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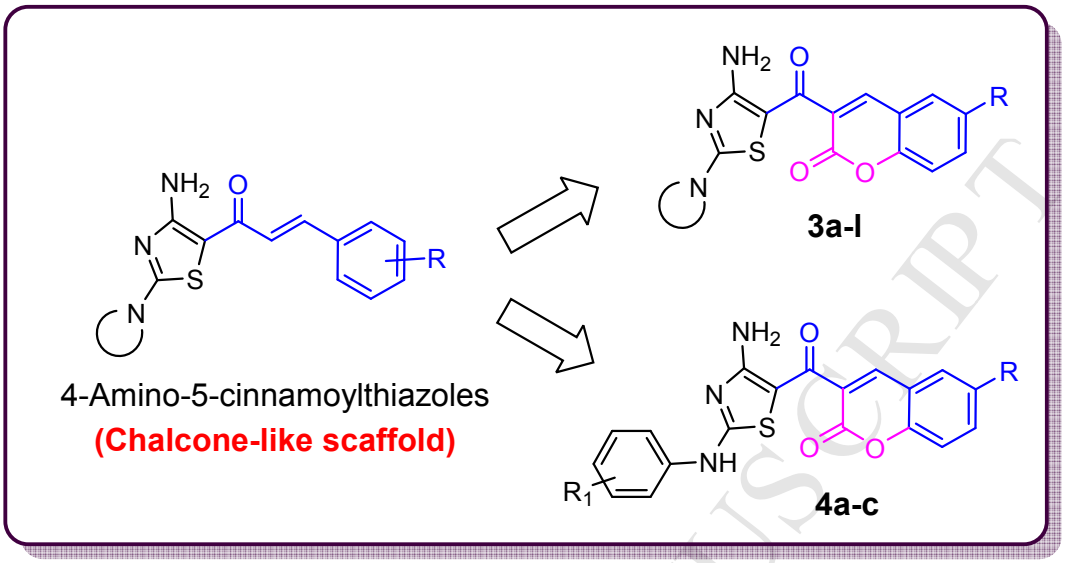
Graphical Abstract



Synthesis and biological evaluation of new coumarins bearing 2,4-diaminothiazole-5-carbonyl moiety

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Abstract: A series of new coumarin-containing compounds **3a-l** and **4a-c** was designed and synthesized based on the chalcone-type 4-amino-5-cinnamoylthiazole scaffold **2**, and screened for their *in vitro* anticancer and antioxidant activities. Representatively, the 2-thiomorpholinothiazole derivative **3k** with IC_{50} values of 7.5-16.9 µg/ml demonstrated good cytotoxic effects against tested cell lines MCF-7, HepG2 and SW480. Further investigation by flow cytometric analysis confirmed that this compound induces apoptotic cell death in MCF-7 cells and cause G1-phase arrest in the cell cycle. Moreover, most of compounds had intrinsic potential for radical scavenging activity and ferric-reducing power as investigated by DPPH and FRAP assays.

Keywords: Anticancer; Antioxidant; coumarin; cytotoxic activity; thiazole

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1. Introduction

Nowadays cancer is recognized as one of the major threats against human health, which in many cases leads to the death of patients [1]. Cancer is a class of diseases resulted from uncontrolled growth and division of cells due to the dysregulation of essential enzymes and proteins in cell division and proliferation [2]. Since chemotherapy is still considered as a main treatment approaches among the others, many attempts have been devoted to find potent anticancer agents [3]. Despite prominent progress in the field of anticancer agents and their cellular and molecular aspects, still many patients suffer from resistance to currently available anticancer drugs and diverse adverse effects [4].

Coumarin (2*H*-chromen-2-one or 2*H*-1-benzopyran-2-one) is a fused heterocyclic system, belongs to flavonoid group of plant secondary metabolite. To date, many coumarin bearing compounds have been reported in the literature with significant anticancer activities, working through different mechanisms. The underlying mechanisms of these compounds appear to depend on the substitution pattern of the coumarin core structure [5,6]. Moreover, hybridization of coumarin with varied pharmacophores led to the diverse pharmacologically active derivatives exhibiting antioxidant, antituberculosis, antinflammatory, anticholinesterase, antiviral, antihyperlipidemic, antimicrobial and antidepressant activities [7].

In the field of medicinal chemistry, 1,3-thiazole is an important five-membered heterocyclic nucleus, found in many biologically active natural and synthetic compounds [8]. Among the thiazole derivatives, 2-aminothiazole is the most widely used pharmacophore for drug design and discovery [9]. Dasatinib is a significant example of 2-aminothiazole containing drugs with anticancer activity [10]. The affinity of 2-aminothiazole derivatives to different targets led to focusing on this class of heterocyclic molecules in the design of new anticancer agents [11-14]. As an example, 2-pyrrolidinyl-4-amino-5-aroyl thiazole **1** (Fig.1) showed substantial antiproliferative activity against broad spectrum of tumor cell lines, with potent antimitotic effects at submicromolar concentrations [15].

Due to importance of chalcone type compounds in the field of anticancer drug discovery [16-22], we have recently described 4-amino-5-cinnamoylthiazoles **2** as chalcone-like anticancer

agents [23]. This series of heterocycle-based chalcones were designed by insertion of a double bound in the benzoylthiazole lead structure **1** (Fig. 1). According to these findings and in continuation of our studies on the synthesis of chromene-based anticancer agents [24-30], herein, we describe coumarin-containing hybrid compounds namely 3-(4-amino-2-(cyclic amino-1-yl)thiazole-5-carbonyl)-2*H*-chromen-2-ones **3** and 3-(4-amino-2-(arylamino) thiazole-5-carbonyl)-2*H*-chromen-2-ones **4** as new anticancer agents. The target compounds **3** and **4** can be considered as confromationally constrained analogs of 4-amino-5-cinnamoylthiazoles **2** (Fig. 1). The effect of different amino groups (arylamino or cyclic amino) at C-2 of thiazole core was investigated for structure-activity relationship analysis.

2. Chemistry

The final compounds **3a-l** and **4a-c** were prepared through synthetic pathways shown in Scheme 1. The commercially available 3-acetylcoumarins **5** were brominated using Br_2 in $CHCl_3$ to give bromoacetyl derivatives **6**. In order to prepare compounds **3a-l**, dimethyl *N*-cyanodithioimidocarbonate (**7**) was treated with appropriate cyclic amine and sodium sulfide to produce intermediates **8**, which subsequently reacted with 3-(bromoacetyl)coumarins **6** in *N*,*N*-dimethyl formamide (DMF). After TLC monitoring and upon consumption of the bromoacetyl derivatives **6**, the precipitated solid was filtered and purified by column chromatography to obtain final compounds **3a-l**. On the other hand, the reaction between phenylisothiocyanates **9** and cyanamide in the presence of sodium methoxide afforded intermediate **10**. The resulting intermediate **10** was then treated with 3-(bromoacetyl)coumarin **6a** to give 3-(4-amino-2-(arylamino)thiazole-5-carbonyl)-2*H*-chromen-2-ones (**4a-c**).

3. Results and discussion

3.1. Antiproliferative activity

The antiproliferative activity of compounds **3a-l** and **4a-c** was investigated against breast carcinoma (MCF-7), human colon adenocarcinoma (SW480) and human liver cancer (HepG2) cell lines using MTT assay. The results are presented as IC_{50} values in comparison with etoposide (Table 1).

As seen in Table 1, all the target compounds have moderate to good activities against all the tested cancer cells, displaying IC_{50} values in the range of 7.5-48.7 µg/ml. Most of compounds showed lower IC_{50} values against MCF-7 compared to other cell lines. In particular, morpholine and thiomorpholine derivatives (**3i** and **3k**) with IC_{50} values of 9.8 and 7.5 µg/ml found to be the most potent compounds against MCF-7 cells. Besides these derivatives, compounds **3a-c**, **3e**, **3f**, **3j** and **3l** showed substantial cytotoxic activity against MCF-7 cell line (IC_{50}s <15 µg/ml). In the case of HepG2, compound **3h** followed by **3k** had superior activity. Compound **3k** with IC_{50} value of 13.0 µg/ml was also the best one against human colon adenocarcinoma cells (SW480).

Based on different amino groups attached to the C-2 of thiazole nucleus, the synthesized compounds could be categorized into 2-(cyclic amino) and 2-anilino series. The structure-activity relationship (SAR) studies revealed that 2-(cyclic amino) derivatives **3** exhibited better cytotoxic effects rather than 2-arylamino analogs **4**. The comparison of unsubstituted-piperidine derivative **3c** with 4-benzylpiperidine analog **3e** revealed that the benzyl moiety can be tolerated or even can improve antiproliferative activity. The six-membered cyclic amine analogs **3c**, **3i**, and **3k** were more potent than five-membered cyclic amine congener **3a** against MCF-7 and HepG2 cells. The effect of bromo- substituent on the coumarin ring depended on the type of cyclic amine attached to the thiazole core and the type of cell line tested. For example, while the bromo analog **3b** was more potent than its parent compound **3a** against MCF-7 and HepG2, but its activity toward SW480 was less than that of unsubstituted congener **3a**. The bromo-substitution in compound **3g** resulted in compound **3h** with highest activity against HepG2 and lesser activity against MCF-7. Replacement of oxygen in morpholine derivative **3i** by sulfur resulted in compound **3k** with greater potency against all tested cell lines. All aniline derivatives **4a-c** exhibited modest activity against all cell lines in the same range. The insertion of methoxy or poly-methoxy groups on aniline moiety cannot improve the cytotoxicity.

3.2. Apoptosis inducing activity

Flow cytometry analysis was performed to determine the mechanism of cell death which observed by the most active compound **3k**, in MCF7 cells by Annexin V-FITC/PI (Propidium iodide) dual staining assay [31]. In this regard, non-treated cells were used as negative control and etoposide was used as standard drug. The cells were treated with two different

concentrations of compound **3k**, 7.5 µg/ml (IC_{50}) and 11.2 µg/ml (1.5 fold IC_{50}) for 48 h. After that, the cells were harvested and stained with FITC Annexin-V and Propidium iodide solutions, respectively and the percentages of apoptotic cells were determined by flow cytometry. As shown in Figure 2, the treatment of MCF-7 cells with compound **3k** at 7.5 and 11.2 µg/ml concentrations resulted in 19.2 and 41.7% of cell apoptosis, whereas non-treated cells and etoposide showed 15.6 and 23%. These results confirmed that the cytotoxicity of compound **3k** is associated with cell apoptosis in a concentration-dependent manner.

3.3. Cell cycle arrest

The cell cycle analysis was performed to investigate the prevention of proliferation in cancer cells (MCF-7 cells) by the most potent compound **3k**. MCF-7 cells were treated with compound **3k** at two concentrations (7.5 and 11.2 µg/ml) for 48 h then the treated cells were harvested, stained with propidium iodide (PI) and analyzed by flow cytometry [32]. The obtained results were compared with non-treated MCF-7 cells, as control. As shown in Figs. 3 and 4, treatment of MCF-7 cells with **3k** at 7.5 and 11.2 µg/ml concentrations increased the percentage of G1-phase cells from 68.5 % (as control group) to 83.9 and 89.3 % respectively. These results confirmed that compound **3k** significantly caused G1-phase arrest in MCF-7 cells.

3.4. In vitro antioxidant activity

Since antioxidant activity has been reported for numerous natural and synthetic coumarins [33], thus the antioxidant activity of newly synthesized coumarin-based compounds **3a-l** and **4a-c** was evaluated by using two distinct method; ferric reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

3.4.1. Ferric-reducing antioxidant power (FRAP)

In this experiment, ferric tripyridyl triazine [TPTZ-Fe(III)] complex is converted into ferrous tripyridyl triazine [TPTZ-Fe(II)] complex and the absorbance of the blue color [TPTZ-Fe(II)] complex was measured at 593 nm [34]. The experiments were conducted at different concentrations (from 5 to 100 µg/ml) and the results were shown by FRAP values and

compared with ascorbic acid as a positive control (Table 2). Based on the obtained results at the lowest concentration (5 µg/ml), a majority of compounds showed higher FRAP values compared to ascorbic acid as the standard antioxidant. Notably, morpholino derivative **3i** and 3,4,5-trimethoxyanilino analog **4c** showed the highest ferric-reducing power at low concentrations. All 6-bromocoumarin derivatives exhibited lower FRAP values compared to unsubstituted counterparts. In the other words, the introduction of bromine at the C-6 of coumarin decreased the reducing power of the synthesized compounds. Compounds with morpholine, piperidine and pyrrolidine moieties at C-2 of the thiazole ring, showed higher antioxidant activities rather than derivatives bearing cyclic amines with pendent groups (phenyl or benzyl).

3.4.2. Radical-scavenging activity on DPPH

The radical scavenging activity of synthesized compounds was measured by DPPH assay. This method evaluates the hydrogen donating ability of synthesized compounds, converting the purple DPPH radical to the yellow hydrazine. The results were illustrated as IC_{50} values and listed in Table 2. The ascorbic acid was used as the positive control for the experiment. As displayed in Table 2, all the tested compounds showed moderate radical scavenging activity with IC_{50} values less than 50 µg/ml with the exception of **3h**. The most active compound was thiomorpholine derivative **3k** with IC_{50} = 23.9 µg/ml which was about 2.5 fold less potent than ascorbic acid. Compounds with morpholine, pyrrolidine, piperidine moieties showed good radical scavenging activity better than other derivatives with larger substituents. Furthermore, the introduction of bromine into the C-6 of coumarin decreased the radical scavenging activity of synthesized compounds. Among the 2-anilino derivatives, the 3,4,5-trimethoxy derivative (**4c**) showed the highest antioxidant activity (IC_{50} =26.3 µg/ml).

4. Conclusions

In conclusion, we have synthesized and evaluated 3-(4-aminothiazole-5-carbonyl)-2*H*-chromen-2-ones **3a-l** and **4a-c** bearing cyclic amine, substituted cyclic amine, aniline or substituted aniline moieties, as antiproliferative and antioxidant agents. In vitro MTT assay of synthesized compounds revealed that most of them had significant cytotoxic activity against MCF-7, HepG2 and SW400 cell lines. The best results were obtained for thiomorpholine

derivative **3k** with IC_{50} values of 7.5-16.9 µg/ml. Flow cytometric analysis on the representative compound **3k** against MCF-7 cells revealed the induction of apoptosis and blockage of the cell proliferation at the G1-phase. Further biological evaluations by DPPH and FRAP assays indicated the potential of target compounds for antioxidant activity.

5. Experimental section

5.1. General chemistry

Starting materials, reagents and solvents were purchased from Merck and Sigma Aldrich companies and were used without any purification. The 3-(bromoacetyl)coumarins **6** were prepared according to the previously reported method [35]. The reactions progress and the purity of the synthesized compounds were monitored by thin-layer chromatography (TLC) using silica gel 250 micron F254 plastic sheets. Flash chromatography was performed for the purification of the synthesized compounds by using 230-400 mesh silica gel and the indicated solvent system. Melting points were determined with a Kofler hot-plate microscope apparatus and are uncorrected. IR spectra were recorded in KBr disks, using a Nicolet FT-IR Magna 550 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz spectrometer using DMSO-d_6 and $CDCl_3$ as solvent. Chemical shifts (δ) are given in ppm relative to tetramethyl silane (TMS) as internal standard and coupling constant (*J*) values are presented in Hz. The elemental analysis for C, H, N was carried out with an Elementar Analysen system GmbH VarioEL.

*5.2. General procedure for the synthesis of compound **3a-l***

An appropriate cyclic amine (1 mmol) was added to the mixture of dimethyl *N*-cyanodithioimidocarbonate **7** (1 mmol) in DMF (5 ml) and heated at 70° C for 1 h. Then, $Na_2S \cdot 9H_2O$ 60% (1 mmol) was added and the mixture was heated for another 2 h. After this time, the reaction mixture was cooled down to 0-5 °C and proper 3-(bromoacetyl)coumarin derivative **6** was added over 10 min. After the consumption of compound **6** (checked by TLC), the reaction mixture was poured into cold water (15 ml). The precipitated solid was separated by filtration, washed with cold water and dried. The crude product was purified by flash chromatography on silica gel using ethyl acetate-petroleum ether as eluent to give corresponding target compound **3**.

*5.2.1. 3-(4-amino-2-(pyrrolidin-1-yl)thiazole-5-carbonyl)-2H-chromen-2-one (**3a**)*

Yield 71%; mp 192-194 °C, IR (KBr, 1/cm): 3333, 2924, 1708, 1609, 1542, 1442, 1351, 1282, 1172, 1101, 1049, 901, 801, 751. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.96 (s, 4H, 2×CH_2 pyrrolidine), 3.56 (bs, 4H, 2×CH_2 pyrrolidine), 7.39 (t, 1H, H-6 coumarin, J = 7.5 Hz), 7.44 (d, 1H, H-8 coumarin, J = 8.0 Hz), 7.67 (t, 1H, H-7 coumarin, J =7.5 Hz), 7.79 (d, 1H, H-5 coumarin, J = 7.5 Hz), 8.07 (bs, 2H, NH_2), 8.22 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 24.88, 49.16, 116.06, 118.22, 124.72, 127.82, 127.96, 129.06, 132.65, 141.48, 153.37, 157.39, 166.34, 168.38, 175.54. MS (m/z, %): 341 (M^+, 100), 324 (29), 284 (21), 173 (25), 97 (27), 55 (19). Anal. calcd for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31. Found: C, 60.01; H, 4.45; N, 12.36.

*5.2.2. 3-(4-amino-2-(pyrrolidin-1-yl)thiazole-5-carbonyl)-6-bromo-2H-chromen-2-one (**3b**)*
Yield 53%; mp 134-136 °C, IR (KBr, 1/cm): 3375, 3269, 1732, 1619, 1558, 1446, 1282, 822, 619. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.86-2.07 (m, 4H, 2×CH_2 pyrrolidine), 3.56 (bs, 4H, 2×CH_2 pyrrolidine), 7.42 (d, 1H, H-8 coumarin, J = 8.5 Hz), 7.82 (d, 1H, H-7 coumarin, J = 8.5 Hz), 8.06 (s, 1H, H-5 coumarin), 8.11 (bs, 2H, NH_2), 8.18 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz,) δ: 24.97, 49.26, 116.31, 118.35, 120.20, 130.21, 131.15, 135.01, 139.70, 140.19, 153.70, 157.03, 166.53, 168.47, 175.01. MS (m/z, %): 421 (M+2, 100), 419 (M^+, 100), 391 (39), 362 (46), 221 (86), 196 (57), 89 (54), 55 (47). Anal. calcd for $C_{17}H_{14}BrN_3O_3S$: C, 48.58; H, 3.36; N, 10.00. Found: C, 48.76; H, 3.24; N, 10.09.

*5.2.3. 3-(4-amino-2-(piperidin-1-yl) thiazole-5-carbonyl)-2H-chromen-2-one (**3c**)*

Yield 66%; mp 186-188 °C, IR (KBr, 1/cm): 3370, 3268, 2942, 1726, 1614, 1569, 1523, 1470, 1437, 1311, 1252, 1164, 1022, 881, 775, 753. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.48-1.71 (m, 6H, 3×CH_2 piperidine), 3.51 (bs, 4H, 2×CH_2 piperidine), 7.30 (t, 1H, H-6 coumarin, J = 7.5 Hz), 7.35 (d, 1H, H-8 coumarin, J = 8.0 Hz), 7.54-7.58 (m, 2H, H-5,7 coumarin), 8.00 (s, 1H, H-4 coumarin). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 23.85, 25.20, 49.17, 116.62, 118.66, 124.56, 127.34, 128.66, 129.67, 132.48, 142.38, 154.21, 158.28, 166.96, 172.56, 176.66. MS (m/z, %): 355 (M^+, 100), 338 (27), 324 (21), 297 (15), 173 (21), 111 (25). Anal. calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.59; H, 4.67; N, 11.70.

*5.2.4. 3-(4-amino-2-(piperidin-1-yl)thiazole-5-carbonyl)-6-bromo-2H-chromen-2-one (**3d**)*

Yield 59%; mp 138-140 °C, IR (KBr, 1/cm): 3437, 3327, 2923, 1709, 1619, 1534, 1464, 1433, 1322, 1241, 1016, 827, 514. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.51-1.68 (m, 6H,

3×CH_2 piperidine), 3.50 (bs, 4H, 2×CH_2 piperidine), 7.42 (d, 1H, H-8 coumarin, *J* = 9.0 Hz), 7.82 (d, 1H, H-7 coumarin, *J* = 9.0 Hz), 8.01 (s, 1H, H-5 coumarin), 8.05 (bs, 2H, NH_2), 8.18 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz,) *δ*: 23.44, 25.36, 48.23, 116.61, 120.44, 121.65, 129.12, 130.64, 134.21, 139.78, 141.39, 152.12, 159.47, 167.35, 170.42, 175.21. MS (m/z, %): 435 (M+2, 100) 433 (M^+, 100), 404 (41), 377 (57), 363 (23), 350 (25), 55 (38), 41 (76). Anal. calcd for $C_{18}H_{16}BrN_3O_3S$: C, 49.78; H, 3.71; N, 9.68. Found: C, 49.90; H, 3.82; N, 9.70.

*5.2.5. 3-(4-amino-2-(4-benzylpiperidin-1-yl)thiazole-5-carbonyl)-2H-chromen-2-one (**3e**)*

Yield 49%; mp: 134-136 °C, IR (KBr, 1/cm): 3446, 3299, 2918, 1717, 1607, 1539, 1447, 1322, 1253, 1169, 755. ^{1}H-NMR ($CDCl_3$, 500 MHz) *δ*: 1.26-1.31 (m, 2H, CH_2 piperidine), 1.55-1.66 (m, 1H, piperidine), 1.72-1.75 (m, 2H, CH_2 piperidine), 2.55-2.57 (m, 2H, CH_2-Ph), 2.97-3.02 (m, 2H, CH_2 piperidine), 4.01-4.06 (m, 2H, CH_2 piperidine), 7.12 (d, 2H, H-2,6 Ph, *J* = 7.5 Hz), 7.20 (t, 1H, H-6 coumain, *J* = 8.5 Hz), 7.26-7.29 (m, 3H, H-3,4,5 Ph), 7.35 (d, 1H, H-8 coumarin, *J* = 8.5 Hz), 7.54 (m, 2H, H-5,7 coumarin), 8.01 (s, 1H, H-4 coumarin). ^{13}C-NMR ($CDCl_3$-d_6, 125 MHz) *δ*: 31.34, 37.67, 42.73, 48.49, 116.68, 118.71, 124.59, 126.21, 127.62, 128.38, 128.69, 129.04, 132.53, 139.54, 142.50, 157.72, 159.53, 166.94, 172.24, 176.80. MS (m/z, %): 445 (M^+, 100), 354 (28), 298 (67), 285 (35), 271 (18), 91 (38), 55 (14). Anal. calcd for $C_{25}H_{23}N_3O_3S$: C, 67.40; H, 5.20; N, 9.43. Found: C, 67.43; H, 5.02; N, 9.49.

*5.2.6. 3-(4-amino-2-(4-benzylpiperazin-1-yl)thiazole-5-carbonyl)-2H-chromen-2-one (**3f**)*

Yield 57%; mp: 128-130 °C, IR (KBr, 1/cm): 3403, 3290, 1724, 1608, 1536, 1466, 1432, 1323, 1246, 1167, 1040, 996, 879, 804, 746. ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 2.35-2.46 (m, 4H, 2×CH_2 piperazine), 3.16-3.29 (m, 2H, CH_2-Ph), 3.32-3.58 (m, 4H, 2×CH_2 piperazine), 7.26 (d, 2H, H-2,6 Ph, *J* = 6.5 Hz), 7.31-7.32 (m, 3H, H-3,4,5 Ph), 7.39 (t, 1H, H-6 coumarin, *J* = 7.5 Hz), 7.43 (d, 1H, H-8 coumarin, *J* = 8.0 Hz), 7.67 (t, 1H, H-7 coumarin, *J* = 7.5 Hz), 7.79 (d, 1H, H-5 coumarin, *J* = 7.5 Hz), 8.03 (bs, 2H, NH_2), 8.23 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz) *δ*: 47.39, 51.45, 61.51, 116.17, 118.21, 124.69, 127.01, 127.98, 128.24, 128.62, 128.86, 129.32, 132.67, 137.54, 141.54, 153.39, 157.37, 166.16, 171.47, 175.93. MS (m/z, %): 446 (M^+, 100), 298 (28), 160 (42), 146 (77), 91 (89), 56 (21). Anal. calcd for $C_{24}H_{22}N_4O_3S$: C, 64.56; H, 4.97; N, 12.55. Found: C, 64.33; H, 5.09; N, 12.50.

*5.2.7. 3-(4-amino-2-(4-phenylpiperazin-1-yl)thiazole-5-carbonyl)-2H-chromen-2-one (**3g**)*

Yield 76%; mp: 136-138 °C, IR (KBr, 1/cm): 3365, 2923, 1712, 1609, 1522, 1463, 1431, 1315, 1229, 1018, 927, 759. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.15-3.26 (m, 4H, 2×CH_2 piperazine), 3.51-3.68 (m, 4H, 2×CH_2 piperazine), 6.82 (t, 1H, H-4 phenyl, J = 7.5 Hz), 6.97 (d, 2H, H-2,6 phenyl, J = 8.0 Hz), 7.23 (t, 2H, H-3,5 phenyl, J = 7.5 Hz), 7.40 (t, 1H, H-6 coumarin, J =7.5 Hz), 7.45 (d, 1H, H-8 coumarin, J = 8.0 Hz), 7.68 (t, 1H, H-7 coumarin, J = 7.5 Hz), 7.81 (d, 1H, H-5 coumarin, J = 7.5 Hz), 8.09 (bs, 2H, NH_2), 8.26 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 47.27, 47.78, 114.36, 116.12, 118.28, 119.57, 124.71, 127.86, 128.32, 129.16, 132.58, 141.70, 150.40, 153.46, 157.40, 166.15, 171.75, 176.08. MS (m/z, %): 432 (M^+, 100), 311 (37), 298 (19), 132 (85), 104 (23), 77 (21). Anal. calcd for $C_{23}H_{20}N_4O_3S$: C, 63.87; H, 4.66; N, 12.95. Found: C, 64.12; H, 4.53; N, 13.11.

*5.2.8. 3-(4-amino-2-(4-phenylpiperazin-1-yl)thiazole-5-carbonyl)-6-bromo-2H-chromen-2-one (**3h**)*

Yield 61%; mp 173-175 °C, IR (KBr, 1/cm): 3438, 3287, 1729, 1600, 1537, 1465, 1440, 1338, 1307, 1232, 1159, 1107, 1023, 924, 820. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.04-3.09 (m, 4H, 2×CH_2 piperazine), 3.66-3.73 (m, 4H, 2×CH_2 piperazine), 6.82 (t, 1H, H-4 phenyl, J = 7.5 Hz), 6.97 (d, 2H, H-2,6 phenyl, J = 8.0 Hz), 7.24 (t, 2H, H-3,5 phenyl, J = 7.5 Hz), 7.43 (d, 1H, H-8 coumarin, J = 8.5 Hz), 7.83 (d, 1H, H-7 coumarin, J = 8.5 Hz), 8.06 (s, 1H, H-5 coumarin), 8.10 (bs, 2H, NH_2), 8.21 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 46.45, 48.23, 114.53, 116.48, 119.64, 119.89, 120.43, 127.45, 129.16, 129.89, 133.74, 137.56, 143.36, 149.47, 152.26, 157.54, 166.48, 171.27, 176.80. MS (m/z, %): 512 (M+2, 100), 510 (M^+, 100), 391 (34), 295 (51), 223 (97), 197 (98), 171 (67), 145 (73). Anal. calcd for $C_{23}H_{19}BrN_4O_3S$: C, 54.02; H, 3.74; N, 10.96. Found: C, 53.99; H, 3.75; N, 10.72.

*5.2.9. 3-(4- amino-2-morpholinothiazole-5-carbonyl)-2H-chromen-2-one (**3i**)*

Yield 62%; mp 172-174 °C, IR (KBr, 1/cm): 3459, 3394, 2922, 1724, 1610, 1533, 1464, 1319, 1270, 1242, 1114, 890, 762. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 3.45-3.56 (m, 4H, 2×CH_2 morpholine), 3.64- 3.82 (m, 4H, 2×CH_2 morpholine), 7.31 (t, 1H, H-6 coumarin, J = 7.5 Hz), 7.36 (d, 1H, H-8 coumarin, J = 8.0 Hz), 7.54-7.59 (m, 2H, H-5,7 coumarin), 8.04 (s, 1H, H-4 coumarin). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 47.86, 65.96, 116.68, 118.63, 124.66, 127.45, 128.77, 129.38, 132.70, 142.93, 154.28, 158.30, 166.52, 173.04, 177.27. MS (m/z, %): 357 (M^+, 100), 298 (44), 167 (31), 149 (87), 97 (48), 86 (87), 57 (63), 43 (51). Anal. calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.11; H, 4.31; N, 11.53.

*5.3.10. 3-(4-amino-2-morpholinothiazole-5-carbonyl)-6-bromo-2H-chromen-2-one (**3j**)*

Yield 67%; mp 138-140 °C, IR (KBr, 1/cm): 3392, 3287, 2923, 1728, 1607, 1531, 1466, 1435, 1320, 1239, 1113, 892, 819. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.44-3.55 (m, 4H, 2×CH_2 morpholine), 3.57-3.86 (m, 4H, 2×CH_2 morpholine), 7.42 (d, 1H, H-8 coumarin, J = 8.5 Hz), 7.81 (d, 1H, H-7 coumarin, J = 8.5 Hz), 8.05 (s, 1H, H-5 coumarin), 8.09 (bs, 2H, NH_2), 8.20 (s, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz,) δ: 45.48, 66.47, 116.94, 119.83, 120.23, 128.92, 131.14, 137.52, 139.48, 140.50, 153.34, 159.71, 166.85, 172.62, 176.12. MS (m/z, %): 437 (M+2, 100), 435 (M^+, 100), 377 (24), 340 (23), 222 (46), 165 (23), 54 (63). Anal. calcd for $C_{17}H_{14}BrN_3O_4S$: C, 46.80; H, 3.23; N, 9.63. Found: C, 46.98; H, 3.14; N, 9.70.

*5.3.11. 3-(4-amino-2-thiomorpholinothiazole-5-carbonyl)-2H-chromen-2-one (**3k**)*

Yield 69%; mp 174-176 °C, IR (KBr, 1/cm): 3444, 1697, 1608, 1531, 1461, 1427, 1351, 1279, 1170, 945, 777, 749. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.56-2.78 (m, 4H, 2×CH_2 thiomorpholine), 3.75-4.03 (m, 4H, 2×CH_2 thiomorpholine), 7.31 (t, 1H, H-6 coumarin, J = 7.5 Hz), 7.36 (d,1H, H-8 coumarin, J = 8.0 Hz), 7.55-7.60 (m, 2H, H-5,7 coumarin), 8.04 (s, 1H, H-4 coumarin). ^{13}C-NMR ($CDCl_3$, 125 MHz,) δ: 26.80, 50.80, 116.71, 118.67, 124.67, 127.23, 128.77, 132.70, 142.95, 155.83, 158.36, 166.62, 172.47, 177.22. MS (m/z, %): 373 (M^+, 100), 356 (18), 298 (87), 228 (26), 173 (38), 129 (22), 69 (29). Anal. calcd for $C_{17}H_{15}N_3O_3S_2$: C, 54.67; H, 4.05; N, 11.25. Found: C, 55.10; H, 4.06; N, 11.29.

*5.3.12. 3-(4-amino-2-thiomorpholinothiazole-5-carbonyl)-6-bromo-2H-chromen-2-one (**3l**)*

Yield 70%; mp 178-180 °C, IR (KBr, 1/cm): 3421, 2924, 1735, 1606, 1536, 1465, 1244, 1190, 1080, 955, 854, 820. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 2.67 (bs, 4H, 2×CH_2 thiomorpholine), 3.80 (bs, 4H, 2×CH_2 thiomorpholine), 7.41 (bs, 1H, H-8 coumarin), 7.81 (bs, 1H, H-7 coumarin), 8.08 (bs, 3H, H-5 coumarin and NH_2), 8.18 (bs, 1H, H-4 coumarin). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 26.87, 51.27, 116.82, 119.35, 120.86, 128.96, 130.44, 137.25, 138.68, 141.56, 153.94, 159.23, 166.70, 170.24, 176.52. MS (m/z, %): 453 (M+2, 100), 451 (M^+, 100), 436 (28), 378 (24), 272 (23), 222 (25), 128 (29), 67 (48). Anal. calcd for $C_{17}H_{14}BrN_3O_3S_2$: C, 45.14; H, 3.12; N, 9.29. Found: C, 45.32; H, 3.21; N, 9.33.

*5.3. General procedure for the synthesis of compounds **4a-c***

Cyanamide (1 mmol) was added to the solution of arylisothiocyanate **9** (1 mmol) in methanol at 0-5 °C. A solution of sodium methoxide (0.8 M), obtained by dissolving sodium (1 mmol)

in MeOH (1.2 ml), was slowly added at 0 °C to the reaction mixture over 10 min. After 2 h, 3-(bromoacetyl)coumarin **6** (1 mmol) was added portion by portion over 10 min and the reaction color turned to yellow. After the consumption of 3-(bromoacetyl)coumarin, the mixture was diluted with cold water (15 ml) and the obtained yellow solid was filtered and washed with cold water three times. The crude product was dried and purified by column chromatography on silica gel using ethyl acetate-petroleum ether as eluent system.

*5.3.1. 3-(4-amino-2-(phenylamino)thiazole-5-carbonyl)-2H-chromen-2-one (**4a**)*

Yield 66%; mp 178-180 °C, IR (KBr, 1/cm): 3275, 2923, 1721, 1606, 1549, 1439, 1171, 755. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.16 (t, 1H, H-4 Ph, J = 7.5 Hz), 7.31-7.36 (m, 6H, H-Ph, H-6,8 coumarin), 7.54-7.60 (m, 2H, H-5,7 coumarin), 8.03 (s, 1H, H-4 coumarin). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 116.09, 117.36, 118.26, 119.11, 123.54, 124.77, 129.14, 131.32, 132.92, 139.33, 140.23, 142.06, 153.02, 159.63, 167.96, 172.62, 176.70. MS (m/z, %): 363 (M^+, 100), 339 (13), 323 (16), 198 (22), 180 (21), 152 (36), 77 (34), 51 (28). Anal. calcd for $C_{19}H_{13}N_3O_3S$: C, 62.80; H, 3.61; N, 11.56. Found: C, 62.66; H, 3.62; N, 11.39.

*5.3.2. 3-(4-amino-2-((4-methoxyphenyl)amino)thiazole-5-carbonyl)-2H-chromen-2-one (**4b**)*

Yield 76%; mp 180-182 °C, IR (KBr, 1/cm): 3424, 1727, 1608, 1546, 1429, 1308, 1248, 1214, 1173, 1081, 1028, 766. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.73 (s, 3H, OCH_3), 6.93 (d, 2H, H-3,5 Ph, J = 8.5 Hz), 7.38-7.45 (m, 4H, H-2,6 Ph, H-6,8 coumarin), 7.67 (t, 1H, H-7 coumarin, J = 7.5 Hz), 7.80 (d, 1H, H-5 coumarin, J = 7.5 Hz), 8.13 (bs, 2H, NH_2), 8.26 (s, 1H, H-4 coumarin), 10.63 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 55.23, 114.32, 115.97, 116.21, 121.80, 124.72, 127.23, 128.65, 129.07, 132.27, 132.74, 141.81, 153.44, 157.37, 159.92, 166.89, 170.94, 176.24. MS (m/z, %): 393 (M^+, 100), 173 (43), 149 (24), 122 (23), 97 (36), 83 (33), 69 (24), 57 (26), 43 (39). Anal. calcd for $C_{20}H_{15}N_3O_4S$: C, 61.06; H, 3.84; N, 10.68. Found: C, 59.88; H, 4.00; N, 10.64.

*5.3.3. 3-(4-amino-2-((3,4,5-trimethoxyphenyl)amino)thiazole-5-carbonyl)-2H-chromen-2-one (**4c**)*

Yield 68%; mp 186-188 °C, IR (KBr, 1/cm): 3413, 3313, 1710, 1607, 1561, 1508, 1457, 1425, 1234, 1178, 1129, 997, 987, 762. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.64 (s, 3H, OCH_3), 3.78 (s, 6H, 2×OCH_3), 6.96 (bs, 2H, H-2,6 Ph), 7.40 (t, 1H, H-6 coumarin, J = 7.5 Hz), 7.45 (d, 1H, H-8 coumarin, J = 8.0 Hz), 7.68 (t, 1H, H-7 coumarin, J = 7.5 Hz), 7.81 (d, 1H, H-5 coumarin, J = 7.5 Hz), 8.18 (bs, 2H, NH_2), 8.29 (s, 1H, H-4 coumarin), 10.69 (s,

1H, NH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 55.83, 59.99, 97.70, 116.22, 118.27, 124.72, 127.32, 128.91, 129.08, 132.77, 133.90, 135.30, 141.97, 152.95, 153.48, 157.38, 167.56, 170.64, 176.51. MS (m/z, %): 453 (M^+, 100), 368 (23), 194 (26), 147 (29), 98 (33), 85 (46), 71 (74), 57 (93), 43 (89). Anal. calcd for $C_{22}H_{19}N_3O_6S$: C, 58.27; H, 4.22; N, 9.27. Found: C, 58.50; H, 4.23; N, 9.31.

5.4. MTT assay

MTT assay was used to evaluate the cytotoxic activity of synthesized compounds (**3a-l** and **4a-c**) against three different tumor cell lines MCF-7, HepG2 and SW400 which were grown in RPMI-1640 medium and DMEM with 10% FBS (Gibco, Milano, Italy). All the cell lines were purchased from National Cell Bank of Iran (Pastor Institute, Tehran, Iran). Different concentrations of target compounds were prepared by dissolving synthetic compounds in DMSO. Each wells of a 96-well microtiter plate had 190 µl of complete medium containing 8 × 10^3 cells, incubated at 37°C in a humidified 5% CO_2 incubator overnight. The cells were treated with different concentrations of test compounds and further incubated at 37°C for 48 h. After that, the cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and the absorbance of each well was measured by microplate reader (Bio-Rad microplate reader, Model 680) at 570 nm wavelengths [36]. The negative control was 0.1% DMSO. The reference drug etoposide was used as positive control. The data were expressed by IC_{50} which means the compound concentration required to inhibit cell proliferation by 50% and were depicted as the mean± SE from the dose-response curves of at least three independent experiments.

5.5. Analysis of cellular apoptosis

Annexin V-FITC/PI (Propidium iodide) dual staining assay was used to determine the induction of apoptosis by the most potent compound **3k**, in comparison with etoposide (tested at IC_{50} concentration). MCF-7 cells were seeded into 6-well plates and incubated overnight at 37 °C under 5% CO_2. The cells were treated with two concentrations of test compound **3k** at IC_{50} and 1.5×IC_{50} concentrations and incubated for 48 h. Then, the treated cells were trypsinized, washed with PBS twice and centrifuged at 1200 rpm to collect the cells. After that, 500 µl of binding buffer and 5 µl of Annexin V-FITC and PI were added to suspend

cells. After 5−15 min incubation at room temperature in dark, cell apoptosis was analyzed by flow cytometry (FACS Calibur Bectone-Dickinson) [31].

5.6. Cell cycle assessment

Propidium iodide (PI) staining assay was used to investigate the cell cycle distributions in the MCF-7 cells by flow cytometry analysis. PI can bind to DNA and emit a fluorescent, the intensity of which is in proportion to the DNA content. The cells were treated with IC_{50} and $1.5\times IC_{50}$ concentrations of test compound (**3k**), after 48 h, the treated cells were trypsinized, washed with PBS and centrifuged at 1000 rpm for 5 min. After incubation with PBS, the obtained MCF-7 cells were fixed with 70% cold ethanol (-20 °C). The fixed cells were washed with PBS, treated with RNase A (0.1 mg/ml) and incubated for half an hour, sequentially treated with 50 mg/ml of PI and incubated for 15 min more. The cell cycle distribution was calculated using a Novocyte flow cytometer (ACEA Biosciences) and the data were analyzed by NovoExpress 1.1.0 software. All the experiments were performed on three samples in parallel [32].

5.7. FRAP assay

FRAP assay was used as a standard method to evaluate antioxidant activity of target compounds. This experiment measured the reduction capacity of compounds which converting ferric tripyridyl triazine (Fe (III)-TPTZ) complex into a blue color ferrous tripyridyl triazine (Fe (II)-TPTZ) complex at low pH. An 1 ml TPTZ solution (10 mM) in 40 mM HCl was mixed with 1 ml $FeCl_3.6H_2O$ solution (20 mM) and 10 ml acetate buffer (300 mM) at pH =3.6 to make Fe (II)-TPTZ (2,4,6-tripyridyl-s-triazine) reagent. 20 µl of sample solution in different concentration was mixed with 150 µl of Fe (II)-TPTZ reagent and incubated at 37°C for 25 min. The absorbance of mixture was measured at 593 nm. The ascorbic acid was used as the standard solution. The results were expressed in µM equivalent to $FeSO_4.7H_2O$ by calculating from calibration curve [37].

5.8. DPPH radical scavenging activity

The ability of target compounds to capture free radicals was evaluated by DPPH radical scavenging method [38]. Different concentrations of synthesized compounds were prepared by dissolving in DMSO. The working solution was obtained by mixing 0.5 mM stock solution of DPPH in methanol to obtain an absorbance of 9.1±0.02 units at 517 nm using the spectrophotometer. A mixture of 20 µl of each sample solution and 250 µl of DPPH in methanol was incubated at room temperature for 30 min in dark. Then the absorbance of the mixture was measured at 517 nm. The control group had the same content without any compound and the ascorbic acid solution was used as standard. The experiments were carried out in triplicates. The average values were used to determine the radical scavenging activity as the percentage of inhibition according to the following equation: % inhibition = [(absorbance of control- absorbance of sample)/absorbance of control]× 100. The data were exhibited as IC_{50} which define as compound concentration required capturing free radicals by 50%.

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Captions:

Figure 1. Design of compounds **3** and **4** based on the thiazole-derived lead **1**, as new anticancer agents.

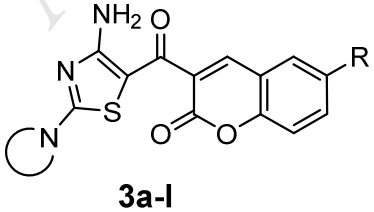
Figure 2. Flow cytometric analysis of MCF-7 cells treated with compounds **3k**. (A) Non-treated cells as negative control group; (B) treated with etoposide as positive control; (C) treated with **3k** at IC_{50} concentration (7.5 µg/ml); (D) treated with **3k** at $1.5 \times IC_{50}$ concentration (11.2 µg/ml).

Figure 3. Cell cycle analysis of MCF-7 cells by flow cytometric analysis. (A) Non-treated cells as control group; (B) treated with **3k** at IC_{50} concentration (7.5 µg/ml); (C) treated with **3k** at $1.5\times IC_{50}$ concentration (11.2 µg/ml)

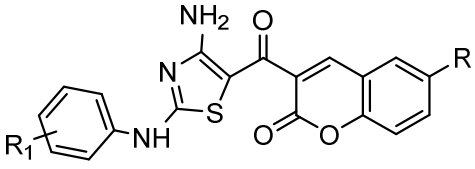
Figure 4. Quantitative analysis of cell cycle distributions; (1) Non-treated cells as control group; (2) treated with **3k** at IC_{50} concentration (7.5 µg/ml); (3) treated with **3k** at $1.5\times IC_{50}$ concentration (11.2 µg/ml)

Scheme 1. Synthesis of compounds **3a-l** and **4a-c**. *Reagents and conditions*: a) Br_2, $CHCl_3$, rt.; b) appropriate cyclic amine, DMF, 70 °C, 1 h; c) $Na_2S\cdot 9H_2O$ 60%, 70 °C, 2 h; d) 0-5 °C, 2 h; e) NH_2CN, CH_3OH, 0-5 °C, 1h; f) Na/CH_3OH, 0-5 °C, 2h; g) 0-5 °C, 2h.

Table 1
In vitro cytotoxic activity (IC_{50} values, µg/ml) of compounds **3a-l** and **4a-c** against cancer cell lines.



3a-l

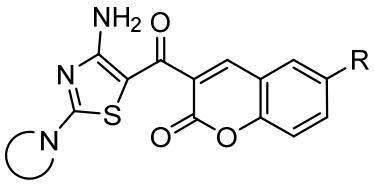


4a-c

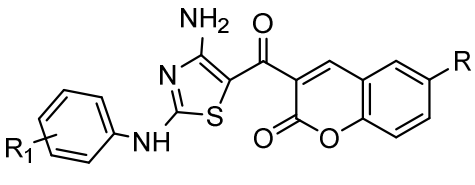
Compound	A	R	R_1	MCF-7	HepG2	SW480

3a	pyrrolidin-1-yl	H	-	14.1 ± 2.3	40 ± 0.7	13.5 ± 3.1
3b	pyrrolidin-1-yl	Br	-	10.9 ± 3.4	27.7 ± 1.8	33.4 ± 2.1
3c	piperidin-1-yl	H	-	10.7 ± 0.9	37.1 ± 3.2	16.3 ± 1.4
3d	piperidin-1-yl	Br	-	29.1 ± 1.2	19.8 ± 2.3	18.3 ± 0.6
3e	4-benzylpiperidin-1-yl	H	-	11.1 ± 1.7	21.7 ± 1.1	14.5 ± 3.1
3f	4-benzylpiperazin-1-yl	H	-	11.6 ± 2.4	32 ± 0.6	23.4 ± 2.3
3g	4-phenylpiperazin-1-yl	H	-	17.9 ± 3.2	34.9 ± 1.5	22.5 ± 0.7
3h	4-phenylpiperazin-1-yl	Br	-	28.6 ± 2.6	12.2 ± 2.3	17.1 ± 1.3
3i	morpholino	H	-	9.8 ± 0.8	34.8 ± 2.6	20.5 ± 1.9
3j	morpholino	Br	-	14.6 ± 1.2	19.3 ± 2.1	48.7 ± 0.9
3k	thiomorpholino	H	-	7.5 ± 0.7	16.9 ± 0.7	13.0 ± 0.6
3l	thiomorpholino	Br	-	12.5 ± 1.3	29.7 ± 3.1	44.5 ± 4.3
4a	-	H	H	26.6 ± 2.1	20.6 ± 2.1	19.2 ± 0.9
4b	-	H	4-MeO	30.3 ± 0.5	23.1 ± 1.3	20.9 ± 2.3
4c	-	H	3,4,5-$(MeO)_3$	24.2 ± 1.6	26.8 ± 0.8	24.4 ± 2.5
Etoposide				3.1 ± 0.6	6.2 ± 0.5	7.7 ± 1.5

Table 2
In vitro antioxidant activity of compounds **3a-l** and **4a-c** determined by ferric-reducing antioxidant power (FRAP) and DPPH methods

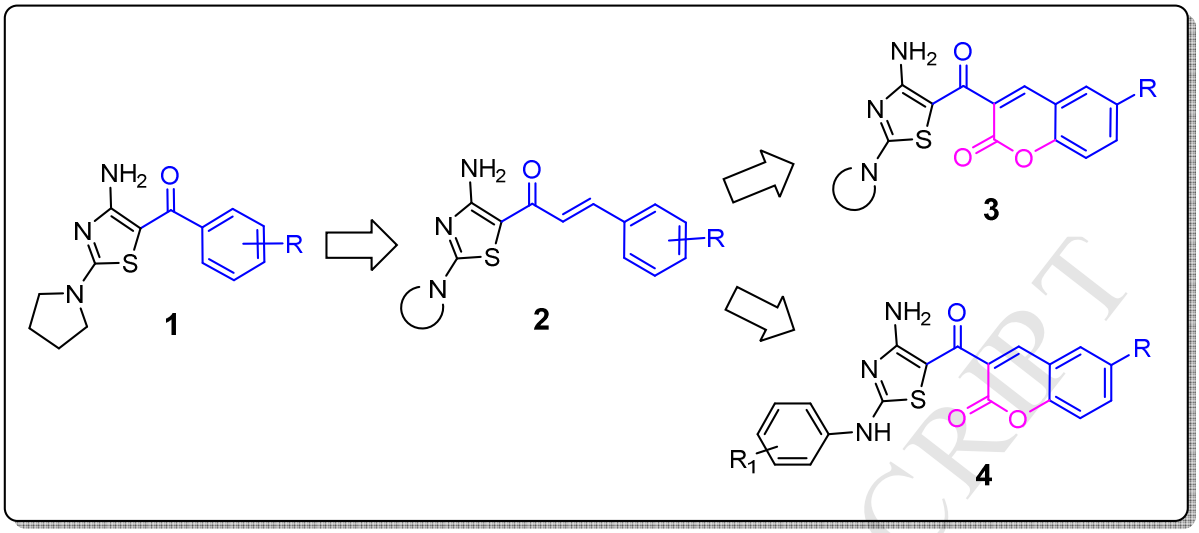


3a-l



4a-c

Compound	FRAP values at different concentrations						DPPH (IC_{50}, µg/ml)
	5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	100 µg/ml	
3a	93.3±0.6	116.5±1.6	155.3±0.7	272.5±3.9	275.8±3.5	542.3±3.4	28.8±1.3
3b	72.3±2.3	97.5±1.7	102.8±1.2	147.5±2.2	205.5±0.6	243.3±1.3	31.7±0.8
3c	103.5±4.6	134.5±3.8	237 ±2.5	445 ±2.6	463.5±1.9	676 ±2.5	27.3±2.1
3d	88.8±2.4	118.8±4.1	151.3±2.7	194.3±2.8	243.3±1.6	243.8±3.5	46.6±1.4
3e	54.3±3.9	111.3±1.3	136.5±3.6	179.8±2.7	236.8±3.8	263.3±3.2	37.7±1.2
3f	88.5±2.4	134±2.5	197.5±0.3	226.5±3.5	228.8±2.2	443.8±0.7	31.7±2.8
3g	91±1.7	108±3.1	154±1.9	217±1.3	326±2.7	400±2.2	38.3±0.9
3h	63.5±2.1	72.3±1.4	73.3±2.1	82±1.4	98.3±4.1	121.3±2.3	>50
3i	110.8±5.6	142 ±0.4	205.3±3.4	344.8±3.5	383.8±1.8	605.3±1.4	25.8±1.5
3j	76.55±2.4	84.3±3.8	110 ±2.1	162.3±4.2	211.5±3.1	247.8±1.6	34.6±0.9
3k	76.3±3.5	86.3±0.9	148±2.3	266±3.8	454.8±2.9	564.5±2.4	23.9±1.4
3l	57±0.7	70.5±0.7	78.5±3.1	94.3±2.3	164±1.5	171.3±3.1	27.7±1.8
4a	65.5±3.7	92.5±3.8	158.8±2.7	176.3±1.1	246.8±3.6	289.8±4.8	49.9±3.7
4b	88.3±2.1	121.3±0.7	184.1 ±3.5	220 ±3.1	300±3.3	350.3±3.4	39.7±1.6
4c	131.8±4.4	173.3±1.6	270.5±4.7	346.8±1.7	372.3±1.7	372.3±3.8	26.3±1.3
Ascorbic acid	74.9±3.4	123.6±3.1	215.8±1.7	309.7±0.6	397.4±3.7	484±2.3	9.6±1.5



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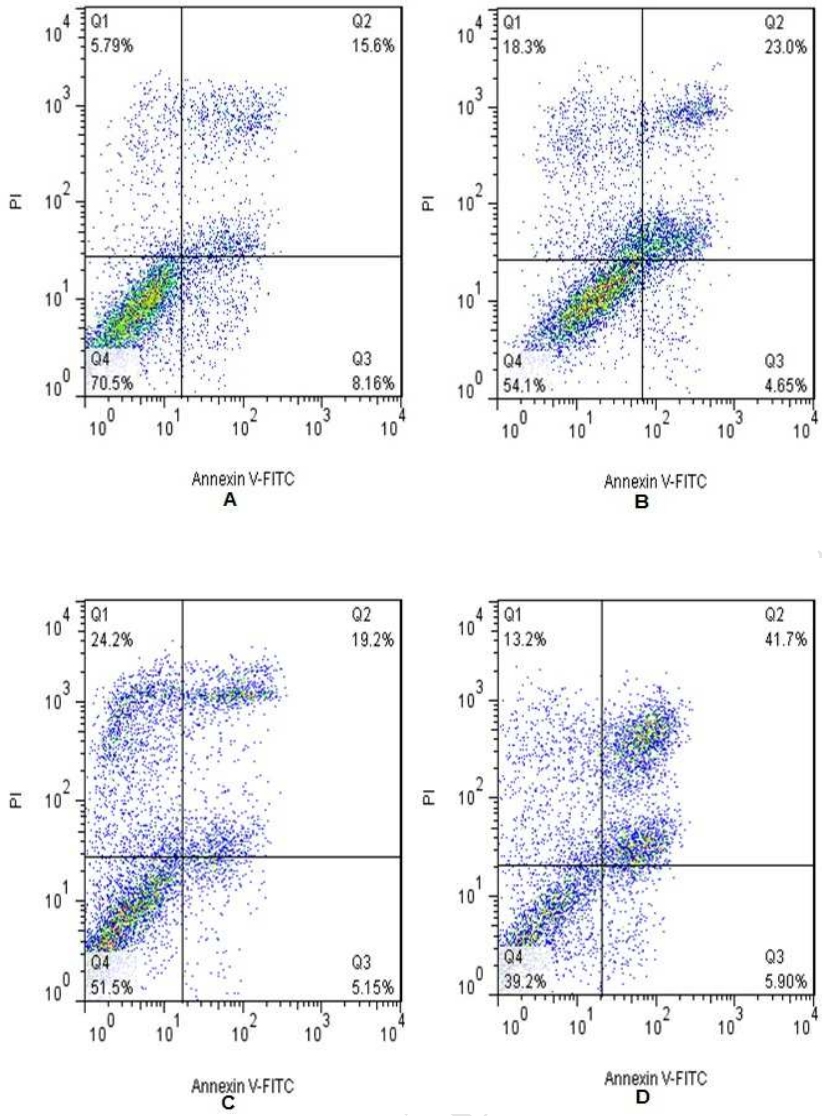

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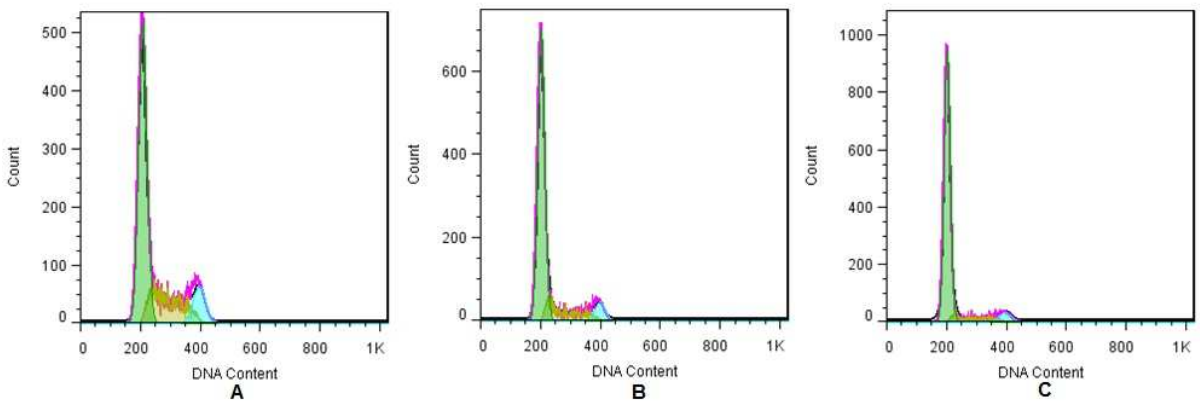

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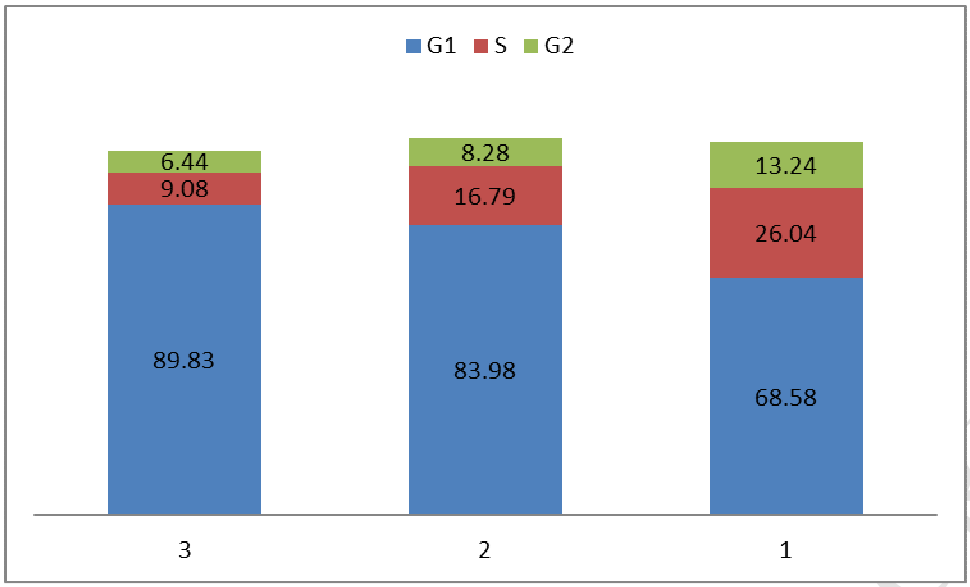

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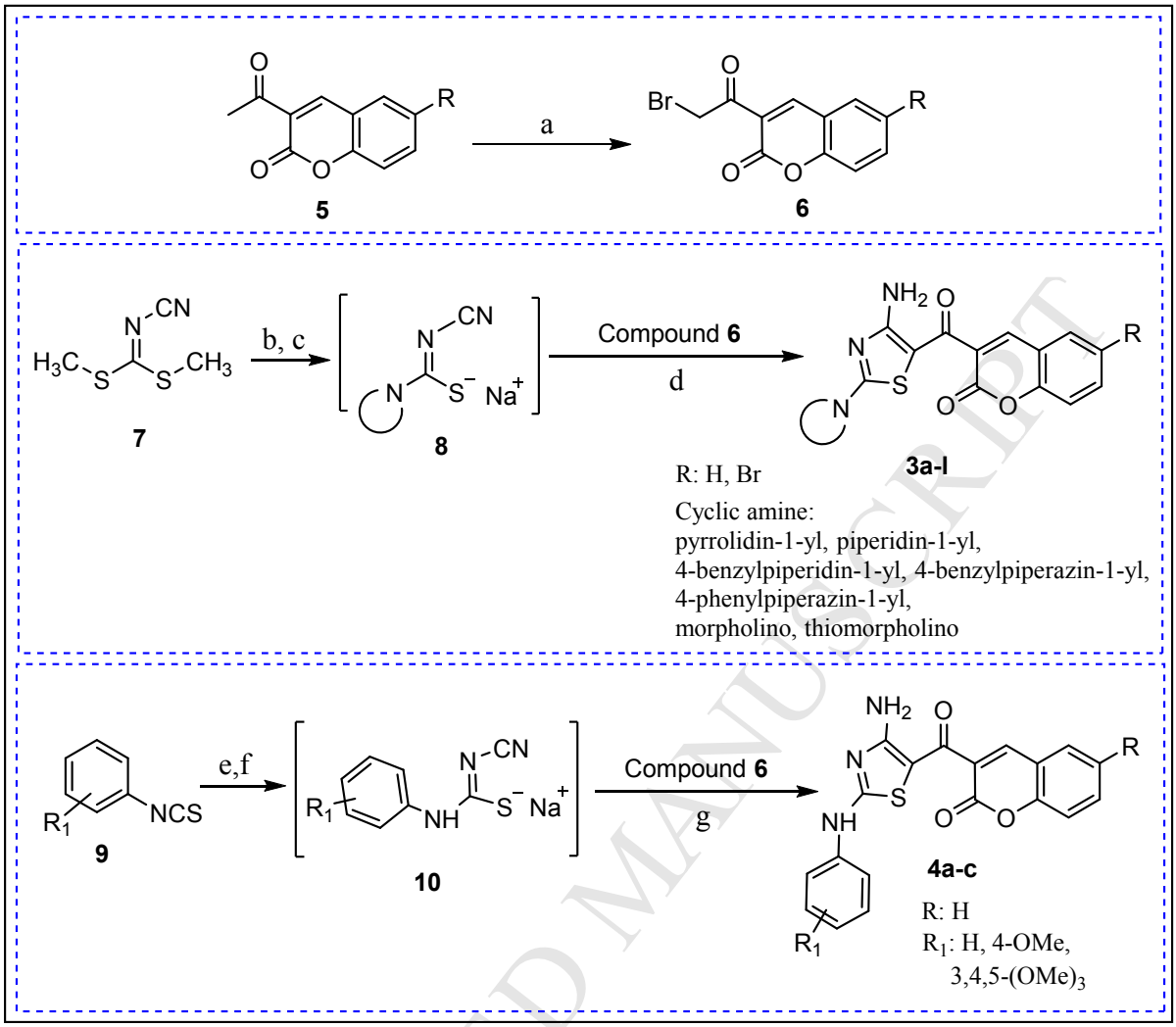

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Research Highlights

- New coumarin-containing compounds **3a-l** and **4a-c** was synthesized.
- All compounds were screened for their anticancer and antioxidant activities.
- Compound **3k** can induce apoptotic and causes G1-phase arrest in the cell cycle.
- DPPH and FRAP assays confirmed the antioxidant potential of synthesized compounds.