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Design, Synthesis, and Biological Evaluation of Coumarin–Triazole Hybrid Molecules as Potential Antitumor and Pancreatic Lipase Agents

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The design, synthesis, and investigation of antitumor and anti-lipase activities of some coumarin–triazole hybrid molecules are reported. The synthesis of these hybrid molecules was performed under microwave irradiation and conventional heating procedures. The newly synthesized hybrid molecules were investigated as inhibitors against four tumor cell lines (BT20 human breast carcinoma, SK-Mel 128 melanoma, DU-145 prostate carcinoma, and A549 lung carcinoma) and porcine pancreatic lipase (PPL). Most of these compounds showed notable antitumor activities against the tested tumor cell lines, and compounds **8i** and **8l** showed the best anti-lipase activity of $99.30 \pm 0.56\%$ and $99.85 \pm 1.21\%$, respectively, at a concentration of $10\,\mu M$.

Keywords: Antitumor / Coumarin / Hybrid molecule / Lipase inhibition / Triazol-3-one

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Introduction

Cancer comprises a group of diseases that include abnormal cell growth over other parts of the human body. Cancer is a big health problem and it is the second frequent cause of death after heart diseases. It is characterized by uncontrolled division and spread of abnormal cells in human body. Although chemotherapy is widely used for various cancer treatments, the toxicity and resistance of chemotherapeutic drugs make it a necessity to design more selective drugs for cancer therapy. For instance, the synthesis of effective novel anticancer drugs is one of the most important targets of modern medicinal chemistry [1–3].

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Overweight and obesity are globally the fifth leading risks for death. Every year, about 2.8 million adults die from diseases caused by obesity. Obesity and obesity-related diseases like diabetes and coronary heart diseases are the major problem in the world. An important way for obesity is treatment to develop inhibitors of food digestion. Inhibition of pancreatic lipase and the associated reduction of lipid absorption is an attractive approach for the discovery of potent agents. Orlistat is the only drug used as a pancreatic lipase inhibitor. Unfortunately, it has some unpleasant side effects on human body like oily stools, oily spotting and flatulence [4–6].

Coumarins (2*H*-chromen-2-one) are generally obtained from plants and consists of a class of phenolic compounds. Thirteen hundred coumarin derivatives have been identified from plants, bacteria, and fungi. In the year of 1820, coumarin was first obtained as a natural product and nearby 150 species of coumarin were found in 30 diverse plant families such as Umbelliferae, Oleaceae, Clusiaceae, Guttiferae, Rutaceae [7]. Coumarin and its derivatives show rare side effects on human body [8]. Also, they are known to exhibit many pharmacological activities like antioxidant [9], anticoagulant [10],

antibacterial [11], antitubercular [12], antifungal [13], anti-inflammatory [14], and anticancer [15]. In addition to these, coumarin is regularly used as an important nucleus in medicinal chemistry. Warfarin and acenocoumarol (Fig. 1) are anticoagulant agents that are known as vitamin K antagonists, and are normally used in the prevention of thrombosis and thromboembolism [16].

Past researches have demonstrated that coumarin hydrazones have been investigated by researchers in recent years due to the fact that they have pharmacologically powerful properties [17, 18]. On the other hand, Nasr et al. [19] reported *in vitro* anticancer activity of some coumarin hydrazide-hydrazone derivatives and found that one of the compounds could be a potent anticancer drug to overcome drug resistance in cancer.

Triazole ring is an important five-membered heterocycle structure for designing new bioactive molecules. This electron-rich heterocycle easily binds to various types of enzymes and receptors. Therefore, triazol containing compounds show a broad spectrum of biological activities [20]. One of the isomers of this heterocycle is 1,2,4-triazoles and they have been known to possess various biological activities such as antimicrobial [21], anti-inflammatory [22], and anticancer activities [23]. Some of the examples of triazoles are in medical use, like ribavirin (antiviral) [24, 25], fluconazole (antifungal) [26], vorozole, and letrozole (nonsteroidal competitive aromatase inhibitor) [20] (Fig. 2).

Molecular modification is considered as an effective method for developing new drug candidates [27]. Molecular hybridization is among the molecular modification methods for achieving new molecules which have better pharmacokinetic and pharmacodynamic properties. In recent years, this method has become a powerful tool for obtaining more bioactive compounds. Considering the promising activity of coumarin and triazole moieties, the use of these heterocycles in one molecule may result in the formation of more bioactive compounds. Hence, we decided to use the mentioned moieties in a molecular hybridization approach to design new potential antitumor and anti-lipase agents.

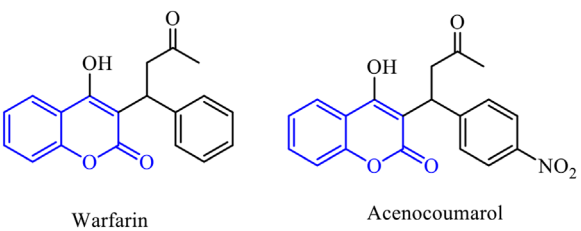


Figure 1. Structures of warfarin and acenocoumarol.

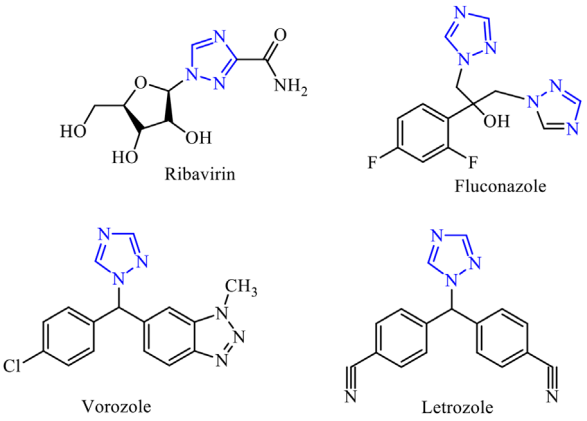


Figure 2. Structures of some drugs bearing 1,2,4-triazole.

Results and discussion

Chemistry

The synthesis of target hybride molecules *N′*-(1,2,4-triazol-1-yl)acetyl coumarin-3-carbohydrazides (**8**) was performed by reaction of 2-(3-alkyl/aryl-4-amino-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetohydrazides (**5a–f**) with 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-ones (**7a–c**). Firstly, compounds **1a–f** were prepared according to the related literature [28]. Then, these compounds were reacted with ethyl carbazate in ethanol to obtain corresponding hydrazone derivatives (**2a–f**). Next, compounds **2a–f** were reacted with hydrazine monohydrate in water to synthesize 4-amino-1,2,4-triazol-3-one derivatives (**3a–f**). Ester derivatives of compounds **3a–f** were prepared by the reaction of compounds **3a–f** and ethyl bromoacetate in the presence of sodium ethoxide (NaOEt) in ethanol. Afterwards, these compounds were treated with hydrazine monohydrate in *n*-butanol to obtain 2-(3-alkyl/aryl-4-amino-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetohydrazides (**5a–f**) [29], which are the first intermediate for the synthesis of target compounds.

The synthesis of second intermediate 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-ones (**7a–c**) was performed in three steps. First, coumarin-3-carboxylic acid derivatives (**6a–c**) were prepared by the reaction of the respective salicylic aldehydes and 2,2-dimethyl-1,3-dioxane-4,6-dione in ethanol containing pyridine. Then, these compounds were reacted with 1*H*-benzotriazole in the presence of $SOCl_2$ in dichloromethane to prepare 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-ones (**7a–c**), which are the second intermediate of the target compounds.

The synthesis of hybrid molecules resulted in the formation of more bioactive compounds. This type of molecules can offer the advantages of a combination therapy with improved pharmacological properties [30, 31]. Our literature surveys have revealed that benzotriazole is an easy leaving group and

this group offers many advantages to synthesize new hybrid molecules [29, 32]. Thus, we used this chemical transformation method to synthesize the target compounds (**8a–r**) by the reaction of compounds **5a–f** and **7a–c** (Scheme 1). This last reaction step was performed under microwave irradiation and using conventional heating procedure, and their results were compared.

The spectral results of the target compounds **8a–r** correspond to the proposed structures. In the ^{1}H NMR spectra, two NH signals (exchangeable with D_2O) were observed at about 11.00 and 10.60 ppm and NH_2 signal (exchangeable with D_2O) was observed at about 5.30 ppm. The NCH_2 signal was shown at about 4.45 ppm. In the ^{13}C NMR spectra, C=O signals were shown at about 165.0, 160.0, 159.0, and 153.0 ppm as two hydrazides, coumarin C-2, triazole C-3, and the C=N signal was observed at about 147.0 ppm. In addition, all compounds showed the proposed molecular ion signals in their LC-MS spectra.

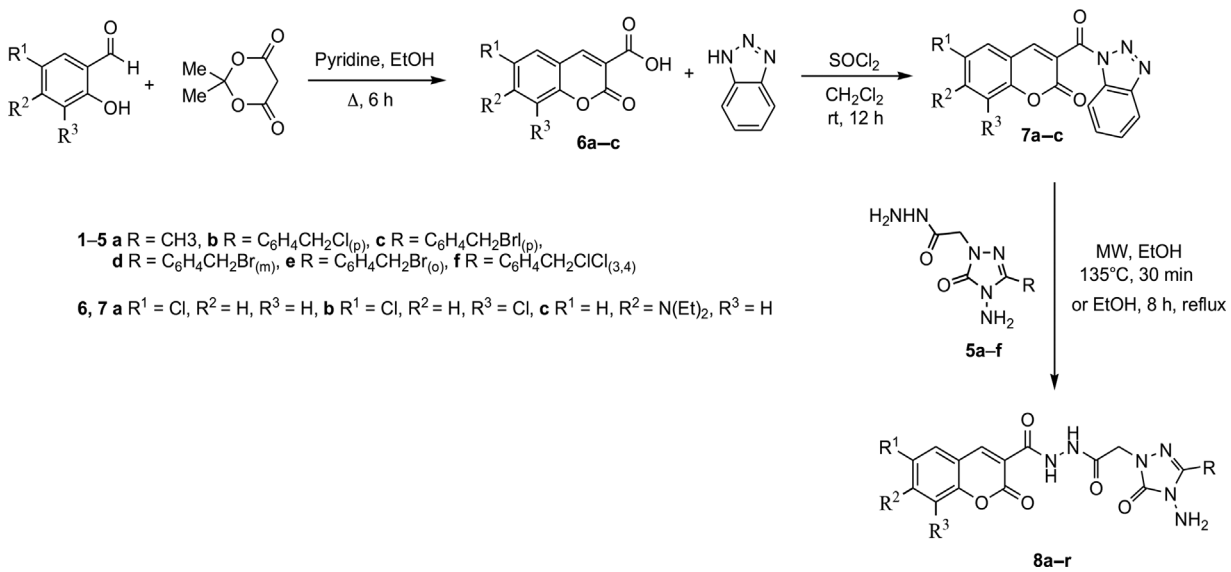
Biological activity

Cytotoxicity

The compounds **8a–r** were screened for *in vitro* anticancer activity against normal human fibroblasts and four human tumor cell lines: BT20 human breast carcinoma, SK-Mel 128 melanoma, DU-145 prostate carcinoma, and A549 lung carcinoma, in 24-h drug exposure assays using cisplatin as reference. The synthesized compounds' cytotoxicity properties were determined using tetrazolium dye reduction assay [33–35]. The cytotoxic activities of compounds are displayed in Table 1. As shown in Table 1, the tested compounds showed moderate to good activity in the *in vitro* antitumor screening expressed by the CC_{50} values. Compounds **8e**, **8f**, **8h**, **8i**, **8k**, **8m**, **8p**, and **8r** showed notable anticancer activity against tested cell line. The compounds **8f**, **8h**, **8i**, **8k**, **8p**, and **8r** were found to be more potent against human breast cancer cell line than cisplatin. Compound **8f** was found to be more potent against human prostate cancer cell line than cisplatin. Also, compounds **8h** and **8i** were found to be more potent against human lung cancer cell line than cisplatin. The antitumor activity of the compounds against human breast cancer cell line can be arranged in this order compared to cisplatin **8k** > **8i** > **8h** > **8p** > **8f** > **8r** > cisplatin > **8m** > **8e**. For prostate cell line this order was determined as **8f** > cisplatin > **8e**. The antitumor activity of the synthesized compounds against human lung cancer cell line can be stated as **8h** > **8i** > cisplatin > **8r** > **8m** > **8e**. Among the tested compounds, compounds **8k** and **8p** inhibited selectively (SI = 5.2 and 2.7) human breast cancer cell line, and compound **8m** inhibited selectively human lung cancer cell line (SI = 2.6). All the tested compounds showed no activity against melanoma.

Lipase inhibition assay

All compounds were evaluated with regard to pancreatic lipase activity and most of compounds showed anti-lipase activities at various concentrations (Table 2). Among the tested compounds **8i** and **8l** showed the best anti-lipase activity. These compounds inhibited pancreatic lipase by $99.30 \pm 0.56\%$ and $99.85 \pm 1.21\%$ at concentration of 10 μM, respectively (Table 2). Orlistat, known as a pancreatic lipase inhibitor used as an anti-obesity drug, showed inhibitory effect by $99.88 \pm 0.43\%$ at concentration of 300 nM ($IC_{50} = 0.41 \pm 0.01$ nM). Compound **8i** and **8l** IC_{50} values were calculated as 2.64 ± 0.33 μM and 1.80 ± 0.08 μM, respectively.



Scheme 1. Synthetic route of compounds **8a–r**.

Table 1. The antitumor activity of synthesized compounds against different tumor and normal cell lines.

Compd.	CC_{50} (Cytotoxicity 50: μg/mL); SI (Selectivity index)								
	BT-20	SI	DU-145	SI	SK-MEL128	SI	A549	SI	HFC
8a	32.6	0.2	30.1	0.2	6.8	1.0	15.9	0.4	6.5
8b	11.6	1.0	16.8	0.7	10.5	1.1	14.7	0.8	12.0
8c	15.6	0.9	3.9	3.5	18.4	0.7	25.1	0.5	13.7
8d	10.2	0.8	14.8	0.5	8.6	0.9	6.4	1.2	7.7
8e	27.6	1.2	4.1	8.2	18.7	1.8	13.8	2.4	33.3
8f	13.9	2.6	3.7	9.9	12.3	3.0	28.1	1.3	36.6
8g	23.1	1.1	36.0	0.7	12.5	2.1	23.0	1.1	26.0
8h	10.0	3.1	17.5	1.8	32.7	1.0	7.5	4.2	31.1
8i	7.3	3.6	6.1	4.4	20.6	1.3	8.1	3.3	26.3
8j	31.7	1.1	24.3	1.4	29.4	1.2	37.9	0.9	35.0
8k	6.4	5.2	10.9	3.0	36.7	0.9	12.7	2.6	33.3
8l	12.8	0.7	12.9	0.7	12.1	0.8	11.3	0.8	9.3
8m	11.1	1.4	3.1	4.9	7.5	2.1	6.0	2.6	15.4
8n	19.0	0.9	17.4	1.0	18.9	0.9	16.7	1.1	17.8
8o	10.6	0.3	6.4	0.5	4.1	0.8	5.2	0.6	3.3
8p	3.6	2.7	10.1	1.0	6.1	1.6	6.8	1.5	9.9
8q	6.9	1.3	4.6	1.9	10.1	0.9	11.6	0.8	8.9
8r	10.9	2.4	6.7	3.9	18.8	1.4	9.2	2.9	26.2
Cisplatin	5.8	2.0	1.4	8.4	0.7	15.4	3.8	3.0	11.5

Synthesized compounds **8i** and **8l** have a potential to be alternatives to orlistat.

Conclusion

In this work, a new series of novel coumarin–triazol hybrid molecules was designed and synthesized by using microwave irradiation and conventional heating techniques. It was seen that the synthesis of these compounds by microwave irradiation method has supplied advantages on reaction yields and time for us. These compounds were screened for antitumor and anti-lipase activities. The anti-lipase activity results indicate that compounds **8i** and **8l** showed the best anti-lipase activity by 99.30 ± 0.56% and 99.85 ± 1.21% at concentration of 10 μM, respectively. Antitumor activity of the synthesized compounds was investigated against four tumor cell lines (BT20 human breast carcinoma, SK-Mel 128 melanoma, DU-145 prostate carcinoma, and A549 lung carcinoma). The results of this activity show that compounds **8f**, **8h**, **8i**, **8k**, **8q**, and **8r** were found to be more active than standard cisplatin against BT20 human breast carcinoma tumor cells, compound **8f** was found to be more active than standard cisplatin against DU-145 prostate carcinoma and compounds **8h** and **8i** were found to be more active than standard cisplatin against A549 lung carcinoma. Most of the hybrid molecules showed more selectivity index (SI) value than cisplatin. It means that the hybrid molecules have more selectivity to show effects on cancer cells than normal human cells. Based on the biological activity, these results indicate that coumarin–triazol hybrid molecules show more activity than coumarin (**6a–c**, **7a–c**) and triazol (**3a–f**, **4a–f**, **5a–f**) derivatives. These results have shown that combination of triazol (**5a–f**) and coumarin (**7a–c**) has a positive effect on biological activity of this type of molecules.

Table 2. Inhibitory effects of selected synthesized compounds (synthesized compounds at final concentration of 10 μm).

Compounds	% Inhibition	IC_{50} (μM)
8b	80.74 ± 5.60	5.78 ± 3.08
8c	96.19 ± 1.37	3.94 ± 1.43
8d	81.96 ± 3.96	3.47 ± 0.22
8f	95.07 ± 2.73	3.29 ± 0.03
8h	99.38 ± 0.18	4.11 ± 1.10
8i	97.81 ± 2.03	3.45 ± 0.21
8j	99.30 ± 0.56	2.64 ± 0.33
8k	99.04 ± 2.29	6.91 ± 0.51
8l	99.85 ± 1.21	1.80 ± 0.08
8n	82.93 ± 6.45	4.96 ± 1.81
8p	90.63 ± 1.27	4.36 ± 0.10
Orlistat	99.88 ± 0.43	0.41 ± 0.01 (nM)

Orlistat (at final concentration of 0.3 μm) was used as positive control.

Experimental

Chemistry

General

All reaction progress was monitored by TLC on silica gel plates (Merck 60, F_{254}, 0.2 mm). The melting points were determined

on capillary tubes on Stuart SMP30 melting point apparatus and uncorrected. ^{1}H and ^{13}C NMR spectra (400 and 100 MHz, respectively) were obtained using a Varian-Mercury (tetramethylsilane as internal standard): chemical shifts are expressed in δ values (ppm). The mass spectra were recorded on Agilent 1260 Infinity series accurate-mass time-of-flight (TOF) LC/MS spectrometer. All experiments were carried out in microwave process vials (30 mL) with control of the temperature by infrared detection temperature sensor. It was monitored by a computer and maintained constant at a constant value by a discrete modulation of delivered microwave power. After completion of the reaction, the vial was cooled to 60 °C via air jet cooling. The compounds **5a–f** and **7a–c** were prepared according to the literature procedure [29].

The ^{1}H NMR, ^{13}C APT, and LC-MS spectra and the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*Synthesis of compounds **7a–c***

These compounds were synthesized according to the literature procedure [29].

*3-(1H-Benzotriazol-1-ylcarbonyl)-6-chloro-2H-chromen-2-on (**7a**)*

Yield: 2.20 g (63%), m.p.: 248–249°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 8.74 (1H, s, H-4); 8.29–8.05 (2H, m, Ar-H); 8.05 (1H, d, J = 2.4 Hz, Ar-H); 7.98–7.79 (2H, m, Ar-H); 7.68–7.64 (1H, m, Ar-H); 7.64 (1H, d, J = 8.0 Hz, Ar-H). ^{13}C APT (DMSO-d_6), δ, ppm: 162.8 (C=O); 157.3 (C=O coumarin C-2); 153.2 (coumarin C-3); 146.9 (coumarin C-4); 145.9; 134.4; 131.7; 130.9; 129.5; 127.5; 122.6; 120.7; 119.3; 119.1; 114.2 (Ar-C). LC-MS, *m/z*: 348.0203 [M (Cl^{35})+Na], 350.0156 [M (Cl^{37})+Na].

*3-(1H-Benzotriazol-1-ylcarbonyl)-6,8-dichloro-2H-chromen-2-on (**7b**)*

Yield: 2.34 g (65%), m.p.: 263–264°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 8.76 + 8.67 (1H, s, coumarin C-4); 8.29 (1H, s, coumarin H-7), 8.27 (1H, s, coumarin H-5), 8.11–7.99 (2H, m, Ar-H), 7.85 (1H, t, J = 7.2 Hz, Ar-H), 7.67 (1H, t, J = 7.2 Hz, Ar-H). ^{13}C APT (DMSO-d_6), δ, ppm: 163.9 (C=O); 158.1 (C=O coumarin C-2); 152.4 (coumarin C-3); 148.7 (coumarin C-4); 146.8; 138.4; 133.1; 132.4; 129.9; 128.4; 123.1; 121.9; 116.0; 1114.1; 112.0 (Ar-C). LC-MS, *m/z*: 359.0207 [M (Cl^{35})(Cl^{35})], 361.0153 [M (Cl^{35})(Cl^{37})].

*3-(1H-Benzotriazol-1-ylcarbonyl)-7-diethylamino-2H-chromen-2-on (**7c**)*

Yield: 2.46 g (68%), m.p.: 210–211°C (lit. 212–214°C [36]).

*Synthesis of compounds **8a–r***

Conventional method: A solution of compounds **5a–f** (0.01 mol) in ethanol (15 mL) and compounds **7a–c** (0.011 mol) was taken in a round-bottom flask. The mixture was refluxed for 8 h. After the completion of the reaction, the mixture was cooled to room temperature and the product appeared as a white solid. It was filtrated and washed with ethanol to obtain the pure product.

Microwave method: Compounds **5a–f** (0.01 mol) and compounds **7a–c** (0.011 mol) were taken in a microwave process vial and dry ethanol (5 mL) was added. Then, mixture was irradiated in microwave at 135°C for 30 min at 300 W maximum power. After the completion of the reaction, the mixture was taken in the beaker with hot ethanol, and a product appeared as a white solid. It was filtrated and washed with ethanol to obtain the pure product.

*N′-[(4-Amino-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-6-chloro-2-oxo-2H-chromen-3-carbohydrazide (**8a**)*

Yield: 2.37 g (60% for microwave), 1.50 g (38% for conventional), m.p.: 265–266°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.04 (1H, s, NH); 10.60 (1H, s, NH); 8.78 (1H, s, H-4); 8.08 (1H, d, J = 2.4 Hz, Ar-H); 7.74 (1H, dd, J = 8.8 Hz, J = 2.4 Hz, Ar-H); 7.26 (1H, d, J = 8.8 Hz, Ar-H); 5.25 (2H, s, NH_2); 4.42 (2H, s, NCH_2); 2.10 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.7 (C=O); 159.8 (C=O); 158.7 (CO coumarin); 153.7 (coumarin C-3); 152.9 (C=O triazole); 147.1 (coumarin C-4); 145.6 (C=N); 134.2; 129.5; 120.2; 119.6; 118.7 (Ar-C); 46.4 (NCH_2); 11.1 (CH_3). LC-MS, *m/z*: 393.0325 [M (Cl^{35})+H]$^+$, 395.0692 [M (Cl^{37})+H]$^+$, 415.0596 [M (Cl^{35})+Na]$^+$, 417.0529 [M (Cl^{37})+Na]$^+$.

*N′-{[4-Amino-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6-chloro-2-oxo-2H-chromen-3-carbohydrazide (**8b**)*

Yield: 3.18 g (63% for microwave), 2.27 g (45% for conventional), m.p.: 259–260°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.02 (1H, s, NH); 10.62 (1H, s, NH); 8.81 (1H, s, H-4); 8.11 (1H, d, J = 2.4 Hz, Ar-H); 7.77 (1H, dd, J = 9.2 Hz, J = 2.4 Hz, Ar-H); 7.54 (1H, d, J = 9.2 Hz, Ar-H); 7.35 (2H,m, Ar-H); 7.29 (2H, m, Ar-H); 5.30 (2H, s, NH_2); 4.45 (2H, s, NCH_2); 3.88 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (C=O); 159.8 (CO); 158.8 (C=O); 153.8 (coumarin C-3); 152.9 (CO triazole); 147.2 (CN); 147.0 (coumarin C-4); 135.2; 134.2; 313.8; 131.1; 129.6; 129.3; 128.7; 120.1; 119.7; 118.7 (Ar-C); 46.5 (NCH_2); 30.2 (CH_2). LC-MS, *m/z*: 503.0605 [M (Cl^{35})+H]$^+$, 505.0577 [M (Cl^{37})+H]$^+$, 525.0418 [M (Cl^{35})+Na]$^+$, 527.0392 [M (Cl^{37})+Na]$^+$.

*N′-{[4-Amino-3-(4-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6-chloro-2-oxo-2H-chromen-3-carbohydrazide (**8c**)*

Yield: 3.57 g (65% for microwave), 2.47 g (45% for conventional), m.p.: 281–282°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.02 (1H, s, NH); 10.62 (1H, s, NH); 8.81 (1H, s, H-4); 8.10 (1H, d, J = 2.4 Hz, Ar-H); 7.77 (1H, dd, J = 9.2 Hz, J = 2.4 Hz, Ar-H); 7.53 (1H, d, J = 9.2 Hz, Ar-H); 7.35 (2H,d, J = 8.4 Hz, Ar-H); 7.23 (2H, d, J = 8.4 Hz, Ar-H); 5.30 (2H, s, NH_2); 4.45 (2H, s, NCH_2); 3.68 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (C=O); 159.8 (C=O); 158.7 (C=O coumarin); 153.8 (coumarin C-3); 152.9 (C=O triazole); 147.2 (CN); 147.0 (coumarin C-4); 135.7; 134.2; 131.6; 131.5; 129.6; 129.3; 120.3; 120.0; 119.7; 118.7 (Ar-C); 46.5 (NCH_2); 30.2 (CH_2). LC-

MS, *m/z*: 547.0603 [M (Cl^{35})(Br^{79})+H]$^+$, 549.0147 [M (Cl^{35})(Br^{81})+H]$^+$, 551.3242 [M (Cl^{37})(Br^{81})+H]$^+$.

N′-{[4-Amino-3-(3-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6-chloro-2-oxo-2H-chromen-3-carbohydrazide ***(8d)***

Yield: 3.40 g (62% for microwave), 2.63 g (48% for conventional), m.p.: 279–280°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.02 (1H, s, NH); 10.62 (1H, s, NH); 8.81 (1H, s, H-4); 8.11 (1H, d, *J* = 2.4 Hz, Ar-H); 7.77 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, Ar-H); 7.53 (1H, d, *J* = 8.8 Hz, Ar-H); 7.46–7,40 (2H,m, Ar-H); 7.28–7.23 (2H, m, Ar-H); 5.32 (2H, s, NH_2); 4.45 (2H, s, NCH_2); 3.90 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (C=O); 159.8 (CO); 158.8 (C=O coumarin); 153.8 (coumarin C-3); 152.9 (C=O triazole); 147.1 (coumarin C-3); 147.0 (C=N); 139.0; 134.2; 131.9; 130.9; 130.0; 129.5; 129.3; 128.3; 122.0; 120.0; 119.7; 118.7 (Ar-C); 46.5 (NCH_2); 30.3 (CH_2). LC-MS, *m/z*: 547.0187 [M (Cl^{35})(Br^{79})+H]$^+$, 549.0171 [M (Cl^{35})(Br^{81})+H]$^+$, 551.0141 [M (Cl^{37})(Br^{81})+H]$^+$.

N′-{[4-Amino-3-(2-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6-chloro-2-oxo-2H-chromen-3-carbohydrazide ***(8e)***

Yield: 3.18 g (58% for microwave), 2.30 g (42% for conventional), m.p.: 258–259°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.99 (1H, s, NH); 10.60 (1H, s, NH); 8.80 (1H, s, H-4); 8.12 (1H, d, *J* = 2.0 Hz, Ar-H); 7.77 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, Ar-H); 7.60–7.53 (2H, m, Ar-H); 7.34–7.17 (3H,m, Ar-H); 5.34 (2H, s, NH_2); 4.43 (2H, s, NCH_2); 3.99 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (C=O); 159.8 (C=O); 158.8 (CO coumarin); 153.9 (coumarin C-3); 153.0 (C=O triazole); 147.0 (coumarin C-4); 146.4 (CN); 135.5; 134.2; 132.9; 131.4; 129.6; 129.4; 129.3; 128.3; 124.4; 120.1; 119.8; 118.7 (Ar-C); 46.5 (NCH_2); 31.3 (CH_2). LC-MS, *m/z*: 547.0180 [M (Cl^{35})(Br^{79})+H]$^+$, 549.0161 [M (Cl^{35})(Br^{81})+H]$^+$, 551.0127 [M (Cl^{37})(Br^{81})+H]$^+$.

N′-{[4-Amino-3-(3,4-dichlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6-chloro-2-oxo-2H-chromen-3-carbohydrazide ***(8f)***

Yield: 3.39 g (63% for microwave), 2.64 g (49% for conventional), m.p.: 289–290°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.02 (1H, s, NH); 10.61 (1H, s, NH); 8.80 (1H, s, H-4); 8.11 (1H, d, *J* = 2.8 Hz, Ar-H); 7.75 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, Ar-H); 7.54–7.49 (3H, m, Ar-H); 7.24 (1H, dd, *J* = 8.4 Hz, *J* = 2,0 Hz, Ar-H); 5.32 (2H, s, NH_2); 4.46 (2H, s, NCH_2); 3.91 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (C=O); 159.8 (C=O); 158.8 (C=O coumarin); 153.9 (coumarin C-3); 152.9 (CO triazole); 147.0 (coumarin C-4); 146.8 (CN); 137.4; 134.2; 131.3; 131.2; 130.9; 129.8; 129.7; 129.5; 129.3; 120.1; 119.6; 118.7 (Ar-C); 46.5 (NCH_2); 29.9 (CH_2). LC-MS, *m/z*: 537.0319 [M (Cl^{35})(Cl^{35})(Cl^{35})+H]$^+$, 539.0292 [M (Cl^{37})(Cl^{35})(Cl^{35})+H]$^+$, 541.0263 [M (Cl^{37})(Cl^{37})+H]$^+$.

N′-[(4-Amino-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide ***(8g)***

Yield: 3.05 g (68% for microwave), 1.66 g (37% for conventional), m.p.: 290–291°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.98 (1H, s, NH); 10.55 (1H, s, NH); 8.79 (1H, s, H-4); 8.11 (1H, d, *J* = 2.4 Hz, Ar-H); 8.07 (1H, d, *J* = 2.4 Hz, Ar-H); 5.25 (2H, s, NH_2); 4.40 (2H, s, NCH_2); 2.07 (3H, s, CH_3). ^{13}C APT (DMSO-d_6), δ, ppm: 164.8 (CO); 158.9 (C=O); 158.6 (C=O coumarin); 153.8 (C=O triazole); 148.8 (CN); 146.6 (coumarin C-4); 145.6 (coumarin C-3); 133.4; 129.2; 128.7; 121.3; 121.1; 120.7 (Ar-C); 46.4 (NCH_2); 11.1 (CH_3). LC-MS, *m/z*: 427.0419 [M (Cl^{35})(Cl^{35})+H]$^+$, 429.0391 [M (Cl^{37})(Cl^{37})+H]$^+$, 449.0258 [M (Cl^{35})(Cl^{35})+Na]$^+$, 451.0230 [M (Cl^{35})(Cl^{37})+Na]$^+$, 453.3526 [M (Cl^{37})(Cl^{37})+Na]$^+$.

N′-{[4-Amino-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide ***(8h)***

Yield: 3.45 g (64% for microwave), 2.59 g (48% for conventional), m.p.: 303–304°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.99 (1H, s, NH); 10.59 (1H, s, NH); 8.79 (1H, s, H-4); 8.11 (1H, d, *J* = 2.4 Hz, Ar-H); 8.07 (1H, d, *J* = 2.4 Hz, Ar-H); 7.30 (2H, d, *J* = 6.4 Hz, Ar-H); 7.27 (2H, d, *J* = 6.4 Hz, Ar-H); 5.30 (2H, s, NH_2); 4.44 (2H, s, NCH_2); 3.88 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.7 (C=O); 158.8 (CO); 158.6 (C=O coumarin); 153.8 (C=O triazole); 148.8 (C=N); 147.2 (coumarin C-3); 146.6 (coumarin C-4); 135.2; 133.4; 131.8; 131.1; 129.2; 128.7; 128.6; 121.3; 121.1; 120.7 (Ar-C); 46.5 (NCH_2); 30.1 (CH_2). LC-MS, *m/z*: 537.0244 [M (Cl^{35})(Cl^{35})(Cl^{35})+H]$^+$, 539.0221 [M (Cl^{37})(Cl^{35})(Cl^{35})+H]$^+$, 541.0188 [M (Cl^{37})(Cl^{37})(Cl^{35})+H]$^+$.

N′-{[4-Amino-3-(4-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide ***(8i)***

Yield: 3.71 g (64% for microwave), 1.86 g (32% for conventional), m.p.: 299–300°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.99 (1H, s, NH); 10.55 (1H, s, NH); 8.79 (1H, s, H-4); 8.11 (1H, d, *J* = 2.0 Hz, Ar-H); 8.07 (1H, d, *J* = 2.0 Hz, Ar-H); 7.48 (2H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, Ar-H); 7.20 (2H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, Ar-H); 5.29 (2H, s, NH_2); 4.44 (2H, s, NCH_2); 3.86 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 165.7 (C=O); 164.7 (C=O); 158.9 (C=O coumarin); 153.9 (C=O triazole); 148.8 (C=N); 147.2 (coumarin C-3); 146.6 (coumarin C-4); 135.7; 133.4; 131.7; 131.5; 129.2; 128.7; 121.3; 121.2; 120.7; 120.3 (Ar-C); 46.5 (NCH_2); 30.2 (CH_2). LC-MS, *m/z*: 580.9700 [M (Br^{79})(Cl^{35})(Cl^{35})+H]$^+$, 582.9681 [M (Br^{81})(Cl^{35})(Cl^{35})+H]$^+$, 584.9653 [M (Br^{81})(Cl^{37})(Cl^{35})+H]$^+$.

N′-{[4-Amino-3-(3-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide ***(8j)***

Yield: 3.89 g (67% for microwave), 3.19 g (55% for conventional), m.p.: 302–303°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.00 (1H, s, NH); 10.56 (1H, s, NH); 8.79 (1H, s, H-4); 8.10 (1H, s, Ar-H); 8.06 (1H, s, Ar-H); 7.46–7.40 (2H, m, Ar-H); 7.27–7.20 (2H, m, Ar-H); 5.32 (2H, s, NH_2); 4.46 (2H, s, NCH_2); 3.88 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.7 (C=O); 158.8 (CO); 158.6 (C=O coumarin); 153.8 (C=O triazole); 148.8 (C=N); 147.1 (coumarin C-3); 146.6 (coumarin C-4); 139.0; 133.5; 131.9; 130.9; 130.0; 129.2; 128.7; 128.4; 122.1; 121.3; 121.1; 120.7 (Ar-C); 46.6 (NCH_2); 30.3 (CH_2). LC-MS, *m/z*: 580.9766

[M (Br^{79})(Cl^{35})(Cl^{35})+H]$^+$, 582.9742 [M (Br^{81})(Cl^{35})(Cl^{35})+H]$^+$, 584.9613 [M (Br^{81})(Cl^{37})(Cl^{35})+H]$^+$.

*N′-{[4-Amino-3-(2-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide (**8k**)*

Yield: 2.79 g (48% for microwave), 1.80 g (31% for conventional), m.p.: 286–287°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.99 (1H, s, NH); 10.56 (1H, s, NH); 8.78 (1H, s, H-4); 8.10 (1H, d, J = 2.0 Hz, Ar-H); 8.04 (1H, d, J = 2.0 Hz, Ar-H); 7.58 (1H, d, J = 7.6 Hz, Ar-H); 7.31–7.28 (2H, m, Ar-H); 7.20–7.17 (1H, m, Ar-H); 5.32 (2H, s, NH_2); 4.46 (2H, s, NCH_2); 3.88 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.7 (C=O); 158.8 (C=O); 158.6 (C=O coumarin); 153.9 (C=O triazole); 148.7 (C=N); 146.7 (coumarin C-3); 146.5 (coumarin C-4); 135.5; 133.4; 132.9; 131.4; 129.4; 129.2; 128.6; 128.2; 124.4; 121.4; 121.2; 120.6 (Ar-C); 46.6 (NCH_2); 31.3 (CH_2). LC-MS, *m/z*: 580.9760 [M (Br^{79})(Cl^{35})(Cl^{35})+H]$^+$, 582.9745 [M (Br^{81})(Cl^{35})(Cl^{35})+H]$^+$, 584.9715 [M (Br^{81})(Cl^{37})(Cl^{35})+H]$^+$.

*N′-{[4-Amino-3-(3,4-dichlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-6,8-dichloro-2-oxo-2H-chromen-3-carbohydrazide (**8l**)*

Yield: 2.74 g (48% for microwave), 2.63 g (46% for conventional), m.p.: 310–311°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.01 (1H, s, NH); 10.56 (1H, s, NH); 8.79 (1H, s, H-4); 8.10 (1H, d, J = 2.4 Hz, Ar-H); 8.04 (1H, d, J = 2.0 Hz, Ar-H); 7.54–7.51 (2H, m, Ar-H); 7.26 (1H, d, J = 9.4 Hz, Ar-H); 5.32 (2H, s, NH_2); 4.46 (2H, s, NCH_2); 3.91 (2H, s, CH_2). ^{13}C APT (DMSO-d_6), δ, ppm: 164.6 (CO); 158.8 (C=O); 158.6 (C=O coumarin); 153.9 (CO triazole); 148.7 (C=N); 146.8 (coumarin C-3); 146.6 (coumarin C-4); 137.4; 133.4; 131.3; 131.2; 131.1; 130.9; 129.8; 129.2; 128.7; 121.3; 121.1; 120.7 (Ar-C); 46.6 (NCH_2); 29.9 (CH_2). LC-MS, *m/z*: 570.9900 [M (Cl^{35})(Cl^{35})(Cl^{35})(Cl^{35})+H]$^+$, 572.9872 [M (Cl^{37})(Cl^{35})(Cl^{35})(Cl^{35})+H]$^+$, 574.9841 [M (Cl^{37})(Cl^{37})(Cl^{35})(Cl^{35})+H]$^+$.

*N′-[(4-Amino-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8m**)*

Yield: 2.23 g (52% for microwave), 1.50 g (35% for conventional), m.p.: 246–247°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.91 (1H, s, NH); 10.51 (1H, s, NH); 8.67 (1H, s, H-4); 7.71 (1H, d, J = 8.8 Hz, Ar-H); 6.81 (1H, d, J = 8.0 Hz, Ar-H); 6.61 (1H, s, Ar-H); 5.25 (2H, s, NH_2); 4.39 (2H, s, NCH_2); 3.84 (4H, q, $2CH_2$); 2.09 (3H, s, CH_3); 1.12 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.4 (C=O); 161.8 (C=O); 160.1 (CO coumarin); 157.8 (Ar-C); 153.8 (coumarin C-3); 153.3 (CO triazole); 148.7 (coumarin C-4); 145.6 (CN); 132.3; 110.8; 108.1; 108.0; 96.4 (Ar-C); 46.3; 44.8 ($2CH_2$); 12.8 ($2CH_3$); 11.1. LC-MS, *m/z*: 430.1878 [M+H]$^+$, 452.1728 [M+Na]$^+$.

*N′-{[4-Amino-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8n**)*

Yield: 3.45 g (64% for microwave), 1.99 g (37% for conventional), m.p.: 263–264°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.93 (1H, s, NH); 10.52 (1H, s, NH); 8.66 (1H, s, H-4); 7.70 (1H, d, J = 9.2 Hz, Ar-H); 7.35–7.27 (4H, m, Ar-H); 6.80 (1H, dd, J = 9.2 Hz, J = 2.8 Hz, Ar-H); 6.60 (1H, d, J = 2.4 Hz, Ar-H); 5.30 (2H, s, NH_2); 4.43 (2H, s, NCH_2); 3.87 (2H, s, CH_2); 3.47 (4H, q, $2CH_2$); 1.10 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.3 (C=O); 161.8 (C=O); 160.1 (CO coumarin); 157.8 (Ar-C); 153.8 (coumarin C-3); 153.2 (C=O triazole); 148.7 (coumarin C-4); 147.2 (CN); 135.3; 132.3; 131.8; 131.1 (2C); 128.8 (2C); 110.8; 108.1; 96.3 (Ar-C); 46.5 (NCH_2); 44.8 ($2CH_2$); 30.2 (CH_2); 12.8 ($2CH_3$). LC-MS, *m/z*: 540.35 [M (Cl^{35})+H]$^+$, 562.38 [M (Cl^{35})+Na]$^+$,564.33 [M (Cl^{37})+Na]$^+$.

*N′-{[4-Amino-3-(4-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8o**)*

Yield: 3.27 g (56% for microwave), 1.93 g (33% for conventional), m.p.: 259–260°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.93 (1H, s, NH); 10.52 (1H, s, NH); 8.67 (1H, s, H-4); 7.70 (1H, d, J = 7.2 Hz, Ar-H); 7.48 (2H, d, J = 8.8 Hz, Ar-H); 7.23 (2H, d, J = 8.8 Hz, Ar-H); 6.80 (1H, dd, J = 8.0 Hz, J = 2.0 Hz, Ar-H); 6.61 (1H, d, J = 2.0 Hz, Ar-H); 5.29 (2H, s, NH_2); 4.43 (2H, s, NCH_2); 3.85 (2H, s, CH_2); 3.47 (4H, q, $2CH_2$); 1.14 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.3 (C=O); 161.8 (C=O); 160.1 (C=O coumarin); 157.8 (Ar-C); 153.9 (coumarin C-3); 153.2 (C=O triazole); 148.7 (coumarin C-4); 147.1 (CN); 135.7; 132.3; 131.7; 131.5; 120.3; 110.8; 108.1; 96.3 (Ar-C); 46.5 (NCH_2); 44.8 ($2CH_2$); 30.3 (CH_2); 12.8 ($2CH_3$). LC-MS, *m/z*: 584.1273 [M (Br^{79})+H]$^+$, 586.1257 [M (Br^{81})+H]$^+$.

*N′-{[4-Amino-3-(3-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8p**)*

Yield: 3.79 g (65% for microwave), 1.87 g (32% for conventional), m.p.: 257–258°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.00 (1H, s, NH); 10.59 (1H, s, NH); 8.74 (1H, s, H-4); 7.77 (1H, d, J = 6.8 Hz, Ar-H); 7.53 (1H, s, Ar-H); 7.49–7.47 (1H, m, Ar-H); 7.33–7.31 (2H, m, Ar-H); 6.88 (1H, d, J = 6.8 Hz, Ar-H); 6.74 (1H, s, Ar-H); 5.39 (2H, s, NH_2); 4.45 (2H, s, NCH_2); 3.96 (2H, s, CH_2); 3.54 (4H, q, $2CH_2$); 1.19 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.3 (C=O); 161.8 (C=O); 160.1 (C=O coumarin); 157.8 (Ar-C); 153.9 (coumarin C-3); 153.2 (CO triazole); 148.7 (C=N); 147.0 (coumarin C-4); 139.0; 132.3; 131.9; 130.9; 130.0; 128.3; 120.0; 110.8; 108.1; 96.4 (Ar-C); 46.5 (NCH_2); 44.8 ($2CH_2$); 30.3 (CH_2); 12.8 ($2CH_3$). LC-MS, *m/z*: 584.1319 [M (Br^{79})+H]$^+$, 586.1305 [M (Br^{81})+H]$^+$.

*N′-{[4-Amino-3-(2-bromobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8q**)*

Yield: 2.80 g (48% for microwave), 1.63 g (28% for conventional), m.p.: 260–261°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.91 (1H, s, NH); 10.52 (1H, s, NH); 8.66 (1H, s, H-4); 7.69 (1H, d, J = 8.8 Hz, Ar-H); 7.60 (1H, d, J = 8.8 Hz, Ar-H); 7.29–7.18 (4H, m, Ar-H); 6.80 (1H, d, J = 7.6 Hz, Ar-H); 6.59 (1H, s, Ar-H); 5.33 (2H, s, NH_2); 4.43 (2H, s, NCH_2); 3.99 (2H, s, CH_2); 3.46 (4H, q, $2CH_2$); 1.11 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.2

(C=O); 161.8 (C=O); 160.1 (C=O coumarin); 157.8 (Ar-C); 153.9 (coumarin C-3); 153.2 (C=O triazole); 148.7 (C=N); 146.4 (coumarin C-4); 135.5; 132.9; 132.2; 131.4; 129.3; 128.2; 124.3; 110.8; 108.1; 96.4 (Ar-C); 46.5 (NCH_2); 44.8 ($2CH_2$); 31.3 (CH_2); 12.8 ($2CH_3$). LC-MS, *m/z*: 584.1324 [M (Br^{79})+H]$^+$, 586.1309 [M (Br^{81})+H]$^+$.

*N′-{[4-Amino-3-(3,4-dichlorobenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-7-diethylamino-2-oxo-2H-chromen-3-carbohydrazide (**8r**)*
Yield: 3.40 g (59% for microwave), 2.19 g (38% for conventional), m.p.: 286–287°C, ^{1}H NMR (DMSO-d_6), δ, ppm: 11.92 (1H, s, NH); 10.50 (1H, s, NH); 8.67 (1H, s, H-4); 7.71 (1H, d, J = 9.2 Hz, Ar-H); 7.56–7.51 (2H, d, Ar-H); 7.25 (1H, dd, J = 8.0 Hz, J = 1.6 Hz, Ar-H); 6.82 (1H, d, J = 8.8 Hz, Ar-H); 6.61 (1H, s, Ar-H); 5.29 (2H, s, NH_2); 4.43 (2H, s, NCH_2); 3.91 (2H, s, CH_2); 3.48 (4H, q, $2CH_2$); 1.11 (6H, t, $2CH_3$). ^{13}C APT (DMSO-d_6), δ, ppm: 164.3 (C=O); 161.8 (CO); 160.2 (C=O coumarin); 157.9 (Ar-C); 153.8 (coumarin C-3); 153.3 (CO triazole); 148.7 (coumarin C-4); 146.8 (C=N); 137.4; 132.3; 131.4; 131.2; 130.9; 129.8; 129.7; 110.8; 108.1; 96.4 (Ar-C); 46.5 (NCH_2); 44.8 ($2CH_2$); 29.8 (CH_2); 12.8 ($2CH_3$). LC-MS, *m/z*: 574.1347 [M (Cl^{35})(Cl^{35})+H]$^+$, 576.1320 [M (Cl^{37})(Cl^{35})+H]$^+$.

Biological activity

Cytotoxic activity assay
Antitumor activities of compounds **8a–r** *in vitro* were determined colorimetrically using the tetrazolium salt (MTT) assay. Tumor cells (BT20 human breast carcinoma, SK-Mel 128 melanoma, DU-145 prostate carcinoma, and A549 lung carcinoma) were grown in RPMI 1640 (Lonza, USA) medium supplemented with 10% fetal calf serum (FCS) and gentamycin/fungizon. Human fibroblast cells were grown in α-MEM (Lonza, USA). Cells (1×10^5 per mL) were seeded into a 96-well plate, then the compounds studied were added in a concentration gradient, and the final concentrations were maintained at 100, 31.6, 10, 3.16, and 1 μg/mL, respectively. The plate was maintained at 37°C in a humidified atmosphere of 5% CO_2 and incubated for 48 h. Antineoplastic cisplatin was used as positive control. After incubation, media were replaced on 100 μL fresh RPMI-1640 1% FCS and antibiotics for 30 min at 37°C in a humidified atmosphere of 5% CO_2. Then, 10 μL MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide) solution of an 5 mg/mL concentration was added to each well. After incubation for 3 h at 37°C, media were removed and 100 μL DMSO was added to all wells for solubilization of formazan crystal. Measurement of the absorbance of the solutions, related to the number of live cells, was carried out using a microplate reader (Tecan, Sunrise) at 570 nm. Cell cytotoxicity (CC_{50}) values were calculated by GraphPad Prism software using dose–response curves. The SI is defined as the ratio of the CC_{50} obtained from the experiments on normal cells to that obtained from cancer cells. As the SI demonstrates the differential activity of the compound, the greater the SI value is, the more selective the compound is [37, 38].

Lipase inhibition assay
The inhibitory effects of those compounds were evaluated against porcine pancreatic lipase (Applichem, Germany) (15 ng/mL). Lipase activity assay was done according to Kurihara et al. [39]. The lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. Briefly, compounds were mixed with porcine pancreatic lipase (PPL) 1:3 (v/v) and incubated for 30 min. The microtiter plates containing 50 μL 0.1 mM 4-MU oleate, 25 μL diluted compound-lipase solution, 25 μL dH_2O and assay buffer (13 mM Tris-HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$, pH 8.0) were incubated at 37°C for 20 min. After incubation, in order to stop the reaction, 0.1 mL 0.1 M pH 4.2 citrate buffer was added to the reaction mixture. The amount of 4-methylumbelliferone released by the lipase was measured by using a spectrofluorometer (SpectraMax M5, Molecular Devices) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The inhibitory activity of those compounds and orlistat (Xenical, Hoffman, La Roche, Segrate, Italy), an inhibitor control of pancreatic lipase, were measured at various concentrations. Residual activities were calculated by comparing to control without inhibitor. The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that gives 50% inhibition of maximal activity.

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