**16**-**18**) exhibited higher activity than compounds (**14**, **15**) containing a carbonyl spacer. Among the tested derivatives, compound **16** was the most potent anticancer agent against the three cancer cell lines with IC_{50} values between 0.31 and 5.62 µM. Nevertheless, the trimethoxyphenyl derivative **15** showed the least potency with IC_{50} values between 18.77 and 47.05 µM. It was found that the replacement of the phenyl group in compound **16** with a p-tolyl group in compound **17** resulted in a sharp decrease in the potency,

especially against HT-29 cell line suggesting a possible significant effect of electron-donating substituents on the activity. However, the replacement with a naphthyl moiety as in compound **18** slightly affected the activity against MCF-7 and HT-29 cell lines compared with compound **16**. Of the other substituents, the carbonyl adamantyl bearing analog **14** displayed moderate activity against HT-29 with IC_{50} value of 8.46 µM but with a weak activity against the other used cell lines. These results indicate that the influence of the substitutions on the activity was mainly electronically more than sterically.

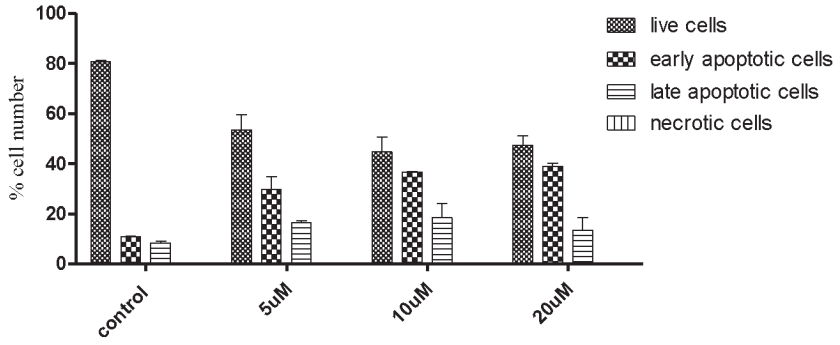


Fig. 4: Phases of staining MCF-7 cells with annexin V FITC/PI treated with compound **16** (24h) to detect apoptosis. Live cells: PI-/annexin V-; Early apoptotic cells: PI-/ annexin V+; Late apoptotic cells: PI+/ annexin V+; Necrotic cells: PI+/ annexin V-. Data shown is mean % ± SD (n = 2). Experiment was repeated 2x.

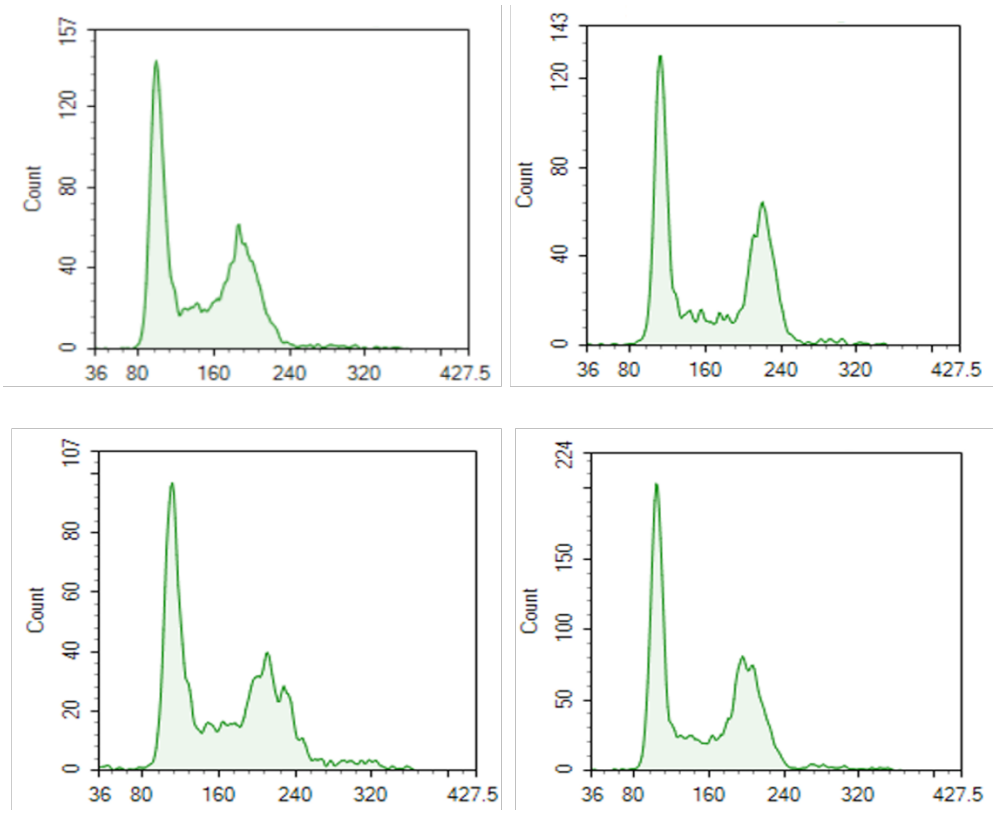


Fig. 5: Graphs showing effect of compound **16** on cell cycle distribution after 24 h treatment in MCF7. X-axis: DNA content of 20,000 events, y axis: % cell number. Top left: 0 µM; top right: 5 µM; bottom left: 10 µM; bottom right: 20 µM.

Annexin V FITC/PI apoptosis assay

Compound **16**, with the highest activity against the three cell lines, was selected for further investigations to explain its mechanism of action. Annexin V FITC/PI assay was used to evaluate whether it can induce apoptosis or not in MCF-7 cells following 24 h treatment. Doses of compound **16** were selected based

on its IC_{50} (x1, x2 and x4, respectively). Compound **16** increased the early apoptotic MCF-7 cell populations in a dose dependent manner, more than three folds compared to control; combined by increase in late apoptosis at 5 and 10 µM; all at expense of live cells which decreased consequently, Figure 4.

Table 1: IC_{50} values of the newly synthesized compounds against three cancer cell lines; MCF-7, HT-29 and A2780 cell lines.

Comp.	Anticancer activity IC_{50} (µM) ± SD		
	MCF-7	A2780	HT-29
14	23.86 ± 1.55	22.68 ± 1.37	8.46 ± 1.31
15	47.05 ± 2.66	18.77 ± 1.82	23.20 ± 2.21
16	5.62 ± 1.33	0.31 ± 0.11	0.47 ± 0.17
17	6.78 ± 1.21	1.79 ± 1.65	16.03 ± 1.68
18	6.02 ± 1.07	0.316 ± 0.16	0.69 ± 0.14
Doxorubicin	1.3 ± 0.8	2.11 ± 0.55	1.53 ± 0.62

Three human cancer cell lines were used; MCF-7 (human breast carcinoma), A2780 (human ovarian cancer) and HT-29 (human colorectal adenocarcinoma). Cells were treated with the test compounds or vehicle for 72 h and cell viability was assessed by MTT assay. Data were reported as mean ± S.D. (n = 6). Doxorubicin was used as a positive control.

Cell cycle analysis

The cell cycle distribution assay of MCF-7 cells treated with compound **16** (24 h), revealed an increase in the sub-G1 pro-apoptotic cells (from 0.30% at 0 µM, to 1.02% and 1.54% at 5 µM and 10 µM, respectively). Compound **16** blocked MCF-7 cells at the G2-M phase in a dose dependent manner (Figure 5 and Table 2) and that was supported by the annexin V assay results.

Table 2: Effect of compound **16** on MCF-7 cell cycle phases (24h).

Cell stage	Control	5 µM	10 µM	20 µM
Sub-G1	0.30%	1.02%	1.54%	0.51%
G1	34.74%	35.07%	31.79%	36.29%
S	28.30%	27.48%	25.85%	22.56%
G2-M	32.74%	33.38%	36.01%	37.55%

CONCLUSION

A new series of spiro[chroman-2,4'-piperidin]-4-one derivatives was synthesized and evaluated as anticancer agents against a panel of human cancer cell lines using MTT assay. Compound **16** with a sulfonyl spacer exhibited the most potent activity with IC_{50} values between 0.31 and 5.62 µM. However, the trimethoxyphenyl derivative **15** was the least potent with IC_{50} values between 18.77 and 47.05 µM. The most active compound **16** could induce more than three folds early apoptosis in MCF-7 cells treated for 24 h. Moreover, it increased MCF-7 cells in the sub-G1 and G2-M cell cycle phases, following the same treatment duration. These compounds could be considered as good leads that need further structural optimization to be developed into potential anticancer agents.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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