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Research paper

Facile synthesis and antiproliferative activity of 7*H*-benzo[7,8]chromeno[2,3-*d*]pyrimidin-8-amines☆

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ABSTRACT

A series of 7*H*-benzo[7,8]chromeno[2,3-*d*]pyrimidin-8-amines **6a-t** were synthesized as new potential antiproliferative agents. The *in vitro* antiproliferative activity evaluation of title compounds using MTT assay revealed that most compounds showed significant activity against tested cancer cell lines (A549, MOLT-4, and HeLa). The 2-fluoro-aniline derivatives **6e** and **6l** were the most active compounds against A549 and MOLT-4 cells, respectively. The benzylamine analog **6h** showed superior activity against HeLa cells. However, compound **6l** with IC_{50} values of 5.2–6.9 μM had the best profile of activity against all tested cell lines. The morphological and flow cytometric analyses showed that compound **6l** can induce apoptosis in the MOLT-4 cells.

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1. Introduction

Cancer is defined as a group of complicated diseases characterized by unregulated proliferation of abnormal cells with capability of metastasizing to healthy tissues [1]. It is a fatal disease responsible for more than 20% of all deaths and stands after the cardiovascular diseases in terms of morbidity and mortality [2]. More than 14 million new cases of cancer have been reported annually around the world. The World Health Organization (WHO) estimates that the worldwide burden of cancer will reach to 22 million per year within the next two decades [3,4]. Anticancer drugs are important tools in the treatment of cancers. However, severe side effects resulting from toxicity of anticancer agents on normal cells remains a substantial drawback in their clinical usefulness [5]. Furthermore, majority of the conventional chemotherapy fails due to drug resistance. Indeed, most of cancer-related deaths are considered to be a result of chemotherapy failure [6]. Accordingly, there is a huge academic and industrial interest to find new chemotherapeutic agents with high potency and optimal selectivity and safety.

In the course of identifying various chemical entities which may serve as new leads for designing new anticancer agents [7–12], our

☆ This work is dedicated to the memory of Prof. Abbas Shafiee (1937–2016).

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interest was focused on the chromene and pyrimidine heterocyclic compounds. The chromene-based heterocyclic compounds are attractive derivatives due to their structural and biological diversity, particularly as natural products [13–16]. Cai and coworkers reported different types of 4-aryl-4*H*-chromenes **I** (Fig. 1) as antiproliferative agents with ability to induce apoptosis [17]. On the other hand, 4-aminopyrimidine derivatives have also gained fame as an important scaffold in different approved chemotherapeutic agents like gefitinib, erlotinib and lapatinib. Particularly, Cai et al. also reported novel 4-aminopyrimidine derivatives **II** (Fig. 1) as potent and efficacious apoptosis inducer anticancer agents [18].

In the light of the above mentioned findings, we designed a novel series of chromenopyrimidine derivatives **6a-t** (Fig. 1) as hybrids of 4-aryl-4*H*-chromenes **I** and 4-aminopyrimidines **II**. Thus we describe here, the synthesis and biological activity of 7*H*-benzo[7,8]chromeno[2,3-*d*]pyrimidin-8-amines **6a-t** as new potential anticancer agents.

2. Chemistry

The synthesis of target compounds **6a**–**t** was outlined in Scheme 1. Several procedures were screened for the synthesis of 2-amino-4-phenyl-4*H*-benzo[*h*]chromene-3-carbonitrile derivatives **4a**–**c** via three-component condensation of α-naphthol (**1**), malononitrile (**2**) and appropriate benzaldehyde **3a-c** (Supplementary material). The best result was obtained in the presence of piperidine in refluxing ethanol. Compounds **4a**–**c** were converted to imines **5a**–**c** by reacting with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA). Finally, the cyclization of intermediates **5a**–**c** with different amines was also screened under several conditions to obtain target compounds **6a**–**t** (Supplementary material). The best result was obtained in the presence of acetic acid under microwave irradiation.

3. Results and discussion

The inhibitory activities (IC_{50} values, μM) of compounds **6a**–**t** against proliferation of cancer cell lines A549, MOLT-4, and HeLa were listed in Table 1.

All compounds showed significant activity against cancer cells proliferation at micromolar range. Among them, the 2-fluoro-aniline derivatives **6e** (5.4 ± 0.2 μM) and **6l** (5.2 ± 0.02 μM) were the most active compounds against A549 and MOLT-4 cells, respectively. It should be noted that the IC_{50} values of all 2-fluoro-aniline congeners towards all cell lines were less than 13 μM, indicating substantial cytotoxic profile of fluoro-aniline derivatives. Moreover their 4-fluoro- regioisomer (compound **6m**) had good activity against all cell lines (IC_{50}s = 6.8–10.5 μM).

A survey on the IC_{50}s of test compounds against HeLa cells revealed that the benzylamine derivative **6h** had superior activity (IC_{50} = 5.9 ± 1.4 μM). In addition, the unsubstituted aniline derivative **6a** with IC_{50} value of 6.4 ± 0.3 μM was equipotent as **6a** towards HeLa cells. According to the activities of the other compounds having benzylamine or aniline substitution, it can be concluded that the introduction of substituent on aniline or benzylamine moiety diminished the activity against HeLa cells. Moreover, increasing the distance between aromatic phenyl group and nitrogen of amine dramatically decrease the activity as seen in compounds **6j** and **6k**. The data indicated that derivatives having substitution on R^2 group and 2,5-dimethoxy substituent on 7-phenyl ring are more potent against HeLa cell. While insertion of additional methoxy group (3,4,5-trimethoxy derivatives) reduces the activity.

The screening data indicated that substitution of 2,5-dimethoxy on 7-phenyl ring is promising for improved activity against MOLT-4. For example, 2,5-dimethoxy analogs **6l** and **6n** were more potent than their corresponding unsubstituted congeners (**6e** and **6c**, respectively). In contrast, the 3,4,5-trimethoxy functionality on the 7-phenyl ring (as was found in compounds **6q-t**) could not improve the cytotoxic activity. In the 2,5-dimethoxyphenyl series, the order of activity by considering the substituent on aniline moiety was as follow: 2-F > 4-F > 4-OMe > 4-Cl > 4-Br. The di-substituted congeners (**6f**, **6g**) were more potent than mono-substituted compounds **6b-e** against MOLT-4 cell line.

Generally it was revealed that 2-fluoro derivatives expressed desired activity on all cell lines. After cytotoxic screening of designed compounds, the representative compound **6l** with IC_{50} value of 5.2 μM against MOLT-4 cell line and compound **6e** with IC_{50} value of 5.4 μM against A549 cell line were selected for further studies. Firstly, compound **6e** and **6l** were studied morphologically to identify their potential for induction of apoptosis in A549 and MOLT-4 cells. The fluorescence microscopy after double staining with acridine orange/ethidium bromide demonstrated that compound **6e** and **6l** can reduce cell viability and induce apoptosis in A549 and MOLT-4 cells (Fig. 2 and Fig. 3).

In the next step, flow cytometry analysis with annexin V-FITC/PI double staining was carried out to confirm apoptosis induction by the compounds **6e** and **6l**. The results of flow cytometric analysis showed that exposure of A549 and MOLT-4 cells to the IC_{50} concentration of compound **6e** and **6l** respectively, resulted in apoptosis. As depicted in Fig. 4, about 47% of cells were at the early stage of apoptosis after 24 h treatment, while 44% of them underwent late stage of apoptosis. According to Fig. 5, it was revealed that about 35% of A549 cells were at the early stage of apoptosis after 24 h treatment while, only 3% of them were dead.

4. Conclusion

We designed and synthesized a new series of 7*H*-benzo[7,8]chromeno[2,3-*d*]pyrimidin-8-amines as potential anticancer

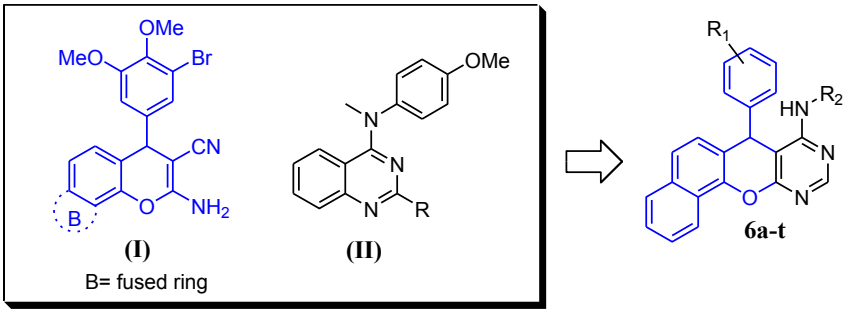
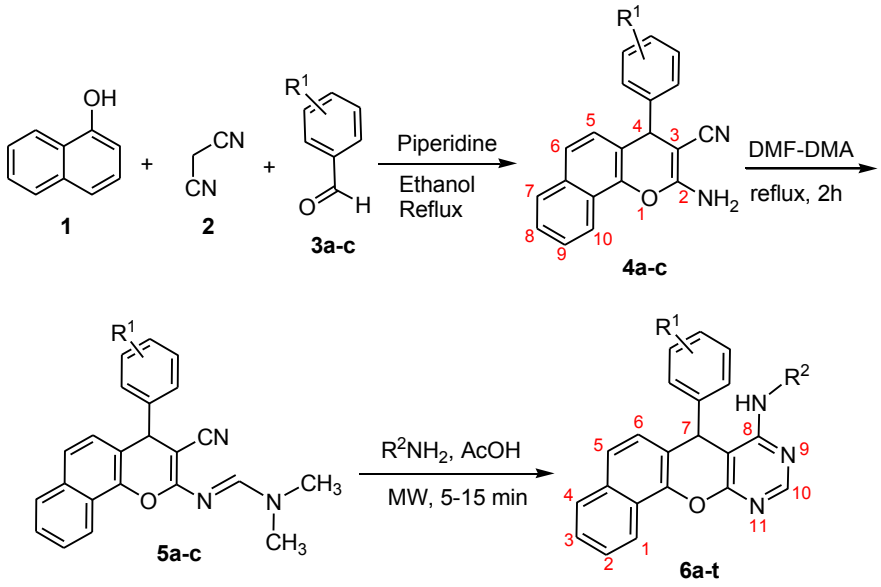


Fig. 1. Design of new compounds **6a-t** as anticancer agents.



Scheme 1. Synthesis of compounds **6a-t**.

agents. The inhibitory activities of compounds **6a**–**t** against proliferation of cancer cells A549, MOLT-4, and HeLa demonstrated the substantial potency of compounds. Particularly, 2-fluoro-aniline derivative **6l** showed better profile of activity against tested cell lines (IC_{50} values = 5.2–6.9 μM). The focused SAR study on the 7-phenyl, 7-(2,5-dimethoxyphenyl), and 7-(3,4,5-trimethoxyphenyl) series revealed that the introduction of 2-fluoroanilino on the 8 position associated with improvement of cytotoxicity. The morphological and flow cytometric analyses showed that compound **6l** can induce apoptosis in the MOLT-4 cells.

5. Experimental

All commercially available reagents were purchased from Merck AG or Aldrich and used without further purification. TLC was conducted on silica gel 250 μm. Melting points were measured on the Buchi Melting point B-540. FT-IR spectra were run on a Bruker, Eqinox 55 spectrometer (ATR). ^{1}H NMR spectra were recorded on a Bruker 400 or 500 MHz NMR instruments. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and Hertz, respectively. The experiments were performed using a microwave oven (ETHOS 1600, Milestone) with a power of 600 W specially designed for an organic synthesis and modified with a condenser and mechanical stirrer.

5.1. General procedure for the synthesis of 2-amino-4-aryl-4H-benzo[h]chromene-3-carbonitrile (*4a-c*)

A mixture of benzaldehyde or 2,5-dimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde (1 mmol), malononitrile (1 mmol), α-naphthol (1 mmol) and three drop of piperidine was refluxed in ethanol for 2–4 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled, filtered and washed with cold ethanol to obtain white solid.

5.1.1. 2-Amino-4-phenyl-4H-benzo[h]chromene-3-carbonitrile (*4a*)

White solid; yield 91%; mp 208–210 °C; IR (KBr, cm^{-1}) ν_{max}: 3465, 3318, 2200, 1660, 1600 and 1550.^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.76 (s, 2H, NH_2), 4.90 (s, 1H, H-4), 7.07–7.12 (m, 6H, H-5 and Ar-$H_{2,3,4,5,6}$), 7.56–7.66 (m, 3H, H-6, H-8 and H-9), 7.94 (d, J = 8.4 Hz, 1H, H-7), 8.23 (d, J = 8.4 Hz, 1H, H-10). Anal. Calcd for $C_{20}H_{14}N_2O$ (298.34): C, 80.52; H, 4.73; N, 9.39. Found: C, 80.79; H, 4.96; N, 9.69.

5.1.2. 2-Amino-4-(2,5-dimethoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (*4b*)

White solid; yield 89%; mp 216–219 °C; IR (KBr, cm^{-1}) ν_{max}: 3418, 3340, 2190, 1636 and 1590.^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.67 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 4.72 (s, 2H, NH_2), 5.39 (s, 1H, H-4), 6.61 (s, 1H, Ar-H_6), 6.72–6.74 (m, 1H, H-5), 6.87 (d, J = 8.2 Hz, 1H, Ar-H_4), 7.14 (d, J = 8.2 Hz, 1H, Ar-H_3), 7.48–7.56 (m, 3H, H-6, H-8 and H-9), 7.78 (d, J = 7.4 Hz, 1H, H-7), 8.17 (d, J = 7.4 Hz, 1H, H-10). Anal. Calcd for $C_{22}H_{18}N_2O_3$ (358.39): C, 73.73; H, 5.06; N, 7.82. Found: C, 73.50; H, 4.86; N, 7.60.

5.1.3. 2-Amino-4-(3,4,5-trimethoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (*4c*)

White solid; yield 88%; mp 201–202 °C; IR (KBr, cm^{-1}) ν_{max}: 3444, 3336, 2187, 1667 and 1590.^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.77 (s, 6H, 2 × CH_3O), 3.83 (s, 3H, -OCH_3), 4.76 (s, 2H, NH_2), 4.83 (s, 1H, H-4), 6.43 (s, 2H, Ar-$H_{2,6}$), 7.08 (d, J = 8.4 Hz, 1H, H-5), 7.53–7.59 (m, 3H, H-6, H-8 and H-9), 7.82 (d, J = 6.8 Hz, 1H, H-7), 8.20 (d, J = 7.6 Hz, 1H, H-10). Anal. Calcd for $C_{23}H_{20}N_2O_4$ (388.14): C, 71.12; H, 5.19; N, 7.21. Found: C, 71.46; H, 5.39; N, 7.53.

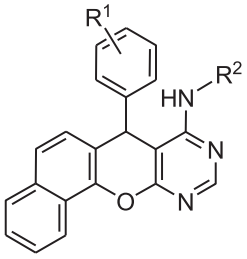
5.2. General procedure for the preparation of compounds *5a-c*

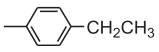
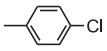
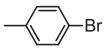
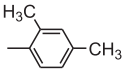
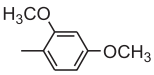
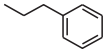
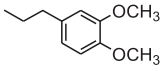
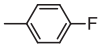
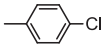
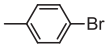
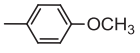
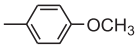
A mixture of compound **4** (10 mmol) in *N,N*-dimethylformamide dimethylacetal (15 ml) was heated under reflux for 3–4 h. After concentration of the reaction mixture, the residue was triturated with diethyl ether to give compounds **5a-c**.

5.2.1. N'-(3-Cyano-4-phenyl-4H-benzo[h]chromen-2-yl)-N,N-dimethylformimidamide (*5a*)

White solid; yield 91%; mp 242–244 °C; IR (KBr, cm^{-1}) ν_{max}: 2191, 1623, 1604 and 1576.^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.17 (s, 3H, -CH_3), 3.22 (s, 3H, -CH_3), 4.95 (s, 1H, H-4), 7.03–7.05 (m, 1H, H-5), 7.27 (br s, 5H, Ar-$H_{2,3,4,5,6}$), 7.48–7.58 (m, 3H, H-6, H-8 and H-9), 7.80 (d, J = 6.8 Hz, 1H, H-7), 8.20 (d, J = 7.2 Hz, 1H, H-10), 8.42 (s, 1H, -N=CH-). Anal. Calcd for $C_{23}H_{19}N_3O$ (353.42): C, 78.16; H, 5.42; N, 11.89. Found: C, 78.46; H, 5.08; N, 11.72.

Table 1
Cytotoxic activities (IC_{50} values, μM, Mean ± SEM) of compounds **6a-t** against cancer cell lines.



Compound	R^1	R^2	A549	MOLT-4	HeLa
6a	H		12.5 ± 2.6	6.6 ± 0.2	6.4 ± 0.3
6b	H		17.4 ± 2.9	17.6 ± 3.1	14.5 ± 1.3
6c	H		13.7 ± 1.8	16.4 ± 1.2	19.9 ± 2.6
6d	H		>100	29.4 ± 3.2	31.6 ± 3.0
6e	H		5.4 ± 0.2	12.8 ± 0.4	10.3 ± 1.9
6f	H		11.2 ± 1.6	8.9 ± 0.2	14.6 ± 2.3
6g	H		10.9 ± 0.5	6.5 ± 0.1	15.9 ± 2.2
6h	H		10.9 ± 2.2	12.7 ± 1.3	5.9 ± 1.4
6i	H		9.6 ± 1.8	11.7 ± 1.0	15.4 ± 2.6
6j	H		68.5 ± 4.4	16.9 ± 1.2	50.2 ± 2.8
6k	H		18.9 ± 2.6	14.5 ± 1.7	43.5 ± 3.4
6l	2,5-$(OCH_3)_2$		6.3 ± 0.9	5.2 ± 0.02	6.9 ± 1.1
6m	2,5-$(OCH_3)_2$		8.4 ± 1.3	6.8 ± 0.2	10.5 ± 1.7
6n	2,5-$(OCH_3)_2$		14.8 ± 2.1	7.4 ± 1.4	15.9 ± 3.0
6o	2,5-$(OCH_3)_2$		26.8 ± 2.4	20.4 ± 1.1	16.7 ± 2.3
6p	2,5-$(OCH_3)_2$		7.2 ± 1.8	6.9 ± 1.5	10.6 ± 1.7
6q	3,4,5-$(OCH_3)_3$		18.6 ± 4.9	9.8 ± 2.9	20.7 ± 4.5
6r	3,4,5-$(OCH_3)_3$		46.5 ± 5.4	33.5 ± 4.2	>100
6s	3,4,5-$(OCH_3)_3$		26.5 ± 3.7	10.4 ± 1.2	44.6 ± 4.9
6t	3,4,5-$(OCH_3)_3$		22.2 ± 3.0	14.5 ± 1.5	33.2 ± 3.2
Cisplatin	–	–	3.9 ± 0.2	4.6 ± 0.7	3.2 ± 0.4

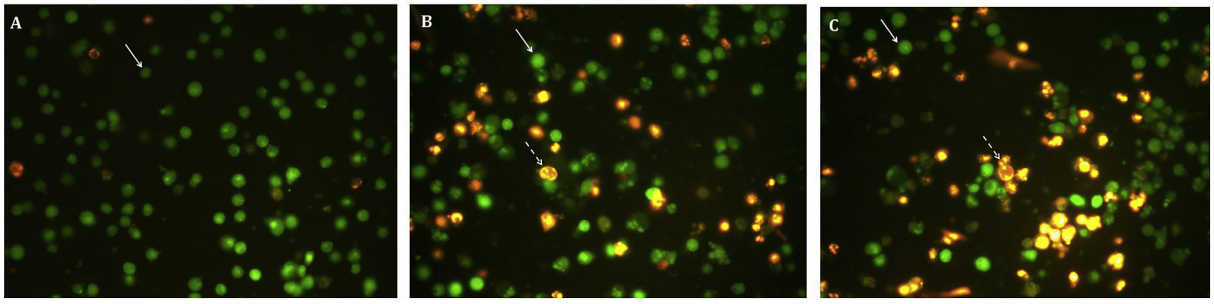


Fig. 2. Morphological analysis of MOLT-4 cells by double staining method. a) Control condition; b) cells treated with IC_{50} concentration of Cisplatin c) cells treated with IC_{50} concentration of compound **6l** for 24 h. White arrow indicates live cells, dash arrow shows apoptosis. The images of cells were taken with a fluorescence microscope at 400×.

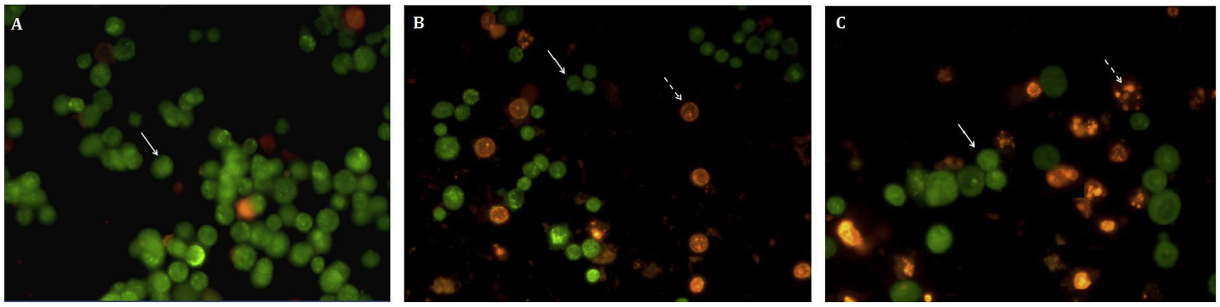


Fig. 3. Morphological analysis of A549 cells by double staining method. a) Control condition; b) cells treated with IC_{50} concentration of Cisplatin c) cells treated with IC_{50} concentration of compound **6e** for 24 h. White arrow indicates live cells, dash arrow shows apoptosis.

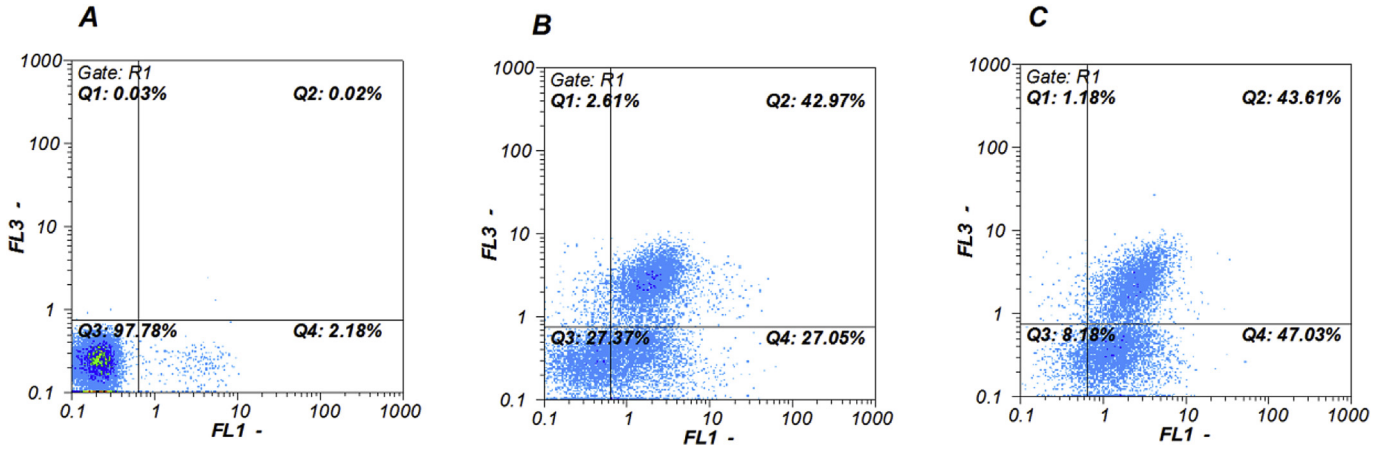


Fig. 4. Flow cytometric analysis of MOLT-4 cell line treated with compound **6l**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. The cells treated with a) DMSO 1% (negative control); b) IC_{50} concentration of Cisplatin; C) IC_{50} concentration of compound **6l**.

*5.2.2. N'-(3-Cyano-4-(2,5-dimethoxyphenyl)-4H-benzo[h] chromen-2-yl)-N,N-dimethylformimidamide (**5b**)*

White solid; yield 90%; mp 223–225 °C; IR (KBr, cm^{-1}) ν_{max}: 2924, 2195, 1625, 1607 and 1588.^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.18 (s, 3H, $-CH_3$), 3.22 (s, 3H, $-CH_3$), 3.65 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-OCH_3$), 5.51 (s, 1H, H-4), 6.67–6.71 (m, 2H, H-5 and Ar-H_6), 6.86 (d, J = 8.4 Hz, 1H, Ar-H_4), 7.14–7.17 (m, 1H, Ar-H_3), 7.45–7.55 (m, 3H, H-6, H-8 and H-9), 7.82 (d, J = 6.8 Hz, 1H, H-7), 8.17 (d, J = 6.8 Hz, 1H, H-10), 8.41 (s, 1H, -N=CH-). Anal. Calcd for $C_{25}H_{23}N_3O_3$ (413.47): C, 72.62; H, 5.61; N, 10.16. Found: C, 72.90; H, 5.36; N, 10.47.

*5.2.3. N'-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-4H-benzo[h] chromen-2-yl)-N,N-dimethylformimidamide (**5c**)*

White solid; yield 89%; mp 252–254 °C; IR (KBr, cm^{-1}) ν_{max}: 2191, 1632, 1604 and 1588.^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.18 (s, 3H, $-CH_3$), 3.23 (s, 3H, $-CH_3$), 3.79 (s, 6H, 2 × CH_3O), 3.81 (s, 3H, $-OCH_3$), 4.90 (s, 1H, H-4), 6.45 (s, 2H, Ar-$H_{2,6}$), 7.07 (d, J = 7.6 Hz, 1H, H-5), 7.51–7.59 (m, 3H, H-6, H-8 and H-9), 7.81 (d, J = 7.6 Hz, 1H, H-7), 8.18 (d, J = 7.6 Hz, 1H, H-10), 8.43 (s, 1H, -N=CH-). Anal. Calcd for $C_{26}H_{25}N_3O_4$ (443.49): C, 70.41; H, 5.68; N, 9.47. Found: C, 70.18; H, 5.37; N, 9.84.

*5.3. General procedure for the preparation of compounds **6a-t***

A mixture of compound **5** (0.5 mmol) and appropriate amine (1 mmol) in acetic acid (2 ml) was irradiated with microwaves at 700 W for 5–15 min. Then, water was added to the reaction mixture and the crude product was extracted with

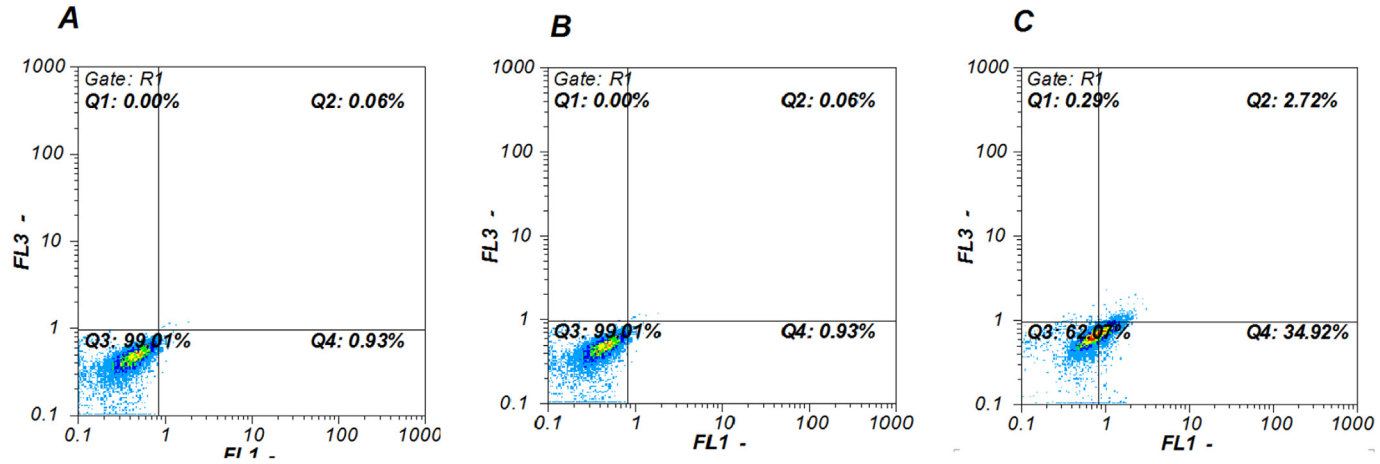


Fig. 5. Flow cytometric analysis of A549 cell line treated with compound **6e**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. The cells treated with a) DMSO 1% (negative control); b) IC_{50} concentration of Cisplatin; C) IC_{50} concentration of compound **6e**.

dichloromethane. After evaporation of organic solvent under reduced pressure, the residue was crystallized from ethanol to give pure compound **6**.

*5.3.1. N,7-Diphenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6a**)*

Yellow solid; yield 89%; mp 242–244 °C; IR (KBr, cm^{-1}) ν_{max}: 3406, 1655, 1633, 1592; ^1H NMR (500 MHz, $CDCl_3$) δ: 5.25 (s, 1H, H-7), 7.11–7.13 (m, 2H, Ar-$H_{2,6}$), 7.22–7.23 (m, 3H, Ar-$H_{3,5}$ and *N*-Ar-H_4), 7.29–7.32 (m, 2H, *N*-Ar-$H_{3,5}$), 7.35–7.37 (m, 1H, Ar-H_4), 7.42–7.43 (m, 4H, H-6, *N*-Ar-$H_{2,6}$ and NH), 7.50–7.58 (m, 2H, H-3 and H-5), 7.60–7.64 (m, 1H, H-2), 7.77 (d, J = 8.0 Hz, 1H, H-4), 8.51–8.54 (m, 2H, H-1 and H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.9, 121.0, 121.2, 121.6, 121.9, 124.1, 124.2, 125.7, 126.0, 126.7, 126.8, 126.9, 127.4, 128.0, 128.2, 128.4, 128.9, 129.3, 143.3, 157.1, 159.6. Anal. Calcd for $C_{27}H_{19}N_3O$ (401.46): C, 80.78; H, 4.77; N, 10.47. Found: C, 80.45; H, 4.98; N, 10.16.

*5.3.2. N-(4-Ethylphenyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6b**)*

Yellow solid; yield 86%; mp 233–235 °C; IR (KBr, cm^{-1}) ν_{max}: 3422, 1645, 1591, 1562 and 1511; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.21 (t, J = 7.6 Hz, 3H, -CH_3), 2.61 (q, J = 7.6 Hz, 2H, -CH_2), 5.38(s, 1H, H-7), 7.08 (d, J = 8.5 Hz, 1H, H-6), 7.13 (s, 4H, *N*-Ar-$H_{3,5}$ and Ar-$H_{2,6}$), 7.31–7.33 (m, 2H, Ar-H_4 and NH), 7.37–7.39 (m, 4H, Ar-$H_{3,5}$ and *N*-Ar-$H_{2,6}$), 7.49 (d, J = 8.5 Hz, H-5), 7.51–7.53 (m, 1H, H-3), 7.56–7.59 (m, 1H, H-2), 7.74 (d, J = 8.0 Hz, 1H, H-4), 8.41 (s, 1H, H-10), 8.49 (d, J = 8.2 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 15.6, 28.3, 40.7, 96.9, 116.8, 121.7, 121.9, 122.2, 123.9, 124.5, 125.7, 126.7, 127.0, 127.4, 128.2, 128.3, 128.4, 129.8, 133.3, 135.5, 140.7, 143.1, 156.5, 159.7. Anal. Calcd for $C_{29}H_{23}N_3O$ (429.5): C, 81.09; H, 5.40; N, 9.78. Found: C, 81.38; H, 5.06; N, 9.52.

*5.3.3. N-(4-Chlorophenyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6c**)*

Yellow solid; yield 92%; mp 206–208 °C; IR (KBr, cm^{-1}) ν_{max}: 3418, 1649, 1631, 1606 and 1587; ^1H NMR (500 MHz, $CDCl_3$) δ: 5.28 (s, 1H, H-7), 6.60 (s br, 1H, NH), 7.13 (d, J = 8.6 Hz, 1H, H-6), 7.18–7.26 (m, 5H, *N*-Ar-$H_{2,6}$ and Ar-$H_{2,4,6}$), 7.33–7.46 (m, 4H, *N*-Ar-$H_{3,5}$ and Ar-$H_{3,5}$), 7.51 (d, J = 8.6 Hz, 1H, H-5), 7.54 (t, J = 7.6, 1H, H-3), 7.60 (t, J = 7.6 Hz, 1H, H-2), 7.77 (d, J = 8.0 Hz, 1H, H-4), 8.50–8.53 (m, 2H, H-1 and H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.6, 121.8, 122.4, 124.4, 125.6, 126.1, 126.3, 126.8, 127.1, 127.4, 128.2, 128.5, 128.9, 129.2, 129.9, 133.3, 143.0, 143.3, 156.4, 159.3, 161.1, 161.6. Anal. Calcd for $C_{27}H_{18}ClN_3O$ (435.90): C, 74.39; H, 4.16; N, 9.64. Found: C, 74.69; H, 4.44; N, 9.95.

*5.3.4. N-(4-Bromophenyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6d**)*

Yellow solid; yield 87%; mp 237-239 °C; IR (KBr, cm^{-1}) ν_{max}: 3407, 1645, 1608, 1586 and 1570; ^1H NMR (500 MHz, $CDCl_3$) δ: 5.27 (s, 1H, H-7), 7.13–7.16 (m, 2H, *N*-Ar-$H_{2,6}$), 7.35–7.40 (m, 4H, *N*-Ar-$H_{3,5}$ and Ar-$H_{3,5}$), 7.42–7.44 (m, 4H, Ar-$H_{2,4,6}$ and NH), 7.50 (d, J = 8.0, 1H, H-6), 7.54–7.56 (m, 2H, H-2 and H-3), 7.58–7.60 (m, 1H, H-5), 7.77 (d, J = 8.0 Hz, 1H, H-4), 8.51–8.53 (m, 2H, H-1 and H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 29.6, 96.1, 121.9, 122.7, 122.9, 124.5, 125.6, 126.0, 126.9, 127.0, 127.4, 127.5, 127.8, 128.0, 128.2, 128.6, 128.9, 130.0, 131.8, 132.3, 143.3, 143.4, 159.3. Anal. Calcd for $C_{27}H_{18}BrN_3O$ (480.36): C, 67.51; H, 3.78; N, 8.75. Found: C, 67.82; H, 3.46; N, 8.39.

*5.3.5. N-(2-Fluorophenyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6e**)*

Yellow solid; yield 89%; mp 225–227 °C; IR (KBr, cm^{-1}) ν_{max}: 3421, 1648, 1625, 1594; ^1H NMR (500 MHz, $CDCl_3$) δ: 5.23 (s, 1H, H-7), 6.81–6.82 (m, 1H, NH), 6.97–7.07 (m, 2H, *N*-Ar-$H_{4,6}$), 7.11–7.15 (m, 1H, *N*-Ar-$H_{3,5}$), 7.27–7.32 (m, 1H, Ar-H_4), 7.36–7.39 (m, 2H, Ar-$H_{3,5}$), 7.42–7.43 (m, 2H, Ar-$H_{2,6}$), 7.49–7.54 (m, 2H, H-5 and H-6), 7.59–7.62 (m, 1H, H-3), 7.76 (d, J = 8.2 Hz, 1H, H-4), 8.34–8.39 (m, 1H, H-2), 8.54 (d, J = 8.4 Hz, 1H, H-1), 8.57 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.8, 98.4, 114.7, 114.9, 117.0, 121.8, 122.2, 122.3, 123.7, 123.8, 124.3, 124.4, 125.7, 126.7, 127.0, 127.4, 128.1, 128.2, 129.7, 133.3, 142.8, 143.4, 152.0, 154.0, 156.6, 159.2, 162.2. Anal. Calcd for $C_{27}H_{18}FN_3O$ (419.45): C, 77.31; H, 4.33; N, 10.02. Found: C, 77.70; H, 4.64; N, 10.37.

*5.3.6. N-(2,4-Dimethylphenyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6f**)*

Yellow solid; yield 89%; mp 187–188 °C; IR (KBr, cm^{-1}) ν_{max}: 3410, 1645, 1589, 1562 and 1509; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.64 (s, 3H, -CH_3), 2.29 (s, 3H, -CH_3), 5.25 (s, 1H, H-7), 6.93 (s, 1H, *N*-Ar-H_3), 7.01 (d, J = 8.0 Hz, 1H, *N*-Ar-H_6), 7.16 (d, J = 8.5 Hz, 1H, H-6), 7.19 (d, J = 8.0 Hz, 1H, *N*-Ar-H_5), 7.31–7.39 (m, 6H, NH and Ar-$H_{2,3,4,5,6}$), 7.51 (d, J = 8.5 Hz, 1H, H-5), 7.54–7.55 (m, 1H, H-2), 7.61 (t, J = 8.0 Hz, 1H, H-3), 7.77 (d, J = 8.0 Hz, 1H, H-4), 8.44 (s, 1H, H-10), 8.54 (d, J = 8.5 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 17.6, 20.9, 38.5, 97.5, 119.5, 121.2, 123.7, 124.6, 126.7, 126.9, 127.1, 127.3, 127.5, 128.1, 129.1, 131.2, 133.2, 134.2, 134.9, 135.3, 144.4, 144.5, 156.9,

160.5, 162.7. Anal. Calcd for $C_{29}H_{23}N_3O$ (429.51): C, 81.09; H, 5.40; N, 9.78. Found: C, 81.38; H, 5.73; N, 9.47.

5.3.7. N-(2,4-Dimethoxyphenyl)-7-phenyl-7H-benzo[7,8]chromeno [2,3-d]pyrimidin-8-amine (**6g**)

Yellow solid; yield 83%; mp 206–207 °C; IR (KBr, cm^{-1}) ν_{max}: 3399, 1604, 1564, 1528; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.71 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3), 5.22(s, 1H, H-7), 6.43 (d, J = 2.6 Hz, 1H, *N*-Ar-H_3), 6.48–6.50 (dd, J = 9.0 Hz, J = 2.6 Hz, 1H, *N*-Ar-H_5), 6.96 (s, 1H, NH), 7.17–7.19 (d, J = 9.0 Hz, 1H, *N*-Ar-H_6), 7.27–7.28 (m, 1H, Ar-H_4), 7.35 (t, J = 7.6 Hz, 2H, Ar-$H_{3,5}$), 7.43–7.44 (m, 2H, Ar-$H_{2,6}$), 7.46–7.53 (m, 2H, H-3 and H-6), 7.59 (t, J = 7.6 Hz, 1H, H-2), 7.76 (d, J = 8.2 Hz, 1H, H-5), 8.23 (d, J = 8.8 Hz, 1H, H-4), 8.52 (s, 1H, H-10), 8.54 (d, J = 8.2 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.7, 55.5, 57.1, 97.6, 98.7, 103.7, 117.3, 121.7, 121.9, 123.9, 124.1, 125.8, 126.6, 126.8, 127.3, 127.8, 128.3, 129.3, 133.2, 143.2, 143.5, 149.9, 156.1, 156.8, 159.3, 162.1. Anal. Calcd for $C_{29}H_{23}N_3O_3$ (461.51): C, 75.47; H, 5.02; N, 9.10. Found: C, 75.74; H, 5.36; N, 9.37.

5.3.8. N-Benzyl-7-phenyl-7H-benzo[7,8]chromeno[2,3-d] pyrimidin-8-amine (**6h**)

Yellow solid; yield 82%; mp 210–211 °C; IR (KBr, cm^{-1}) ν_{max}: 3426, 1645, 1589, 1569; ^1H NMR (500 MHz, $CDCl_3$) δ: 4.48 (dd, J = 14.9 Hz, J = 4.7 Hz, 1H, -CH_2), 4.72 (dd, J = 14.9 Hz, J = 4.7 Hz, 1H, -CH_2), 5.11 (s, 2H, NH and H-7), 6.85–6.86 (m, 2H, *N*-Ar-$H_{2,6}$), 7.09 (d, J = 8.5, 1H, H-6), 7.20–7.24 (m, 3H, *N*-Ar-$H_{3,4,5}$), 7.26–7.32 (m, 5H, Ar-$H_{2,3,4,5,6}$), 7.47 (d, J = 8.5 Hz, 1H, H-5), 7.51–7.54 (m, 1H, H-3), 7.58–7.61 (m, 1H, H-2), 7.75 (d, J = 8.1 Hz, 1H, H-4), 8.44 (s, 1H, H-10), 8.52 (d, J = 8.5 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.5, 45.3, 95.9, 117.0, 121.9, 123.9, 124.0, 125.8, 126.6, 126.8, 127.0, 127.35, 127.39, 127.9, 128.0, 128.6, 129.6, 133.2, 137.9, 143.5, 143.6, 157.0, 161.2, 161.7. Anal. Calcd for $C_{28}H_{21}N_3O$ (415.49): C, 80.94; H, 5.09; N, 10.11. Found: C, 80.63; H, 5.38; N, 9.86.

5.3.9. N-(2-Chlorobenzyl)-7-phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6i**)

Yellow solid; yield 83%; mp 196–198 °C; IR (KBr, cm^{-1}) ν_{max}: 3418, 1650, 1608, 1586; ^1H NMR (500 MHz, $CDCl_3$) δ: 4.63 (dd, J = 15.5 Hz, J = 5.5 Hz, 1H, -CH_2), 4.75 (dd, J = 15.5 Hz, J = 5.5 Hz, 1H, -CH_2), 5.11 (s, 1H, H-7), 5.26 (br s, 1H, NH), 5.26 (br s, 1H, NH), 6.84 (d, J = 6.7 Hz, 1H, *N*-Ar-H_6), 7.07 (t, 1H, J = 7.7 Hz, Ar-H_4), 7.10 (d, J = 8.5 Hz, 1H, H-6), 7.15–7.18 (m, 1H, *N*-Ar-H_5), 7.24–7.26 (m, 1H, *N*-Ar-H_3), 7.30–7.35 (m, 5H, Ar-$H_{2,3,5,6}$ and *N*-Ar-H_4), 7.46 (d, J = 8.5 Hz, 1H, H-5), 7.50–7.52 (m, 1H, H-3), 7.57–7.60 (m, 1H, H-2), 7.73 (d, J = 8.1 Hz, 1H, H-4), 8.40 (s, 1H, H-10), 8.51 (d, J = 8.5 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.3, 43.1, 96.1, 116.9, 121.9, 123.8, 124.2, 125.7, 126.7, 126.8, 126.9, 127.3, 128.0, 128.7, 128.9, 129.1, 129.4, 129.6, 133.2, 133.3, 135.3, 143.2, 143.3, 143.5, 156.1, 161.0. Anal. Calcd for $C_{28}H_{20}ClN_3O$ (449.93): C, 74.74; H, 4.48; N, 9.34. Found: C, 74.99; H, 4.80; N, 9.63.

5.3.10. N-Phenethyl-7-phenyl-7H-benzo[7,8]chromeno[2,3-d] pyrimidin-8-amine (**6j**)

Yellow solid; yield 74%; mp 194–195 °C; IR (KBr, cm^{-1}) ν_{max}: 3426, 1593, 1610, 1570 and 1496; ^1H NMR (500 MHz, $CDCl_3$) δ: 2.73–2.78 (m, 2H, -$NCH_2C\underline{H}_2$), 3.70–3.72 (m, 1H, -$NC\underline{H}_2$), 3.80–3.83 (m, 1H, -$NC\underline{H}_2$), 4.87 (s, 1H, H-7), 7.03 (d, J = 8.5 Hz, 1H, H-6), 7.07 (d, J = 6.6 Hz, 2H, *N*-Ar-$H_{2,6}$), 7.10–7.12 (m, 3H, *N*-Ar-$H_{3,4,5}$), 7.23–7.26 (m, 3H, Ar-$H_{3,5}$ and NH), 7.27–7.32 (m, 3H, Ar-$H_{2,4,6}$), 7.46 (d, J = 8.5 Hz, 1H, H-5), 7.51–7.53 (m, 1H, H-3), 7.57–7.60 (m, 1H, H-2), 7.74 (d, J = 8.1 Hz, 1H, H-4), 8.45 (s, 1H, H-10), 8.50 (d, J = 8.5 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 111.3, 111.5, 117.2, 121.9, 124.0, 125.7, 126.5, 126.8, 127.3, 127.7, 128.7, 129.4, 130.7, 133.2, 138.4, 143.2, 147.8, 149.2, 156.7, 161.2, 161.6. Anal. Calcd for $C_{29}H_{23}N_3O$ (429.51): C, 81.09; H, 5.40; N, 9.78. Found: C, 81.46; H, 5.69; N, 9.99.

5.3.11. N-(2,4-Dimethoxyphenethyl)-7-phenyl-7H-benzo[7,8] chromeno[2,3-d]pyrimidin-8-amine (**6k**)

Yellow solid; yield 82%; mp 154–156 °C; IR (KBr, cm^{-1}) ν_{max}: 3407, 1643, 1605,1586 and 1570; ^1H NMR (500 MHz, $CDCl_3$) δ: 2.72 (t, J = 6.5 Hz, 2H, *N*-CH_2-$C\underline{H}_2$), 2.82–2.84 (m, 1H, *N*-$C\underline{H}_2$), 3.61–3.63 (m, 1H, *N*-$C\underline{H}_2$), 3.84 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 4.75 (br s, 1H, NH), 4.85 (s, 1H, H-7), 6.60–6.62 (m, 2H, *N*-Ar-$H_{3,5}$), 6.82 (d, J = 8.5 Hz, 1H, H-6), 7.03 (d, J = 8.6 Hz, 1H, *N*-Ar-H_6), 7.07–7.09 (m, 2H, Ar-$H_{3,5}$), 7.20–7.22 (m, 3H, Ar-$H_{2,4,6}$), 7.45 (d, J = 8.5 Hz, 1H, H-5), 7.50–7.53 (m, 1H, H-3), 7.58 (t, J = 8.0 Hz, 1H, H-2), 7.74 (d, J = 8.0 Hz, 1H, H-4), 8.46 (s, 1H, H-10), 8.50 (d, J = 8.5 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 34.7, 40.5, 42.1, 55.8, 55.9, 96.0, 111.3, 111.5, 117.2, 120.6, 121.9, 123.9, 124.1, 125.7, 126.6, 126.8, 127.3, 127.7, 129.3, 130.7, 133.2, 143.1, 143.6, 147.8, 149.2, 156.6, 161.1, 161.6. Anal. Calcd for $C_{31}H_{27}N_3O_3$ (489.56): C, 76.05; H, 5.56; N, 8.58. Found: C, 76.36; H, 5.45; N, 8.28.

5.3.12. 7-(2,5-Dimethoxyphenyl)-N-(2-fluorophenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amine (**6l**)

Yellow solid; yield 86%; mp 228–230 °C; IR (KBr, cm^{-1}) ν_{max}: 3396, 1648, 1620, 1608 and 1580; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.55 (s, 3H, -OCH_3), 4.12 (s, 3H, -OCH_3), 5.78 (s, 1H, H-7), 6.56 (d, J = 2.7 Hz, 1H, Ar-H_6), 6.71–6.73 (m, 1H, Ar-H_4), 6.74–6.78 (m, 1H, *N*-Ar-H_5), 6.98 (d, J = 8.9 Hz, 1H, Ar-H_3), 7.05 (d, J = 8.4 Hz, 1H, H-6), 7.18 (d, J = 8.0 Hz, 1H, *N*-Ar-H_6), 7.24–7.27 (m, 3H, H-5 and *N*-Ar-$H_{2,4}$), 7.50–7.56 (m, 1H, H-1, H-3), 7.62 (t, J = 7.5 Hz, 1H, H-2), 7.75–7.79 (m, 2H, H-4 and NH), 8.49 (s, 1H, H-10), 8.55 (d, J = 8.4 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 31.5, 55.5, 57.2, 98.8, 108.1, 108.3, 110.1, 110.3, 112.4, 113.5, 115.9, 116.9, 117.7, 121.8, 123.9, 124.5, 125.9, 126.6, 126.9, 127.4, 129.8, 129.9, 133.1, 133.3, 144.7, 148.4, 155.2, 156.1, 159.0, 161.9. Anal. Calcd for $C_{29}H_{22}FN_3O_3$ (479.50): C, 72.64; H, 4.62; N, 8.76. Found: C, 72.95; H, 4.34; N, 8.47.

5.3.13. 7-(2,5-Dimethoxyphenyl)-N-(4-fluorophenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amine (**6m**)

Yellow solid; yield 91%; mp 195–196 °C; IR (KBr, cm^{-1}) ν_{max}: 3384, 1632, 1601, 1516; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.55 (s, 3H, -OCH_3), 4.08 (s, 3H, -OCH_3), 5.78 (s, 1H, H-7), 6.54–6.57 (d, J = 3 Hz, 1H, Ar-H_6), 6.72–6.75 (dd, J = 9.0 Hz, J = 3.0 Hz, 1H, Ar-H_4), 6.97 (d, J = 9.0 Hz, 1H, Ar-H_3), 7.01–7.06 (m, 2H, *N*-Ar-$H_{3,5}$), 7.41–7.44 (m, 2H, *N*-Ar-$H_{2,6}$), 7.52 (d, J = 8.5 Hz, 1H, H-6), 7.53–7.56 (m, 1H, H-3), 7.61–7.64 (m, 1H, H-2), 7.68 (br s, 1H, NH), 7.79 (d, J = 8.1 Hz, 1H, H-5), 7.96 (d, J = 8.1 Hz, 1H, H-4), 8.43 (s, 1H, H-10), 8.56 (d, J = 8.4 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 31.6, 55.5, 57.1, 112.3, 113.5, 115.4, 115.6, 116.2, 117.0, 123.0, 121.8, 123.1, 123.9, 124.4, 124.8, 126.0, 126.6, 126.8, 127.4, 127.7, 133.4, 133.6, 134.9, 144.8, 148.6, 155.1, 156.4, 159.3, 162.4. Anal. Calcd for $C_{29}H_{22}FN_3O_3$ (479.50): C, 72.64; H, 4.62; N, 8.76. Found: C, 72.35; H, 4.34; N, 8.97.

5.3.14. N-(4-Chlorophenyl)-7-(2,5-dimethoxyphenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amine (**6n**)

Yellow solid; yield 90%; mp 215–217 °C; IR (KBr, cm^{-1}) ν_{max}: 3358, 1618, 1591, 1498; ^1H NMR (500 MHz, $CDCl_3$) δ: 3.54 (s, 3H, -OCH_3), 4.09 (s, 3H, -OCH_3), 5.77 (s, 1H, H-7), 6.56 (d, J = 3.0 Hz, 1H, Ar-H_6), 6.71–6.74 (dd, J = 9.0 Hz, J = 3.0 Hz, 1H, Ar-H_4), 6.97 (d, J = 9.0 Hz, 1H, Ar-H_3), 7.05 (d, J = 8.5 Hz, 1H, H-6), 7.26–7.30 (m, 2H, *N*-Ar-$H_{2,6}$), 7.47 (d, J = 8.8 Hz, 2H, *N*-Ar-$H_{3,5}$), 7.51 (d, J = 8.5 Hz, 1H, H-5), 7.53–7.56 (m, 1H, H-3), 7.62 (t, J = 8.0 Hz, 1H, H-2), 7.70 (s, 1H, NH), 7.79 (d, J = 7.8 Hz, 1H, H-4), 8.45 (s, 1H, H-10), 8.55 (d, J = 8.2 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 31.6, 55.5, 57.2, 98.6, 112.4, 113.5, 117.0, 117.7, 121.8, 122.3, 123.9, 124.5, 125.9, 126.6, 126.9, 127.4, 128.7, 128.9, 133.1, 133.3, 137.5, 144.7, 148.5, 155.1, 156.2, 159.1, 162.3. Anal. Calcd for $C_{29}H_{22}ClN_3O_3$ (495.96): C, 70.23; H, 4.47; Cl, 7.15; N, 8.47; Found: C, 70.57; H, 4.62; N, 8.24.

*5.3.15. N-(4-Bromophenyl)-7-(2,5-dimethoxyphenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amine (**6o**)*

Yellow solid; yield 87%; mp 214–215 °C; IR (KBr, cm^{-1}) ν_{max}: 3367, 1645, 1607, 1561; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.55 (s, 3H, -OCH_3), 4.09 (s, 3H, -OCH_3), 5.77 (s, 1H, H-7), 6.56 (d, J = 3.0 Hz, 1H, Ar-H_6), 6.71–6.74 (dd, J = 9.0 Hz, J = 3.0 Hz, 1H, Ar-H_4), 6.97 (d, J = 9.0 Hz, 1H, Ar-H_3), 7.05 (d, J = 8.5 Hz, 1H, H-6), 7.40–7.45 (m, 4H, *N*-Ar-$H_{2,3,5,6}$), 7.51 (d, J = 8.5 Hz, 1H, H-5), 7.52–7.54 (m, 1H, H-3), 7.56–7.62 (m, 1H, H-2), 7.71 (s, 1H, NH), 7.79 (d, J = 8.1 Hz, 1H, H-4), 8.45 (s, 1H, H-10), 8.55 (d, J = 8.4 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 31.5, 55.5, 57.2, 112.4, 113.5, 116.3, 117.0, 117.6, 121.8, 122.6, 123.8, 124.5, 125.9, 126.6, 126.9, 127.4, 131.8, 133.1, 133.3, 138.0, 148.4, 155.1, 156.1, 159.0, 159.5, 162.2. Anal. Calcd for $C_{29}H_{22}BrN_3O_3$ (540.41): C, 64.45; H, 4.10; N, 7.78; Found: C, 64.80; H, 4.38; N, 7.45.

*5.3.16. 7-(2,5-Dimethoxyphenyl)-N-(4-methoxyphenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amine (**6p**)*

Yellow solid; yield 89%; mp 247–249 °C; IR (KBr, cm^{-1}) ν_{max}: 3324, 1629, 1610, 1580; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.55 (s, 3H, -OCH_3), 3.80 (s, 3H, -OCH_3), 4.06 (s, 3H, -OCH_3), 5.77 (s, 2H, H-7 and NH), 6.58 (d, J = 2.9 Hz, 1H, Ar-H_6), 6.72–6.74 (dd, J = 8.9 Hz, J = 2.9 Hz, 1H, Ar-H_4), 6.88 (d, J = 8.9 Hz, 1H, Ar-H_3), 6.96 (d, J = 8.8 Hz, 1H, *N*-Ar-$H_{2,6}$), 7.07 (d, J = 8.4 Hz, 1H, *N*-Ar-$H_{3,5}$), 7.34 (d, J = 8.7 Hz, 1H, H-6), 7.49–7.63 (m, 3H, H-2, H-3 and H-5), 7.79 (d, J = 8.1 Hz, 1H, H-4), 8.40 (s, 1H, H-10), 8.56 (d, J = 8.4 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 30.9, 55.5, 56.9, 112.0, 113.4, 114.1, 114.4, 117.0, 117.9, 120.8, 121.9, 123.5, 124.0, 124.3, 124.4, 124.7, 126.0, 126.1, 126.5, 126.7, 127.4, 127.6, 131.8, 133.3, 148.7, 156.6, 159.6. Anal. Calcd for $C_{30}H_{25}N_3O_4$ (491.54): C, 73.30; H, 5.13; N, 8.55; Found: C, 73.64; H, 4.78; N, 8.31.

*5.3.17. N-Phenyl-7-(3,4,5-trimethoxyphenyl)-7H-benzo[7,8] chromeno[2,3-d]pyrimidin-8-amine (**6q**)*

Cream solid; yield 89%; mp 214–216 °C; IR (KBr, cm^{-1}) ν_{max}: 3425, 1645, 1608, 1592; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.82–3.83 (m, 9H, 3 × CH_3O), 5.17 (s, 1H, H-7), 6.63 (s, 2H, Ar-$H_{2,6}$), 6.70 (s, 1H, NH), 7.08 (t, J = 7.2 Hz, 1H, *N*-Ar-H_4), 7.18 (d, J = 8.7 Hz, 1H, H-6), 7.26–7.32 (m, 4H, *N*-Ar-$H_{2,3,5,6}$), 7.52–7.56 (m, 2H, H-3 and H-5), 7.61 (t, J = 7.5 Hz, 1H, H-2), 7.79 (d, J = 8.0 Hz, 1H, H-4), 8.52–8.54 (m, 2H, H-1 and H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 41.2, 56.3, 60.9, 97.0, 105.2, 116.5, 121.0, 121.9, 123.9, 124.1, 124.2, 125.5, 126.7, 127.0, 127.4, 129.0, 133.4, 138.0, 138.3, 138.9, 154.4, 157.1, 159.8, 162.1. Anal. Calcd for $C_{30}H_{25}N_3O_4$ (491.54): C, 73.30; H, 5.13; N, 8.55. Found: C, 73.14; H, 5.38; N, 8.20.

*5.3.18. N-(4-Methoxyphenyl)-7-(3,4,5-trimethoxyphenyl)-7H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-amine (**6r**)*

White solid; yield 85%; mp 229–231 °C; IR (KBr, cm^{-1}) ν_{max}: 3384, 1631, 1611, 1580; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.71–3.84 (m, 12H, 4 × CH_3O), 5.61 (s, 1H, H-7), 6.69 (s, 2H, Ar-$H_{2,6}$), 6.89–6.91 (m, 2H, *N*-Ar-$H_{3,5}$), 6.99 (br s, 1H, NH), 7.27–7.30 (m, 2H, *N*-Ar-$H_{2,6}$), 7.44–7.54 (m, 3H, H-3, H-5 and H-6), 7.83–7.86 (m, 2H, H-2 and H-4), 8.15 (d, J = 8.4 Hz, 1H, H-1), 8.41 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.1, 54.5, 56.6, 60.5, 96.5, 111.3, 111.5, 120.1, 122.1, 123.9, 124.0, 124.1, 125.5, 126.5, 127.1, 127.2, 129.1, 133.3, 137.5, 137.8, 137.9, 154.1, 156.0, 159.7, 162.1. Anal. Calcd for $C_{31}H_{27}N_3O_5$ (521.56): C, 71.39; H, 5.22; N, 8.06. Found: C, 71.10; H, 5.57; N, 8.34.

*5.3.19. N-Benzyl-7-(3,4,5-trimethoxyphenyl)-7H-benzo[7,8] chromeno[2,3-d]pyrimidin-8-amine (**6s**)*

Yellow solid; yield 83%; mp 167–169 °C; IR (KBr, cm^{-1}) ν_{max}: 3393, 1648, 1589, 1572; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.64 (s, 6H, 2 × CH_3O), 3.80 (s, 3H, -OCH_3), 4.42 (dd, J = 14.3 Hz, J = 5.0 Hz, 1H, *N*-C$\underline{H}_2$), 4.65 (dd, J = 14.3 Hz, J = 5.0 Hz, 1H, *N*-C$\underline{H}_2$), 4.97 (s, 2H, H-7 and NH), 6.44 (s, 2H, Ar-$H_{2,6}$), 6.97–6.99 (m, 2H, *N*-Ar-$H_{2,6}$), 7.09 (d, J = 8.5 Hz, 1H, H-6), 7.27–7.28 (m, 3H, *N*-Ar-$H_{3,4,5}$), 7.48 (d, J = 8.5 Hz, 1H, H-5), 7.52–7.54 (m, 1H, H-3), 7.57–7.60 (m, 1H, H-2), 7.76 (d, J = 8.0 Hz, 1H, H-4), 8.44 (s, 1H, H-10), 8.51 (d, J = 8.4 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 41.0, 45.8, 59.1, 60.8, 95.6, 104.9, 116.6, 121.9, 123.9, 125.68, 125.62, 126.6, 126.9, 127.4, 127.6, 128.7, 133.3, 137.5, 137.8, 139.3, 143.5, 154.1, 157.3, 161.4, 161.6. Anal. Calcd for $C_{30}H_{26}N_3O_4$ (492.55): C, 73.15; H, 5.32; N, 8.53. Found: C, 73.40; H, 5.06; N, 8.84.

*5.3.20. N-(2-Chlorobenzyl)-7-(3,4,5-trimethoxyphenyl)-7H-benzo [7,8]chromeno[2,3-d]pyrimidin-8-amin (**6t**)*

Yellow solid; yield 80%; mp 181–182 °C; IR (KBr, cm^{-1}) ν_{max}: 3397, 1611, 1581, 1562; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.69 (s, 6H, 2 × CH_3O), 3.79 (s, 3H, -OCH_3), 4.60–4.62 (m, 1H, *N*-C$\underline{H}_2$), 4.79–4.68 (m, 1H, *N*-C$\underline{H}_2$), 5.01 (s, 1H, H-7), 5.18 (s, 1H, NH), 6.47 (s, 2H, Ar-$H_{2,6}$), 7.08–7.14 (m, 3H, *N*-Ar-$H_{3,5,6}$), 7.20–7.22 (m, 1H, *N*-Ar-H_4), 7.33 (d, J = 7.5 Hz, 1H, H-6), 7.47–7.58 (m, 3H, H-2, H-3 and H-5), 7.76 (d, J = 7.0 Hz, 1H, H-4), 8.45 (s, 1H, H-10), 8.51 (d, J = 7.9 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 40.9, 43.4, 56.1, 60.8, 95.8, 104.9, 116.6, 121.9, 123.9, 124.0, 125.6, 126.6, 126.9, 127.4, 129.1, 129.5, 129.8, 133.3, 135.2, 139.0, 144.5, 148.5, 153.1, 154.2, 156.8, 156.9, 161.4. Anal. Calcd for $C_{31}H_{26}ClN_3O_4$ (540.01): C, 68.95; H, 4.85; N, 7.78. Found: C, 69.46; H, 5.09; N, 7.47.

5.4. Cell viability assay

The cancer cell lines A549, MOLT-4, and HeLa were obtained from the National Cell Bank of Iran. The cancer cells were maintained in RPMI 1640 medium (supplemented with 10% FBS, and 100 units/mL penicillin G and 100 μg/ml streptomycin) and incubated at 37 °C in humidified air containing 5% CO_2. For preparation of stock solutions, the test compounds **6a-t** were dissolved in DMSO and then diluted with RPMI 1640 medium. The final concentration of DMSO in the culture medium was less than 0.5%.

The cell viability of cancer cell lines (A549, MOLT-4, and HeLa) after exposure to compounds **6a-t** was determined by MTT assay according to the reported method [19]. Cisplatin was used as standard drug. The optical densities of samples were measured at 570 nm with background correction at 655 nm by using Bio-Rad microplate reader (Model 680).

5.5. Acridine orange/ethidium bromide double staining test

In order to evaluate the potential of compounds **6e** and **6l** for induction of apoptosis, the morphological analysis of A549 and MOLT-4 cells was used. Acridine orange/ethidium bromide double staining test was performed according to the previously reported method [20].

5.6. Annexin V-FITC/PI double staining and flow cytometric analysis

The A549 and MOLT-4 cells were treated with the IC_{50} concentrations of compounds **6e** and **6l**, respectively, and incubated for 24 h. Then, the treated cells were collected by centrifugation and resuspended in 500 μl of 1× Binding buffer. The cells were double stained by using Annexin V-FITC Apoptosis Detection Kit (Bio Vision) according to the described protocol by manufacture. Finally, Annexin V-FITC binding was analyzed by flow cytometry (ex = 488 nm; em = 530 nm) using FITC signal detector (FL1) and PI staining by the phycoerythrin emission signal detector (FL2) [18].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.12.037.

References

[1] R.J. Boohaker, M.W. Lee, P. Vishnubhotla, J.M. Perez, A.R. Khaled, The use of therapeutic peptides to target and to kill cancer cells, Curr. Med. Chem. 19 (2012) 3794–3804.
[2] M. Ogris, E. Wagner, Targeting tumors with non-viral gene delivery systems, Drug Discov. Today 7 (2002) 479–485.
[3] M. Creixell, N.A. Peppas, Co-delivery of siRNA and therapeutic agents using nanocarriers to overcome cancer resistance, Nano Today 7 (2012) 367–379.
[4] B.W. Stewart, C.P. Wild, World Cancer Report 2014, Lyon, France, International Agency for Research on Cancer, 2014.
[5] A. Coates, S. Abraham, S.B. Kaye, T. Sowerbutts, C. Frewin, R.M. Fox, M.H. Tattersall, On the receiving end–patient perception of the side-effects of cancer chemotherapy, Eur. J. Cancer Clin. Oncol. 19 (1983) 203–208.
[6] P.D. Eckford, F.J. Sharom, ABC efflux pump-based resistance to chemotherapy drugs, Chem. Rev. 109 (2009) 2989–3011.
[7] B.H. Alizadeh, A. Foroumadi, S. Emami, M. Khoobi, F. Panah, S.K. Ardestani, A. Shafiee, Isochaihulactone analogues: synthesis and anti-proliferative activity of novel dibenzylbutyrolactones, Eur. J. Med. Chem. 45 (2010) 5979–5984.
[8] M. Khoobi, A. Foroumadi, S. Emami, M. Safavi, G. Dehghan, B.H. Alizadeh, A. Ramazani, S.K. Ardestani, A. Shafiee, Coumarin-based bioactive compounds: facile synthesis and biological evaluation of coumarin-fused 1,4-thiazepines, Chem. Biol. Drug Des. 78 (2011) 580–586.
[9] L. Firoozpour, N. Edraki, M. Nakhjiri, S. Emami, M. Safavi, S.K. Ardestani, M. Khoshneviszadeh, A. Shafiee, A. Foroumadi, Cytotoxic activity evaluation and QSAR study of chromene-based chalcones, Arch. Pharm. Res. 35 (2012) 2117–2125.
[10] M. Azizmohammadi, M. Khoobi, A. Ramazani, S. Emami, A. Zarrin, O. Firuzi, R. Miri, A. Shafiee, 2H-chromene derivatives bearing thiazolidine-2,4-dione, rhodanine or hydantoin moieties as potential anticancer agents, Eur. J. Med. Chem. 59 (2013) 15–22.
[11] F. Molaverdi, M. Khoobi, S. Emami, M. Alipour, O. Firuzi, A. Foroumadi, G. Dehghan, R. Miri, F. Shaki, F. Jafarpour, A. Shafiee, Polyoxygenated cinnamoylcoumarins as conformationally constrained analogs of cytotoxic diarylpentanoids: synthesis and biological activity, Eur. J. Med. Chem. 68 (2013) 103–110.
[12] A. Ramazani, M. Khoobi, A. Torkaman, F.Z. Nasrabadi, H. Forootanfar, M. Shakibaie, M. Jafari, A. Ameri, S. Emami, M.A. Faramarzi, A. Foroumadi, A. Shafiee, One-pot, four-component synthesis of novel cytotoxic agents 1-(5-aryl-1,3,4-oxadiazol-2-yl)-1-(1H-pyrrol-2-yl)methanamines, Eur. J. Med. Chem. 78 (2014) 151–156.
[13] M.N. Elinson, A.I. Ilovaisky, V.M. Merkulova, P.A. Belyakov, A.O. Chizhov, G.I. Nikishin, Solvent-free cascade reaction: direct multicomponent assembling of 2-amino-4H-chromene scaffold from salicylaldehyde, malononitrile or cyanoacetate and nitroalkanes, Tetrahedron 66 (2010) 4043–4048.
[14] S.A. Patil, J. Wang, X.S. Li, J. Chen, T.S. Jones, A. Hosni-Ahmed, R. Patil, W.L. Seibel, W. Li, D.D. Miller, New substituted 4H-chromenes as anticancer agents, Bioorg. Med. Chem. Lett. 22 (2012) 4458–4461.
[15] X. Han, J. Luo, F. Wu, X.Y. Hou, G. Yan, M. Zhou, M. Zhang, C. Pu, R. Li, Synthesis and biological evaluation of novel 2,3-dihydrochromeno[3,4-*d*]imidazol-4(1*H*)-one derivatives as potent anticancer cell proliferation and migration agents, Eur. J. Med. Chem. 114 (2016) 232–243.
[16] S. Emami, S. Dadashpour, Current developments of coumarin-based anticancer agents in medicinal chemistry, Eur. J. Med. Chem. 102 (2015) 611–630.
[17] W. Kemnitzer, J. Drewe, S. Jiang, H. Zhang, J. Zhao, C. Crogan-Grundy, L. Xu, S. Lamothe, H. Gourdeau, R. Denis, B. Tseng, S. Kasibhatla, S.X. Cai, Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. 3. Structure-activity relationships of fused rings at the 7,8-positions, J. Med. Chem. 50 (2007) 2858–2864.
[18] N. Sirisoma, A. Pervin, H. Zhang, S. Jiang, J.A. Willardsen, M.B. Anderson, G. Mather, C.M. Pleiman, S. Kasibhatla, B. Tseng, J. Drewe, S.X. Cai, Discovery of N-(4-methoxyphenyl)-N,2-dimethylquinazolin-4-amine, a potent apoptosis inducer and efficacious anticancer agent with high blood brain barrier penetration, J. Med. Chem. 52 (2009) 2341–2351.
[19] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[20] M. Banimustafa, A. Kheirollahi, M. Safavi, S. Kabudanian Ardestani, H. Aryapour, A. Foroumadi, S. Emami Synthesis and biological evaluation of 3-(trimethoxyphenyl)-2(3H)-thiazole thiones as combretastatin analogs, Eur. J. Med. Chem. 70 (2013) 692–702.