Research Article

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Future Medicinal Chemistry

Identification of some benzoxazepines as anticancer agents inducing cancer cell apoptosis

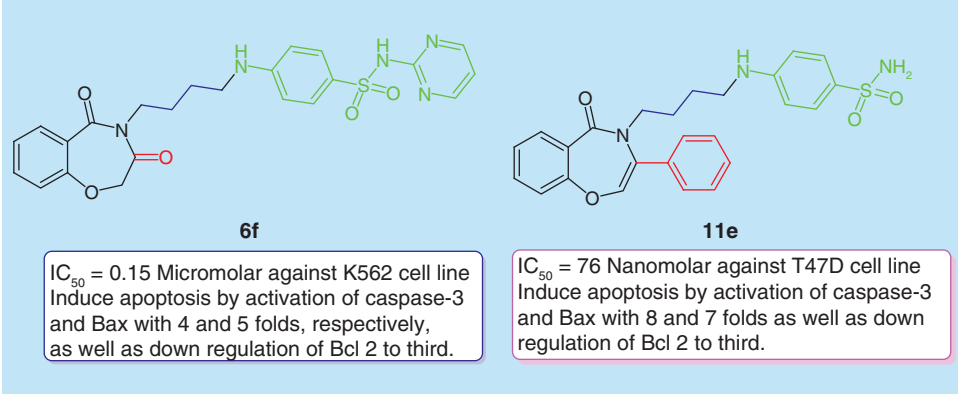
Mohamed S Rashad[1], Hanan H Georgey[1], Riham F George*[,1] & Nagwa M Abdel-Gawad[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

*Author for correspondence: rihamfgeorge@gmail.com

Aim: Using cytotoxic agents with apoptosis induction may represent one of new strategies for cancer treatment to overcome the increased resistance of the disease. **Methodology:** Two series of benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-diones (compounds **5**, **6a–f**) and 3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-ones (compounds **10**, **11a-f**) were synthesized and screened for their cytotoxicity against leukemia K-562 and breast T-47D cancer cell lines as well as normal fibroblasts WI-38. **Results:** The tested compounds revealed good cytotoxicity and selectivity toward cancer cell lines relative to the normal cells, especially compounds **6f**, **10** and **11e, f**. These compounds were screened for cell cycle disturbance and apoptosis induction. They were found to cause PreG1 apoptosis and complete cell growth arrest at G2/M. They induce apoptosis via caspase-3 and Bax activation and downregulation of Bcl2. **Conclusion:** benzo[*f*][1,4]oxazepine represents a scaffold for further optimization to obtain promising anticancer agents.

Graphical abstract:



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Cancer is considered as one of the most urgent problems concerning health worldwide. This may be due to the continual increase in the number of cases every year from different ages and either sex in addition to high death records ranking the second cause of death after the cardiovascular diseases [1]. The greatest danger of this disease resulted from its severity with the tendency of the cancerous cells to resist treatment with the traditional chemotherapeutic agents. This resistance is a result of apoptosis failure with increased death threshold; thus, higher doses of anticancer agents or combination of two or more drugs acting by different mechanisms are needed to overcome this resistance [2,3]. In addition, due to the rapid progress in understanding the molecular pathways involved in cell cycle regulation, there is a motivation to target different steps during the cell cycle in order to control cancer and avoid dangerous side effects observed during traditional chemotherapies [4]. Therefore, apoptosis

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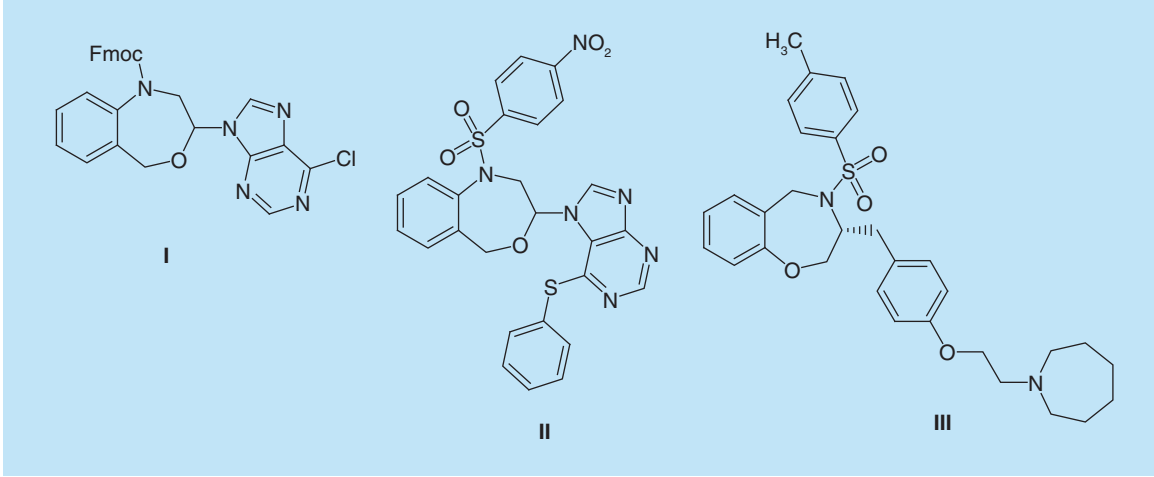

Figure 1. Structure of some anticancer benzoxazepine derivatives.

induction in cancer cells withdraw the attention of medicinal chemists to design small molecules targeting one or more of the key enzymes of apoptosis process in cancer cells and use them as a new weapon against cancer [5]. Caspases are proteases responsible for cleavage of vital proteins important for cells survival, thus, caspase activation can be a sign for apoptosis induction which may be useful during cancer treatment [6]. Furthermore, Bax, the proapoptotic protein and Bcl2, the antiapoptotic protein, can be considered as key factors in the intrinsic apoptosis pathway [7].

Benzoxazepines represent an important core for many bioactive agents exhibiting various biological activities as CNS activity [8–10], immune-modulation [11–14] and anti-inflammatory [15]. Moreover, the benzoxazepine derivatives **I** and **II** showed antiproliferative activity against MCF-7 breast cancer cell line with IC_{50} values 0.67 and 0.86 μM, respectively (Figure 1) [16]. Furthermore, compound **I** was found to induce apoptosis in MCF-7 cell line at the IC_{50} dose level [17]. Additionally, the antiproliferative effect of compound **III** on MCF-7 cells was associated with G_1 cell-cycle arrest followed by apoptosis. It activated the components of both intrinsic and extrinsic pathways of apoptosis characterized by activation of caspase [18].

In this work, two benzoxazepine scaffolds were selected to build the final derivatives, thus, benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-dione (formula A) and 3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-one (formula B) were selected to compare the effect of presence of the hydrophobic phenyl moiety on the obtained activity. The target compounds were intended to have a spacer of four carbon atoms to ensure flexibility between the main core and the side chain. This side chain may be piperidine (compounds **5**, **10**) or different anilines (compounds **6a–f**, **11a–f**) in order to study the influence of these structural modifications on the antiproliferative activity (Figure 2). Furthermore, the cell cycle analysis of the most active compounds was performed to detect if the cytotoxic effect is accompanied by changes in the cell cycle. Moreover, their ability to activate caspase-3 and Bax as well as downregulation of Bcl-2 was investigated to confirm the apoptosis induction.

Experimental

Chemistry

Melting points were carried out by the open capillary tube method using a Stuart (Stone, Staffordshire, UK) apparatus and they were uncorrected. Elemental analyses were performed using FLASH 2000 CHNS/O analyzer, Thermo Scientific at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt. Infrared spectra were done on Schimadzu FTIR 8400 S spectrometer Affinity A1, and expressed in wave number (cm^{-1}), using potassium bromide discs. ^{1}H NMR and ^{13}C NMR spectra were carried out using Bruker High performance Digital FT-NMR spectrometer Avance III 400MHz using $CDCl_3$ or DMSO-d_6 as solvents. The chemical shifts were expressed in δ ppm units using tetramethylsilane as an internal standard. Mass spectrum was carried out on UPLC MS/MS 'waters' 3100 'USA', cone voltage = 65 V at Center for Applied Research and Advanced Studies, Faculty of Pharmacy, Cairo University, Cairo, Egypt. All the reactions were monitored by thin

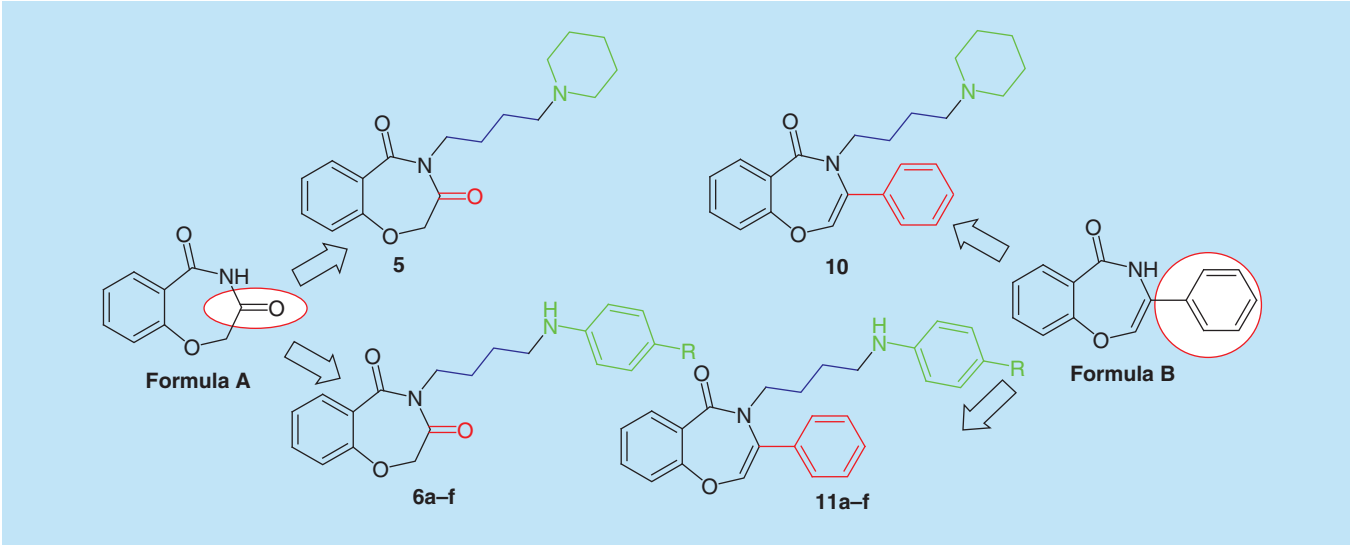

Figure 2. Structure of the proposed compounds 5, 6a–f, 10 and 11a-f.

layer chromatography silica gel F 254 aluminum sheets using dichloromethane: methanol (1:0.1) as the elution solvent system. Compounds **2**, **3**, **4** [8], **7** and **8** [19] were prepared according to the reported procedures.

4-[4-(Piperidin-1-yl)butyl]benzo[f][1,4]oxazepine-3,5(2H,4H)-dione ***(5)***

A mixture of **4** (0.27 g, 1.00 mmol), potassium iodide (0.21 g, 1.20 mmol), piperidine (0.094 g, 1.10 mmol) and potassium carbonate (0.42 g, 3.00 mmol) in dry acetonitrile (10 ml) was heated under reflux for 48 h. The separated solid was removed by filtration, dissolved in ethyl acetate. The organic layer was washed with water, then dried over magnesium sulfate and evaporated under vacuum to give a residue, which was purified by column chromatography over silica gel using mobile-phase dichloromethane:methanol (20:1) to give the titled compound (0.17 g, 54%) as a white solid; mp 160–162°C. IR (KBr) υ cm^{-1}: 3055 (CH aromatic), 2924 (CH aliphatic), 1708 (C=O), 1655 (C=O), 1600, 1485, 1450 (C=C). ^{1}H NMR (C*D*Cl$_3$) δ: 1.74–2.07 (m, 8H, 3CH$_2$ piperidine and CH$_2$ butyl), 2.36–2.39 (m, 2H, CH$_2$ butyl), 2.79 (br s, 2H, *N*CH$_2$ butyl), 3.07–3.12 (m, 2H, *N*CH$_2$ butyl), 3.58–3.60 (m, 2H, *N*CH$_2$ piperidine), 4.00 (t, 2H, *J* = 7.10 Hz, *N*CH$_2$ piperidine), 4.79 (s, 2H, OCH$_{2)}$, 7.10 (d, 1H, *J* = 8.12 Hz, ArH), 7.25 (t, 1H, *J* = 7.66 Hz, ArH), 7.53 (t, 1H, *J* = 7.66 Hz, ArH), 8.14 (d, 1H, *J* = 8.00 Hz, ArH). 13C NMR (C*D*Cl3) δ: 20.9 (CH$_2$ piperidinyl), 21.8 (CH$_2$ butyl), 22.4 (CH$_2$ piperidinyl), 25.2 (CH$_2$ butyl), 43.2 (*N*CH$_2$ butyl), 53.2 (*N*(CH$_2$)$_2$ piperidinyl), 56.9 (*N*CH$_2$ butyl), 74.6 (OCH$_2$), 119.9, 122.8, 124.5, 134.1, 135.2, 159.0 (aromatic C), 165.3 (C=O), 171.1 (C=O). MS (%): 317.21 (M^++1), 140.11 (44), 121.09 (100). Anal. Calcd. for $C_{18}H_{24}N_2O_3$ (316.39): C: 68.33; H: 7.65; N: 8.85. Found: C: 68.59: H: 7.78; N: 9.01 (Figure 3).

General procedure for the synthesis of compounds ***6a–f***

To an equimolar amount of compound **4** (0.27 g, 1.00 mmol) and the appropriate aromatic amine (1.00 mmol) in a tight screw vial with a magnetic bar, tetrabutylammonium bromide (TBAB, 0.50 g) was added and the reaction mixture was heated till melt. The mixture was stirred for 12 h at 70–80°C. After cooling, the mixture was dissolved in dichloromethane and sodium bicarbonate solution was added. The organic layer was separated, washed with water, dried over magnesium sulfate and concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using dichloromethane:methanol (30:1) as an eluent to obtain **6a–f** in a pure form (Figure 3).

4-[4-(Phenylamino)butyl]benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-dione **(6a)**

It was prepared from **4** and aniline (0.094 g, 1.00 mmol) to afford (0.14 g, 43.20%) as a pale yellow semisolid. IR (KBr) υ cm^{-1}: 3398 (NH), 3051, 3020 (CH aromatic), 2927 (CH aliphatic), 1701 (2C=O), 1643 (NH bending), 1604, 1508, 1481 (C=C). ^{1}H NMR (C*D*Cl$_3$) δ: 1.67–1.72 (m, 2H, CH$_2$ butyl), 1.74–1.80 (m, 2H, CH$_2$ butyl), 3.20 (t, 2H, *J* = 6.86 Hz, *N*CH$_2$ butyl), 4.04 (t, 2H, *J* = 7.42 Hz, *N*CH$_2$ butyl), 4.78 (s, 2H, OCH$_2$), 6.66 (d,

2H, *J* = 7.64 Hz, ArH), 6.73 (t, 1H, *J* = 7.30 Hz, ArH), 7.12 (dd,1H, *J* = 0.86, 8.18 Hz, ArH), 7.17–7.21 (m, 2H, ArH), 7.25–7.29 (m, 2H, ArH and NH exchanged with D_2O), 7.52–7.56 (m, 1H, ArH), 8.19 (dd, 1H, *J* = 1.64, 8.08 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) *δ*: 25.5 (CH_2 butyl), 26.4 (CH_2 butyl), 44.1 (*N*CH_2 butyl), 44.3 (*N*CH_2 butyl), 74.6 (OCH_2), 113.8, 118.4, 119.9, 123.0, 124.4, 129.3, 134.1, 135.0, 147.1, 159.0 (aromatic C), 165.3 (C=O), 171.0 (C=O). MS (%): 325.03 (M^++1), 232.06 (50), 147.99 (88). Anal. Calcd. for $C_{19}H_{20}N_2O_3$ (324.37): C: 70.35; H: 6.21; N: 8.64. Found, C: 70.01: H: 6.53; N: 9.01.

4-[4-((4-Fluorophenyl)amino)butyl]benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-dione **(6b)**

It was prepared from **4** and *p*-fluoroaniline (0.111 g, 1.00 mmol) to afford (0.18 g, 52.60%) as a pale brown semisolid. IR (KBr) *υ* cm^{-1}: 3398 (NH), 3074, 3040 (CH aromatic), 2943, 2866 (CH aliphatic), 1705 (2C=O), 1643 (NH bending), 1604, 1516, 1481 (C=C). ^{1}H NMR (C*D*Cl$_3$) *δ*: 1.67–1.76 (m, 2H, CH_2 butyl), 1.79–1.81 (m, 2H, CH_2 butyl), 3.15 (t, 2H, *J* = 6.84 Hz, *N*CH_2 butyl), 4.03 (t, 2H, *J* = 7.42 Hz, *N*CH_2 butyl), 4.77 (s, 2H, OCH_2), 6.57–6.61 (m, 2H, ArH), 6.89 (t, 2H, *J* = 8.74 Hz, ArH), 7.11 (dd,1H, *J* = 0.76, 8.12 Hz, ArH), 7.24–7.28 (m, 2H, ArH and NH exchanged with D_2O), 7.51–7.56 (m, 1H, ArH), 8.18 (dd, 1H, *J* = 1.66, 8.06 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) *δ*: 25.5 (CH_2 butyl), 26.6 (CH_2 butyl), 44.1 (*N*CH_2 butyl), 44.4 (*N*CH_2 butyl), 74.6 (OCH_2), 114.0, 114.1, 115.6, 115.8, 119.9, 123.0, 124.4, 134.1, 135.1, 144.1, 154.8, 157.2, 159.0 (aromatic C), 165.3 (C=O), 171.0 (C=O). Anal. Calcd. for $C_{19}H_{19}FN_2O_3$ (342.36): C: 66.66; H: 5.59; N: 8.18. Found, C: 66.82: H: 5.80; N: 8.31.

4-[4-((4-Methoxyphenyl)amino)butyl]benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-dione **(6c)**

It was prepared from **4** and *p*-anisidine (0.123 g, 1.00 mmol), to afford (0.17 g, 47.90%) as a pale brown semisolid. IR (KBr) *υ* cm^{-1}: 3395 (NH), 3060 (CH aromatic), 2931, 2862 (CH aliphatic), 1705 (2C=O), 1651 (NH bending), 1604, 1516, 1481 (C=C). ^{1}H NMR (DMSO-*d* $_6$) *δ*: 1.50–1.54 (m, 2H, CH_2 butyl), 1.63–1.67 (m, 2H, CH_2 butyl), 2.95 (t, 2H, *J* = 6.86 Hz, *N*CH_2 butyl), 3.63 (s, 1H, OCH_3), 3.90 (t, 2H, *J* = 7.24 Hz, *N*CH_2 butyl), 4.89 (s, 2H, OCH_2), 5.05 (br s, 1H, NH exchanged with D_2O), 6.49 (d, 2H, *J* = 8.88 Hz, ArH), 6.69 (t, 2H, *J* = 8.80 Hz, ArH), 7.17 (d, 1H, *J* = 8.12 Hz, ArH), 7.30 (t, 1H, *J* = 7.60 Hz, ArH), 7.62 (t, 1H, *J* = 7.52 Hz, ArH), 8.10 (dd, 1H, *J* = 1.26, 8.02 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) *δ*: 25.5 (CH_2 butyl), 26.5 (CH_2 butyl), 44.1 (*N*CH_2 butyl), 45.2 (*N*CH_2 butyl), 55.8 (OCH_3), 74.5 (OCH_2), 114.9, 115.2, 119.9, 123.0, 124.4, 134.1, 135.0, 141.3, 152.8, 159.0 (aromatic C), 165.3 (C=O), 171.0 (C=O). MS (%): 355.45 (M^++1), 232.09 (18), 178.15 (100). Anal. Calcd. for $C_{20}H_{22}N_2O_4$ (354.40): C: 67.78; H: 6.26; N: 7.90. Found, C: 67.61: H: 6.22; N: 8.10.

4-[(4-(3,5-Dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepin-4(5*H*)-yl)butyl)amino] benzoic acid **(6d)**

It was prepared from **4**, TBAB (1.00 g) and *p*-amino benzoic acid (0.14 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.16 g, 43.40%) as a white solid; mp 181–183°C. IR (KBr) *υ* cm^{-1}: 3406 (NH), 3067 (CH aromatic), 2939, 2870 (CH aliphatic), 2654–2538 (OH carboxylic), 1708 (2C=O), 1660 (C=O), 1643 (NH bending), 1600, 1523, 1481(C=C). ^{1}H NMR (DMSO-*d* $_6$) *δ*: 1.51–1.58 (m, 2H, CH_2 butyl), 1.61–1.69 (m, 2H, CH_2 butyl), 3.08 (q, 2H, *J* = 6.46 Hz, *N*CH_2 butyl), 3.91 (t, 2H, *J* = 7.12 Hz, *N*CH_2 butyl), 4.89 (s, 2H, OCH_2), 6.42 (t, 1H, *J* = 5.20 Hz, NH exchanged with D_2O), 6.54 (d, 2H, *J* = 8.80 Hz, ArH), 7.16 (d, 1H, *J* = 8.04 Hz, ArH), 7.29 (t, 1H, *J* = 7.58 Hz, ArH), 7.59–7.63 (m, 1H, ArH), 8.10 (dd, 1H, *J* = 1.52, 8.04 Hz, ArH), 11.96 (br s, 1H, OH exchanged with D_2O). ^{13}C NMR (DMSO-*d* $_6$) *δ*: 25.8 (CH_2 butyl), 26.5 (CH_2 butyl), 42.5 (*N*CH_2 butyl), 43.8 (*N*CH_2 butyl), 74.5 (OCH_2), 111.1, 117.1, 120.2, 123.0, 124.5, 131.6, 134.1, 135.6, 153.1, 159.2 (aromatic C), 165.4 (C=O), 168.0 (C=O), 171.3 (C=O). Anal. Calcd. for $C_{20}H_{20}N_2O_5$ (368.38): C: 65.21; H: 5.47; N: 7.60. Found, C: 65.07: H: 5.86; N: 7.79.

4-[(4-(3,5-Dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepin-4(5*H*)-yl)butyl)amino] benzenesulfonamide **(6e)**

It was prepared from **4**, TBAB (1.00 g) and sulfanilamide (0.17 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.16 g, 38.40%) as an off-white solid; mp 72–74°C. IR (KBr) *υ* cm^{-1}: 3391–3350 (NH and NH_2), 3039 (CH aromatic), 2928, 2855 (CH aliphatic), 1705 (2C=O), 1644 (NH bending), 1597, 1516, 1454 (C=C), 1320, 1150 (SO_2). ^{1}H NMR (DMSO-*d* $_6$) *δ*: 1.51–1.54 (m, 2H, CH_2 butyl), 1.63–1.66 (m, 2H, CH_2 butyl), 3.07 (q, 2H, *J* = 6.52 Hz, *N*CH_2 butyl), 3.89 (t, 2H, *J* = 7.12 Hz, *N*CH_2 butyl), 4.89 (s, 2H, OCH_2), 6.10 (s, 1H, ArH) 6.58–6.64 (m, 3H, ArH and NH_2 exchanged with D_2O), 7.16 (d, 1H, *J* = 8.12 Hz, ArH), 7.29 (t, 1H, *J* = 7.60 Hz, ArH), 7.50 (d, 2H, *J* = 8.84 Hz, ArH), 7.61 (td, 1H, *J* = 1.64, 6.72, 8.36 Hz, ArH), 8.09 (dd, 1H, *J* = 1.56, 8.04 Hz, ArH), 10.94 (br s, 1H, NH exchanged with D_2O). ^{13}C NMR (DMSO-*d*

$_6$) δ: 25.7 (CH_2 butyl), 26.4 (CH_2 butyl), 42.4 (*N*CH_2 butyl), 43.7 (*N*CH_2 butyl), 74.5 (OCH_2), 95.7, 111.2, 120.2, 123.0, 124.4, 124.5, 129.2, 134.1, 135.6, 153.0, 158.4, 159.1, 165.5 (aromatic carbons), 170.3 (C=O), 171.3 (C=O). Anal. Calcd. for $C_{19}H_{21}N_3O_5S$ (403.45): C: 56.56; H: 5.25; N: 10.42. Found, C: 56.60: H: 4.90; N: 10.18.

4-[(4-(3,5-Dioxo-2,3-dihydrobenzo[*f*][1,4]oxazepin-4(5*H*)-yl)butyl)amino]-*N*-(pyrimidin-2-yl) benzenesulfonamide **(6f)**

It was prepared from **4**, TBAB (1.00 g) and sulfadiazine (0.25 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.16 g, 33.20%) as a white solid; mp 159–161°C. IR (KBr) υ cm^{-1}: 3379 (2NH), 3039 (CH aromatic), 2954, 2866 (CH aliphatic), 1713 (2C=O), 1643 (NH bending), 1597, 1577, 1481 (C=C), 1327, 1153 (SO_2). ^{1}H NMR (DMSO-*d* $_6$) δ: 1.50–1.56 (m, 2H, CH_2 butyl), 1.61–1.66 (m, 2H, CH_2 butyl), 3.06 (q, 2H, *J* = 6.60 Hz, *N*CH_2 butyl), 3.90 (t, 2H, *J* = 7.14 Hz, *N*CH_2 butyl), 4.89 (s, 2H, OCH_2), 6.54–6.58 (m, 3H, 2ArH and NH exchanged with D_2O), 7.00 (t, 1H, *J* = 4.86 Hz, ArH), 7.16 (dd, 1H, *J* = 0.84, 8.12 Hz, ArH), 7.27–7.31 (m, 1H, ArH), 7.58–7.62 (m, 1H, ArH), 7.66 (d, 2H, *J* = 8.92 Hz, ArH), 8.10 (dd, 1H, *J* = 1.62, 8.06 Hz, ArH), 8.48 (d, 1H, *J* = 4.84 Hz, ArH), 11.27 (br s, 1H, NH exchanged with D_2O). ^{13}C NMR (DMSO-*d* $_6$) δ: 25.7 (CH_2 butyl), 26.4 (CH_2 butyl), 42.5 (*N*CH_2 butyl), 43.8 (*N*CH_2 butyl), 74.5 (OCH_2 butyl), 110.8, 116.0, 120.2, 123.0, 124.5, 125.1, 130.2, 134.1, 135.6, 152.8, 157.7, 158.7, 159.1 (aromatic carbons), 165.4 (C=O), 171.2 (C=O). Anal. Calcd. for $C_{23}H_{23}N_5O_5S$ (481.52): C: 57.37; H: 4.81; N: 14.54. Found, C: 57.67: H: 5.15; N: 14.29.

4-(4-Chlorobutyl)-3-phenylbenzo[f][1,4]oxazepin-5(4H)-one ***(9)***

To a stirred solution of **8** (10.00 g, 42.00 mmol) and 1-bromo-4-chlorobutane (7.95 g, 46.00 mmol) in DMF (100 ml) was added 60% dispersion of sodium hydride in mineral oil (2.00 g, 50.00 mmol) at 0°C. After 1 h, the reaction mixture was poured onto an aqueous solution of citric acid at 0°C, and then the product was extracted with diethyl ether. The extract was washed with brine and dried over magnesium sulfate. The solvent was removed under vacuum to give a residue, which was chromatographed over silica gel using dichloromethane as an eluent to give white solid (12 g, 86.50%); mp 93–95°C. IR (KBr) υ cm^{-1}: 3063, 3028 (CH aromatic), 2943–2870 (Ch aliphatic), 1650 (C=O), 1574–1543 (C=C). ^{1}H NMR (C*D*Cl_3) δ: 1.58–1.65 (m, 2H, CH_2 butyl), 1.68–1.75 (m, 2H, CH_2 butyl), 3.41 (t, 2H, *J* = 6.38 Hz, -CH_2Cl butyl), 3.59 (br s, 1H, *N*CH_2 butyl), 6.60 (s, 1H, OCH=), 6.95 (d, 1H, *J* = 8.08 Hz, ArH), 7.14 (t, 1H, *J* = 7.56 Hz, ArH), 7.21–7.36 (m, 6H, ArH), 7.85 (dd, 1H, *J* = 1.32, 7.76 Hz, ArH). ^{13}C NMR (C*D*Cl_3) δ: 25.4 (CH_2 butyl), 29.3 (CH_2 butyl), 44.3 (ClCH_2 butyl), 44.6 (*N*CH_2 butyl), 119.5, 125.0, 129.9, 127.4, 128.9, 129.1, 132.4, 132.4, 133.2, 134.2, 140.7, 162.1 (aromatic C), 167.4 (C=O). MS (%): 350.22 (M^++Na^{23}), 292.14 (93), 147.22 (64). Anal. Calcd. for $C_{19}H_{18}ClNO_2$ (327.80): C: 69.62; H: 5.53; N: 4.27. Found, C: 69.91; H: 5.74; N: 4.39 (Figure 4).

3-Phenyl-4-[4-(piperidin-1-yl)butyl]benzo[f][1,4]oxazepin-5(4H)-one ***(10)***

It was prepared using the same procedure for synthesis of compound **5** using compound **9** (0.33 g, 1.00 mmol), potassium iodide (0.21 g, 1.20 mmol), piperidine (0.094 g, 1.10 mmol) and potassium carbonate (0.42 g, 3.00 mmol). Compound **10** was obtained as a pale yellow semisolid (0.23 g, 78.80%). IR (KBr) υ cm^{-1}: 3063, 3032 (CH aromatic), 2931, 2854, 2801 (CH aliphatic), 1655 (C=O), 1574, 1454 (C=C). ^{1}H NMR (C*D*Cl_3) δ: 1.44 (br s, 2H, CH_2 piperidine), 1.54–1.64 (m, 8H, $2CH_2$ piperidine and $2CH_2$ butyl), 2.33–2.41 (m, 6H, *N*$(CH_2)_2$ piperidine and *N*CH_2 butyl), 3.65 (br s, 2H, *N*CH_2 butyl), 6.68 (s, 1H, O-CH=), 7.05 (dd, 1H, *J* = 0.78, 8.01 Hz, ArH), 7.24 (td, 1H, *J* = 0.93, 7.50, 8.44 Hz, ArH), 7.30–7.33 (m, 2H, ArH), 7.34–7.39 (m, 3H, ArH), 7.43 (td, 1H, *J* = 1.70, 7.78, 10.82 Hz, ArH), 7.95 (dd, 1H, *J* = 1.64, 7.80 Hz, ArH). ^{13}C NMR (C*D*Cl_3) δ: 23.2 (CH_2 piperidinyl), 24.2 (CH_2 butyl), 25.5 (CH_2 piperidinyl), 26.1 (CH_2 butyl), 45.1 (*N*CH_2 butyl), 54.2 (*N*$(CH_2)_2$ piperidinyl), 58.6 (*N*CH_2 butyl), 119.5, 124.9, 127.0, 127.4, 128.8, 128.9, 132.3, 132.5, 133.1, 134.2, 140.5, 162.1 (aromatic C), 167.3 (C=O). MS (%): 377.26 (M^++1), 292.14 (22), 140.25 (100). Anal. Calcd. for $C_{24}H_{28}N_2O_2$ (376.49): C: 76.56; H: 7.50; N: 7.44. Found, C: 76.82; H: 5.61; N: 7.67 (Figure 4).

General procedure for synthesis of compounds ***11a–f***

These compounds were prepared by the same procedure used for synthesis of **6a–f** using compound **9** (0.33 g, 1.00 mmol) and the appropriate aromatic amine (1.00 mmol), and TBAB (0.50 g). They were purified by column chromatography over silica gel using dichloromethane: methanol (30:1) as an eluent to obtain **11a–f** in a pure form (Figure 4).

3-Phenyl-4-[4-(phenylamino)butyl]benzo[*f*][1,4]oxazepin-5(4*H*)-one **(11a)**

It was prepared from **9** and aniline (0.094 g, 1.00 mmol) to afford (0.18 g, 43.20%) as a pale yellow solid; mp 89–91°C. IR (KBr) υ cm^{-1}: 3372 (NH), 3055, 3024 (CH aromatic), 2924 (CH aliphatic), 1690 (C=O), 1631 (NH bending), 1605, 1516, 1496 (C=C). ^{1}H NMR (C*D*Cl$_3$) δ: 1.67–1.69 (m, 4H, 2CH$_2$ butyl), 3.10 (t, 2H, *J* = 6.42 Hz, *N*CH$_2$ butyl), 3.70 (br s, 2H, *N*CH$_2$ butyl), 5.32 (s, 1H, NH exchanged with D$_2$O), 6.64 (d, 1H, *J* = 7.76 Hz, ArH), 6.70 (s, 1H, OCH=), 6.73 (t, 1H, *J* = 7.34 Hz, ArH), 7.07 (dd, 1H, *J* = 0.80, 8.12 Hz, ArH), 7.16–7.20 (m, 2H, ArH), 7.26 (dd, 2H, *J* = 0.88, 7.52 Hz, ArH), 7.30–7.36 (m, 2H, ArH), 7.38–7.40 (m, 3H, ArH), 7.45 (td, 1H, *J* = 1.76, 7.80, 10.68 Hz, ArH), 7.97 (dd, 1H, *J* = 1.64, 7.76 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) δ: 25.6 (CH$_2$ butyl), 26.0 (CH$_2$ butyl), 43.9 (*N*CH$_2$ butyl), 45.0 (*N*CH$_2$ butyl), 113.3, 117.7, 119.5, 125.0, 127.0, 127.5, 128.9, 129.1, 129.2, 132.4, 132.5, 133.2, 134.3, 140.7, 147.7, 162.1 (aromatic C), 167.5 (C=O). MS (%): 385.44 (M^++1), 292.14 (37), 148.29 (100). Anal. Calcd. for $C_{25}H_{24}N_2O_2$ (384.47): C: 78.10; H: 6.29; N: 7.29. Found, C: 78.40; H: 6.24; N: 6.99.

4-[4-((4-Fluorophenyl) amino)butyl]-3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-one **(11b)**

It was prepared from **9** and *p*-fluoroaniline (0.11 g, 1.00 mmol) to afford (0.18 g, 43.20%) as a pale brown semisolid. IR (KBr) υ cm^{-1}: 3368 (NH), 3059, 3032 (CH aromatic), 2932, 2859 (CH aliphatic), 1670 (C=O), 1636 (NH bending), 1605, 1508, 1454 (C=C). ^{1}H NMR (C*D*Cl$_3$) δ: 1.56–1.57 (m, 4H, 2CH$_2$ butyl), 2.95 (t, 2H, *J* = 6.40 Hz, *N*CH$_2$ butyl), 3.59 (br s, 2H, *N*CH$_2$ butyl), 6.43–6.46 (m, 2H, ArH), 6.59 (s, 1H, OCH=), 6.77 (t, 2H, *J* = 8.74 Hz, ArH), 6.96 (dd, 1H, *J* = 0.70, 8.10 Hz, ArH), 7.14–7.24 (m, 3H, ArH), 7.27–7.30 (m, 2H, ArH), 7.34 (td, 1H, *J* = 1.53, 7.73, 10.68 Hz, ArH), 7.86 (dd, 1H, *J* = 1.66, 7.78 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) δ: 25.6 (CH$_2$ butyl), 25.9 (CH$_2$ butyl), 44.5 (*N*CH$_2$ butyl), 44.9 (*N*CH$_2$ butyl), 114.0, 114.11, 115.5, 115.7, 119.5, 125.0, 127.0, 127.4, 128.9, 129.1, 132.4, 132.4, 133.2, 134.3, 140.6, 144.1, 154.8, 157.2, 162.1 (aromatic C), 167.5 (C=O). Anal. Calcd. for $C_{25}H_{23}FN_2O_2$ (402.46): C: 74.61; H: 5.76; N: 6.96. Found, C: 74.90; H: 5.40; N: 7.18.

4-[4-((4-Methoxyphenyl) amino)butyl]-3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-one **(11c)**

It was prepared from **9** and *p*-anisidine (0.12 g, 1.00 mmol) to afford (0.19 g, 45.80%) as a brown semisolid. IR (KBr) υ cm^{-1}: 3368 (NH), 3059, 3032 (CH aromatic), 2936, 2862 (CH aliphatic), 1680 (C=O), 1636 (NH bending), 1605, 1508, 1454 (C=C). ^{1}H NMR (C*D*Cl$_3$) δ: 1.56–1.57 (m, 4H, 2CH$_2$ butyl), 2.95 (t, 2H, *J* = 6.50 Hz, *N*CH$_2$ butyl), 3.58 (br s, 2H, *N*CH$_2$ butyl), 3.65 (s, 3H, OCH$_3$), 5.21 (s, 1H, NH exchanged with D$_2$O), 6.50 (d, 2H, *J* = 8.88 Hz, ArH), 6.59 (s, 1H, OCH=), 6.67 (d, 2H, *J* = 8.88 Hz, ArH), 6.96 (dd, 1H, *J* = 0.52, 8.08 Hz, ArH), 7.13–7.22 (m, 3H, ArH), 7.27–7.28 (m, 3H, ArH), 7.34 (td, 1H, *J* = 1.46, 7.61, 10.64 Hz, ArH), 7.86 (dd, 1H, *J* = 1.66, 7.78 Hz, ArH). ^{13}C NMR (C*D*Cl$_3$) δ: 25.6 (CH$_2$ butyl), 26.0 (CH$_2$ butyl), 45.0 (*N*CH$_2$ butyl), 53.5 (*N*CH$_2$ butyl), 55.8 (OCH$_3$), 114.8, 114.9, 119.5, 125.0, 127.0, 127.5, 129.0, 129.1, 132.4, 132.5, 133.2, 134.3, 140.6, 141.7, 152.5, 162.1 (aromatic C), 167.4 (C=O). Anal. Calcd. for $C_{26}H_{26}N_2O_3$ (414.50): C: 75.34; H: 6.32; N: 6.76. Found, C: 75.18; H: 6.65; N: 6.70.

4-[(4-(5-Oxo-3-phenylbenzo[*f*][1,4]oxazepin-4(5*H*)-yl)butyl)amino] benzoic acid **(11d)**

It was prepared from **9** and *p*-aminobenzoic acid (0.14 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.18 g, 40.80%) as a white solid; mp 170–172°C. IR (KBr) υ cm^{-1}: 3345 (NH), 2650–2540 (OH carboxylic), 3063 (CH aromatic), 2940 (CH aliphatic), 1663 (C=O), 1624 (NH bending), 1601, 1535, 1454 (C=C). ^{1}H NMR (DMSO-*d*$_6$) δ: 1.53 (s, 4H, 2CH$_2$ butyl), 3.00 (d, 2H, *J* = 5.20 Hz, *N*CH$_2$ butyl), 3.58 (br s, 2H, *N*CH$_2$ butyl), 6.39 (t, 1H, *J* = 5.22 Hz, NH exchanged with D$_2$O), 6.50 (d, 2H, *J* = 8.80 Hz, ArH), 6.95 (s, 1H, OCH=), 7.14 (dd, 1H, *J* = 0.64, 8.12 Hz, ArH), 7.29–7.36 (m, 3H, ArH), 7.38–7.43 (m, 3H, ArH), 7.54 (td, 1H, *J* = 1.49, 7.62, 10.32 Hz, ArH), 7.63 (d, 2H, *J* = 8.76 Hz, ArH), 7.83 (dd, 1H, *J* = 1.60, 7.76 Hz, ArH), 11.96 (s, 1H, OH exchanged with D$_2$O). ^{13}C NMR (C*D*Cl$_3$) δ: 25.5 (CH$_2$ butyl), 25.8 (CH$_2$ butyl), 42.8 (*N*CH$_2$ butyl), 44.8 (*N*CH$_2$ butyl), 111.4, 117.1, 119.6, 125.1, 127.4, 129.0, 129.1, 132.29, 132.33, 132.4, 133.3, 134.2, 140.8, 152.6, 162.1 (aromatic C), 167.6 (C=O), 172.2 (C=O). MS (%): 451.41 (M^++Na^{23}), 429.40 (M^++1), 292.21 (41), 192.12 (78). Anal. Calcd. for $C_{26}H_{24}N_2O_4$ (428.48): C: 72.88; H: 5.65; N: 6.54. Found, C: 73.10; H: 5.65; N: 6.60.

4-[(4-(5-Oxo-3-phenylbenzo[*f*][1,4]oxazepin-4(5*H*)-yl) butyl)amino] benzenesulfonamide **(11e)**

It was prepared from **9**, TBAB (1.0 g) and sulfanilamide (0.17 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.19 g, 41.70%) as a white solid; mp 95–97°C. IR (KBr) υ cm^{-1}: 3383–3350 (NH

and NH_2), 3071 (CH aromatic), 2928, 2859 (CH aliphatic), 1660 (C=O), 1633 (NH bending), 1597, 1516, 1454 (C=C), 1340, 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ: 1.51 (br s, 4H, $2CH_2$ butyl), 2.27 (s, 2H, NCH_2 butyl), 2.98 (d, 2H, J = 5.24 Hz, NCH_2 butyl), 3.56 (br s, 2H, NH_2 exchanged with D_2O), 5.75 (s, 1H, ArH), 6.09 (s, 1H, ArH), 6.54–6.61 (m, 3H, ArH), 6.92 (s, 1H, OCH=), 7.14 (d, 1H, J = 8.16 Hz, ArH), 7.29–7.33 (m, 3H, ArH), 7.37–7.40 (m, 3H, ArH), 7.49 (d, 2H, J = 8.84 Hz, ArH), 7.54 (td, 1H, J = 1.48, 7.62, 10.65 Hz, ArH), 7.82 (dd, 1H, J = 1.60, 7.76 Hz, ArH), 10.93 (s, 1H, NH exchange with D_2O). ^{13}C NMR (DMSO-d_6) δ: 25.5 (CH_2 butyl), 26.0 (CH_2 butyl), 42.2 (NCH_2 butyl), 44.4 (NCH_2 butyl), 95.7, 111.2, 113.1, 120.0, 124.4, 125.6, 127.1, 127.7, 129.1, 129.3, 129.4, 132.4, 132.6, 133.9, 134.0, 141.5, 153.0, 158.4, 161.9, 166.7 (aromatic C), 170.3 (C=O). Anal. Calcd. for $C_{25}H_{25}N_3O_4S$ (463.55): C: 64.78; H: 5.44; N: 9.06. Found, C: 64.70; H: 5.24; N: 9.08.

4-[(4-(5-Oxo-3-phenylbenzo[*f*][1,4]oxazepin-4(5*H*)-yl) butyl)amino]-*N*-(pyrimidin-2-yl) benzenesulfonamide **(11f)**

It was prepared from **9**, TBAB (1.00 g) and sulfadiazine (0.25 g, 1.00 mmol) using water instead of sodium bicarbonate solution to afford (0.19 g, 35.12%) as a white solid; mp 102–104°C. IR (KBr) υ cm^{-1}: 3398 (2NH), 3051, 3020 (CH aromatic), 2927 (CH aliphatic), 1701 (2C=O), 1643 (NH bending), 1604, 1508, 1481 (C=C), 1345, 1154 (SO_2). ^{1}H NMR (DMSO-d_6) δ: 1.43 (br s, 4H, $2CH_2$ butyl), 2.92 (s, 2H, NCH_2 butyl), 3.50 (br s, 2H, NCH_2 butyl), 6.01 (br s, 1H, NH exchanged with D_2O), 6.53 (d, 2H, ArH), 6.58 (d, 1H, ArH), 6.90 (s, 1H, OCH=), 6.94–7.01 (m, 1H, ArH), 7.12–7.15 (m, 1H, ArH), 7.28–7.42 (m, 6H, ArH), 7.53 (td, 1H, J = 1.68, 7.82, 11.52 Hz, ArH), 7.62–7.66 (m, 2H, ArH), 7.82 (dd, 1H, J = 1.50, 7.74 Hz, ArH), 8.47 (t, 2H, J = 4.56 Hz, ArH), 11.52 (s, 1H, NH exchange with D_2O). ^{13}C NMR (DMSO-d_6) δ: 25.5 (CH_2 butyl), 25.6 (CH_2 butyl), 42.2 (NCH_2 butyl), 44.4 (NCH_2 butyl), 110.8, 112.6, 116.0, 120.0, 125.3, 127.2, 127.7, 129.4, 130.1, 130.3, 132.3, 132.6, 134.0, 141.5, 152.8, 153.5, 157.7, 158.7, 161.9 (aromatic C), 166.8 (C=O). Anal. Calcd. for $C_{29}H_{27}N_5O_4S$ (541.62): C: 64.31; H: 5.02; N: 12.93. Found, C: 64.06; H: 5.23; N: 13.24.

Biological activity

In vitro 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity, cell cycle analysis, caspase-3 activation assay, Bax and Bcl2 enzymes assay were performed at VACSERA, Dokki, Cairo, Egypt.

In vitro MTT cytotoxic activity screening

All the final compounds were screened for their cytotoxicity against leukemia K-562 and breast T-47D cancer cell lines as well as normal fibroblasts WI-38 using MTT assay according to the reported procedures [20–22]. Doxorubicin was used as a positive control.

The results were presented in Table 1.

Cell cycle analysis

Compounds **6f**, **10** and **11e, f** were chosen to study their effect on the cell cycle of leukemia K-562 and breast T-47D cell lines, respectively, at their IC_{50} values using standard procedure of flow cytometry [23]. The results were presented in Figure 5 and Table 2.

Apoptosis assay

The Annexin V-fluorescein isothiocyanate (V-FITC)/propidium iodide dual-staining assay was carried out using K-562 and T-47D cancer cells, to quantify the percentage of apoptotic cells according to the reported procedure [24]. The results were exhibited in Figure 6 and Table 3.

Caspase-3 activation assay

The caspase-3 activity in K-562 and T-47D cell lines was detected in the presence of compounds **6f**, **10** and **11e, f**, respectively, at their IC_{50} concentration using Invitrogen ELISA kit Human caspase-3 according to the reported method (Table 4) [25].

Bax & Bcl-2 enzymes assay

These assays were performed using Human Bax ELISA and Invitrogen Zymed® Bcl-2 ELISA kits according to the previously reported procedure (Table 4) [26]. K-562 and T-47D cells were obtained from American Type Culture Collection.

Table 1. Cytotoxic screening results of the tested compounds.

Compound ID	IC_{50} (μM) ± SE† against			Selectivity index relative to K-562	Selectivity index relative to T-47D
	Leukemia K-562	Breast T-47D	Normal fibroblast WI-38		
5	10.85 ± 0.73	5.00 ± 0.18	132.25 ± 5.25	12.19	26.45
6a	1.10 ± 0.05	0.92 ± 0.06	55.14 ± 2.17	50.13	59.93
6b	1.09 ± 0.05	10.40 ± 0.51	23.70 ± 1.24	21.74	2.28
6c	0.68 ± 0.01	1.69 ± 0.08	64.60 ± 2.25	95.00	38.22
6d	8.06 ± 0.21	5.73 ± 0.23	38.96 ± 2.12	4.83	6.80
6e	0.78 ± 0.03	0.84 ± 0.05	55.56 ± 3.19	71.23	66.14
6f	0.15 ± 0.004	1.08 ± 0.02	52.63 ± 2.11	350.87	48.73
10	0.22 ± 0.006	1.34 ± 0.08	24.43 ± 1.34	111.05	18.23
11a	1.23 ± 0.08	5.96 ± 0.31	23.70 ± 1.19	19.27	3.98
11b	0.82 ± 0.04	52.73 ± 4.21	26.33 ± 1.48	32.11	0.50
11c	1.37 ± 0.06	0.67 ± 0.01	35.80 ± 1.61	26.13	53.43
11d	0.58 ± 0.01	0.82 ± 0.01	42.27 ± 1.84	72.88	51.55
11e	0.54 ± 0.01	0.076 ± 0.003	25.08 ± 1.12	46.44	330.00
11f	0.70 ± 0.03	0.085 ± 0.002	24.37 ± 1.16	34.81	286.71

†Values are the mean of three independent replicates ± SE.
SE: Standard error.

Table 2. Cell cycle analysis for compounds 6f, 10 and 11e, f.

Sample ID	G0–G1 (%)	S (%)	G2–M (%)	Apoptosis (%)
Control K-562	62.49	27.95	9.56	1.27
6f (K-562)	44.24	17.45	38.31	15.72
10 (K-562)	48.28	12.80	24.30	14.62
Control T-47D	62.79	28.49	8.72	1.57
11e (T-47D)	36.41	17.50	46.09	17.49
11f (T-47D)	35.28	16.31	48.41	18.47

Table 3. Percentage of viable, necrotic and apoptotic cells in the K-562 and T-47D cell lines after 48 h of induction.

Sample ID	Viable (%)	Early apoptosis (%)	Late apoptosis (%)	Necrosis (%)
Control K-562	96.47	0.42	0.51	0.34
6f (K-562)	82.47	5.84	9.38	2.23
10 (K-562)	82.11	6.23	6.52	1.87
Control T-47D	96.4	0.69	0.34	0.54
11e (T-47D)	80.74	7.23	8.11	2.15
11f (T-47D)	79.37	7.21	8.06	3.20

Table 4. Effect of compounds 6f, 10 and 11e, f on caspase-3, Bax and Bcl-2.

Sample ID	Caspase-3 assay		Bax assay		Bcl-2 assay	
	OD	% fold change of control	OD	% fold change of control	OD	% fold change of control
Control K-562	0.11 ± 0.002	100	0.088 ± 0.003	100	0.477 ± 0.02	100
6f (K-562)	0.46 ± 0.01	418	0.446 ± 0.01	506	0.149 ± 0.005	31
10 (K-562)	0.841 ± 0.02	718	0.475 ± 0.005	540	0.112 ± 0.004	23
Control T-47D	0.073 ± 0.003	100	0.072 ± 0.004	100	0.42 ± 0.03	100
11e (T-47D)	0.598 ± 0.02	819	0.514 ± 0.01	714	0.135 ± 0.01	32
11f (T-47D)	0.565 ± 0.03	773	0.457 ± 0.007	635	0.105 ± 0.007	25

OD: Optical density.

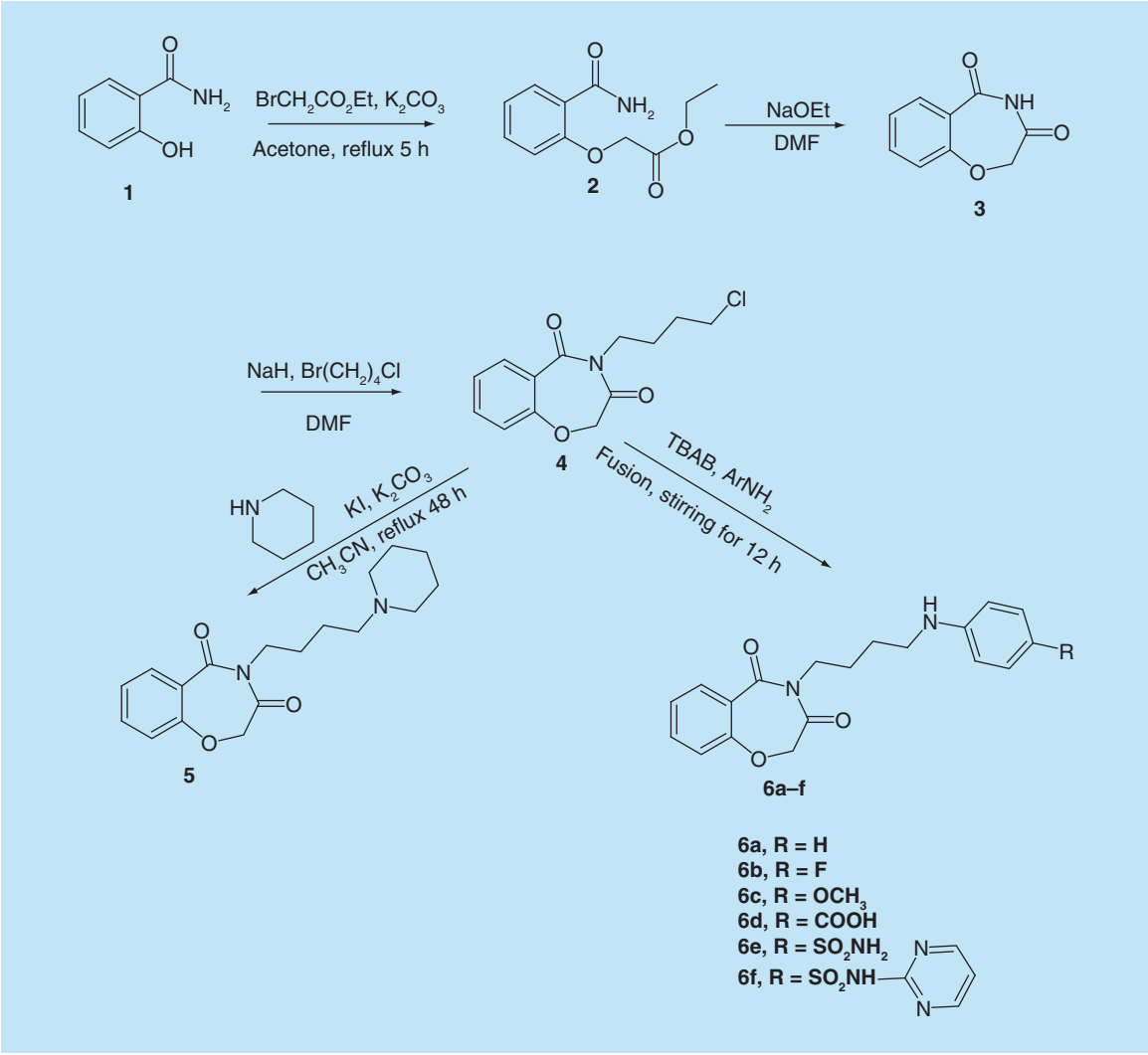

Figure 3. Synthetic pathways for the target compounds 5 and 6a–f.

Results & discussion

Chemistry

Figures 3 and 4 illustrated the adopted synthetic pathways for the target compounds. The first key intermediate, 1,4-benzoxazapine-3,5-dione **3**, was synthesized as reported by *O*-alkylation of salicylamide **1** with ethyl 2-bromoacetate in the presence of potassium carbonate to give **2** that was cyclized with sodium ethoxide to give **3** in an excellent yield (Figure 3) [8]. On the other hand, the second key intermediate **8** was synthesized by *O*-alkylation of salicylamide **1** with phenacyl bromide in the presence of potassium carbonate to yield **7**, which was cyclized with *p*-toluenesulfonic acid monohydrate using Dean stark apparatus to remove all water, which was produced as a side product from the reaction (Figure 4) [19]. Furthermore, compounds **4** or **9** were obtained in a good yield by *N*-alkylation of **3** or **8** with 1-bromo-4-chlorobutane in DMF in the presence of sodium hydride. ^{1}H NMR spectrum of compound **9** revealed the disappearance of NH signal and appearance of aliphatic signals at 1.83–4.04 and 1.58–3.59 ppm, respectively, corresponding to butyl chain in addition to a singlet at 6.60 ppm attributed to olefinic proton (OCH=).

The reaction of the chloro derivatives **4** and **9** with piperidine in dry acetonitrile in the presence of potassium carbonate and a catalytic amount of potassium iodide to promote the reaction afforded the target compounds **5** and **10**, respectively. The structure of the obtained compounds **5** and **10** were confirmed by spectral and elemental data. Their ^{1}H NMR revealed multiplet signals at 1.74–2.07 and 1.54–2.41 ppm corresponding to piperidine moiety, respectively. ^{13}C NMR of compounds **5** and **10** showed additional aliphatic signals at 20.9–23.2, 22.4–25.5 and

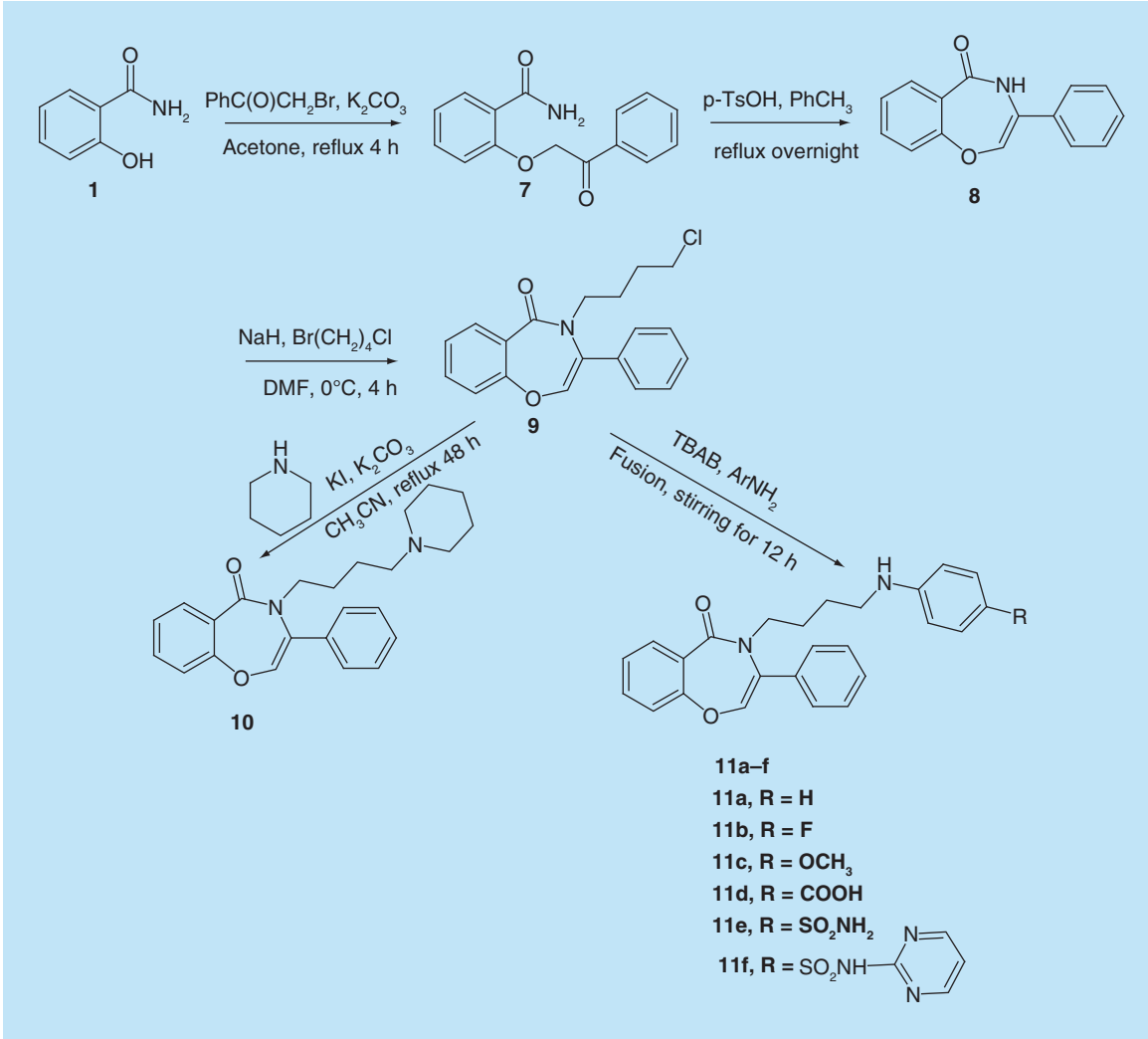

Figure 4. Synthetic pathways for the target compounds 10 and 11a–f.

53.2–54.2 ppm attributed to piperidinyl carbons. Also, their LC MS–MS spectra showed the exact mass (M^++1) at 317.21 and 377.26, respectively.

Moreover, the reaction of **4** and **9** with different aromatic amines in the presence of TBAB as a transfer catalyst resulted in compounds **6a–f** and **11a–f**, respectively. Their structures were confirmed by spectral and elemental data. IR spectra of **6a–f** and **11a–f** showed NH band at 3345–3406 cm^{-1}. ^{1}H NMR of compounds **6a–f** revealed the presence of an exchangeable NH proton and additional aromatic signals at 5.05, 6.58–6.64, 7.24–7.29 ppm, respectively. ^{13}C NMR of **6a–f** confirmed the carbon skeleton due to presence of signals at 25.5–25.8, 26.4–26.6, 42.4–44.1 and 43.7–45.2 ppm corresponding to CH_2, CH_2, NCH_2 and NCH_2 of the butyl linker in addition to signals at 74.5–74.6, 165.3–170.3, 171.0–171.3 ppm corresponding to OCH_2, two C=O, respectively. Furthermore, ^{13}C NMR of **6d** revealed an additional signal at 168.0 ppm due to C=O group of the carboxylic moiety.

On the other hand, ^{1}H NMR spectra of compounds **11a–f** showed additional signals at aromatic region due to the presence of phenyl group at position 3. ^{13}C NMR of compounds **11a–f** showed only one C=O signals at 167.4–170.3 ppm, in addition to aromatic signals of the phenyl group at position 3. Additionally, ^{13}C NMR spectra of compounds **11c** and **11d** showed a signal at 55.8 and 172.2 ppm due to OCH_3 and C=O of the carboxylic acid moiety, respectively.

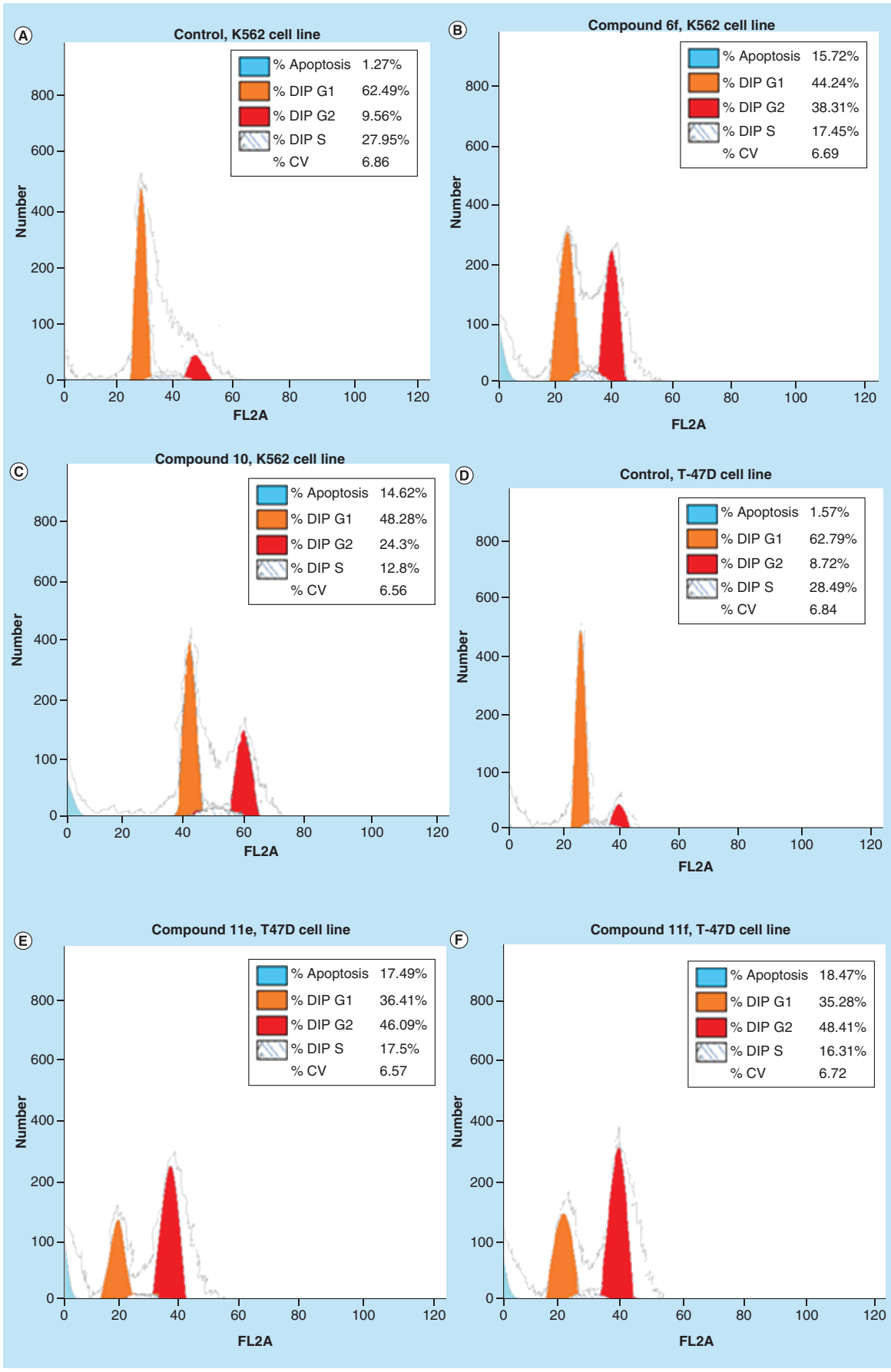

Figure 5. Effect of compounds 6f, 10 and 11e, f on cell cycle progression of K-562 and T-47D cell lines, respectively.

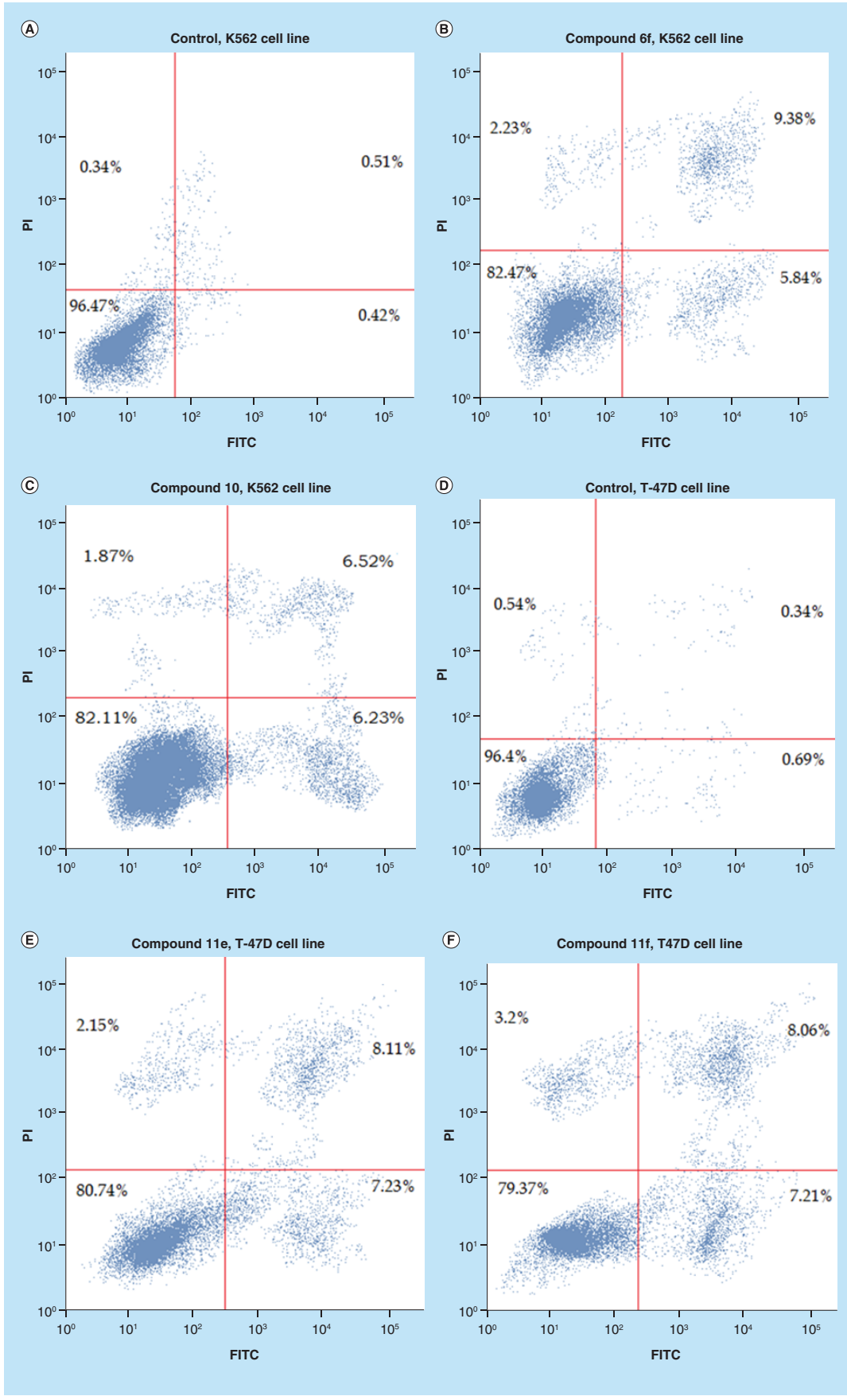

Figure 6. Effect of compounds 6f, 10 and 11e, f on induction of apoptosis in K-562 and T-47D cell lines, respectively.
FITC: Fluorescein isothiocyanate.

Biological activity

In vitro MTT cytotoxic activity screening

All the final compounds **5**, **6a–f**, **10** and **11a–f** were screened for their cytotoxic activity against leukemia K-562 and breast T-47D cancer cell lines as well as normal fibroblast WI-38 [20–22]. The obtained results are summarized in Table 1.

From the obtained results, it was found that the tested compounds showed considerable cytotoxic activity against leukemia K-562 and breast T-47D cell lines with IC_{50} values 0.15–10.85 and 0.076–52.73 μM, respectively. Doxorubicin was used as a positive control and revealed IC_{50} = 0.36 and 0.19 μM, respectively. Additionally, these compounds revealed higher IC_{50} values 23.70–132.25 μM against normal fibroblast WI-38; thus, they can be considered to be selective cytotoxic agents against cancer cell lines and safe toward the normal cells except compound **11b** with small selectivity index relative to T-47D cell line.

Regarding the activity against leukemia K-562 cell line, it can be observed that compound **5** revealed moderate activity (IC_{50} = 10.85 μM) and it was the least active compound compared with either its phenyl congener **10** or substituted aniline derivatives **6a–f**. The phenylamino butyl derivative **6a** was equipotent to the *p*-fluoro congener **6b**. On the other hand, the presence of an electron-donating OCH_3 moiety in compound **6c** resulted in a higher activity than the carboxylic acid derivative **6d**. Furthermore, the pyrimidinyl benzenesulfonamide derivative **6f** exerted higher activity than the benzene sulfonamide analog **6e**. In case of 3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-ones, the piperidinylbutyl derivative **10** showed higher activity than the substituted aniline derivatives **11a–f**. Moreover, the presence of an electron-withdrawing group enhances the cytotoxicity of compounds **11b**, **11d–f** compared with unsubstituted **11a** or the *p*-methoxyphenylamino derivative **11c**.

Concerning the cytotoxicity against the breast T-47D cell line, compound **5** revealed lower activity than its 3-phenyl analog **10**. Additionally, the presence of an electron-withdrawing group in compounds **6b** and **6d** decreased the activity compared with the unsubstituted **6a** or *p*-methoxyphenyl **6c**. On the other hand, the sulfonamide derivatives **6e, f** revealed promising activity despite of its electron-withdrawing power, thus, their activity may be attributed to its tendency to form hydrogen bonding with the target receptor and its hydrophilic character. Moreover, the 3-phenylbenzoxazepinone **11a–f** showed best activity in case of sulfonamide derivatives **11e, f**, followed by *p*-methoxyphenyl **11c**, the benzoic acid derivative **11d** and the unsubstituted one **11a** and the least activity by *p*-fluorophenyl derivative **11b**. Thus, the electronic property of the substituent does not have an absolute effect on the activity.

Cell cycle analysis

The most active compounds against leukemia K-562 and breast T-47D were selected to investigate whether their cytotoxicity involved changes in the cell cycle. Thus, leukemia K-562 and breast T-47D cells were treated with IC_{50} of compounds **6f**, **10** and **11e, f**, respectively, for 48 h, then, the cells were analyzed by flow cytometry and the results were presented in Figure 5 and Table 2. From the results, these compounds increased the accumulation of both cell types at G2/M with significant decrease in the S Phase and G0/G1 in comparison to control. Thus, they resulted in PreG1 apoptosis and complete cell growth arrest at G2/M.

Apoptosis assay

Annexin V-FITC/propidium iodide dual-staining assay was carried out to detect the effect of compounds **6f**, **10** and **11e**, **f** on early and late apoptosis percentages in leukemia K-562 and breast T-47D cancer cell lines, respectively. From the obtained results (Table 3 & Figure 6), it was observed that all compounds increased the apoptosis percentage at early and late stages in both cell lines compared with the control. Therefore, they can be considered as apoptotic inducers in both cell lines.

Caspase-3 activation assay

Compounds **6f**, **10** and **11e, f** were screened for their ability to activate caspase-3 in order to investigate the molecular pathway involved during apoptosis. Leukemia K-562 and breast T-47D cancer cell lines were treated with compounds **6f**, **10** and **11e, f**, respectively, at their IC_{50} values in μM and caspase-3 activation was tested using Invitrogen ELISA human caspase-3 kit. From the results presented in Table 4, compounds **6f** and **10** increased the concentration of caspase-3 as observed from the optical density (OD) values by four and seven folds in K-562, respectively. Similarly, compounds **11e, f** resulted in caspase-3 activation by eight and seven folds in

T-47D compared with control. Therefore, compounds **6f**, **10** and **11e, f** may induce apoptosis through activation of caspase-3.

Bax & Bcl2 enzyme assay

To ensure that compounds **6f**, **10** and **11e, f** induce apoptosis, measuring the level of expression of apoptotic protein Bax and antiapoptotic Bcl2 was performed using Human Bax ELISA and Invitrogen Zymed® Bcl-2 ELISA kits, respectively. From the obtained results (Table 4), it was observed that compounds **6f** and **10** increased the expression of Bax in K-562 cell line by fivefold, additionally, compounds **11e** and **11f** increased Bax concentration in T-47D by seven and six folds compared with the control, respectively. On the other hand, these compounds resulted in downregulation of Bcl2 in both cell lines. Therefore, it can be concluded that compounds **6f, 10** and **11e, f** induce the intrinsic pathway of apoptosis through activation of Bax and downregulation of Bcl2.

Conclusion

Fourteen 1,4-benzoxazepines were synthesized and screened for their cytotoxicity against leukemia K-562 and breast T-47D as well as normal fibroblast WI-38. The tested compounds revealed good activity and selectivity toward the tested cancer cell lines, especially compounds **6f** and **10** with IC_{50} values 0.15 and 0.22 μM, respectively, against K-562 cell line, in addition to compounds **11e, f** with IC_{50} values 0.076, 0.085 μM, respectively against T-47D cells. Moreover, these compounds were found to be apoptotic inducers via activation of caspase-3 and Bax and downregulation of Bcl2. Therefore, benzo[*f*][1,4]oxazepine-*3,5(2H,4H)-dione* and 3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-one can be considered as scaffolds for structural optimization to obtain more potent cytotoxic agents with apoptotic induction property.

Future perspective

Several trends have been emerged to fight cancer through targeting special molecular pathways to avoid resistance and undesirable side effects ensuring better anticancer activity profile. Benzo[*f*][1,4]oxazepin-5(4*H*)-one represents a promising scaffold for building new derivatives with potential cytotoxic activity in addition to disturbing the cell cycle through induction of apoptosis and to get more detailed investigation, several derivatives should be designed to target many biological cascades involved in the incidence of cancer.

Summary points

Chemistry

- Benzo[*f*][1,4]oxazepine-3,5(2*H*,4*H*)-dione and 3-phenylbenzo[*f*][1,4]oxazepin-5(4*H*)-one cores were hybridized with piperidine or different aromatic amines through four carbon spacer to obtain compounds **5**, **10** or **6a–f** and **11a–f**, respectively.

Biological activity

- All compounds were screened for their cytotoxicity against leukemia K-562 and breast T-47D cancer cell lines as well as normal fibroblast WI-38.
- The tested compounds revealed good cytotoxicity and selectivity toward cancer cell lines relative to normal cells, especially compounds **6f**, **10** and **11e, f**.
- These compounds also induced apoptosis through activation of caspase-3, Bax and downregulation of Bcl2.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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