European Journal of Medicinal Chemistry 68 (2013) 103–110

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Polyoxygenated cinnamoylcoumarins as conformationally constrained analogs of cytotoxic diarylpentanoids: Synthesis and biological activity

Fatemeh Molaverdi [a], Mehdi Khoobi [b], Saeed Emami [c], Masoumeh Alipour [a], Omidreza Firuzi [d], Alireza Foroumadi [b], Gholamreza Dehghan [e], Ramin Miri [d], Fatemeh Shaki [f], Farnaz Jafarpour [a], Abbas Shafiee [b,*]

[a] School of Chemistry, College of Science, University of Tehran, Tehran, Iran
[b] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
[c] Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[d] Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[e] Department of Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran
[f] Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

ARTICLE INFO

*Article history:*
Received 3 February 2013
Received in revised form
11 June 2013
Accepted 4 July 2013
Available online 8 August 2013

*Keywords:*
Curcuminoids
Diarylpentanoids
Coumarin
Anti-cancer
Cytotoxic agent
Antioxidant activity

ABSTRACT

A series of polyoxygenated cinnamoylcoumarins was synthesized as conformationally constrained analogs of cytotoxic diarylpentanoids. The title compounds were tested against the viability of human chronic myelogenous leukemia (K562), human acute lymphoblastic leukemia (MOLT-4) and human breast adenocarcinoma (MCF-7) cell lines by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Among them, all 6- or 7-hydroxylated compounds **6a**–**h** exhibited remarkable cytotoxic activity. Particularly, 7-hydroxycoumarin analog **6h** showed good antiproliferative activity against all tested cell lines ($IC_{50}$ values $\leq$ 5.5 μM). The preliminary study with selected compounds **6e** and **6f** showed that reactivity towards mitochondrial thiol compounds cab be considered as cytotoxic mechanism of designed compounds. Furthermore, the antioxidant activity evaluation of synthesized compounds showed that hydroxylated compounds had antioxidative potential at higher concentrations.



## 1. Introduction

Cancer is going to be the leading cause of death worldwide and affects about one third of people during their lives. Despite notable advances have been made in detection, prevention and treatment of cancer diseases, but one-half of cancer patients do not respond to therapy or relapse following initial response [1]. However, chemotherapy is still one of the primary bases for the treatment of cancer diseases. Due to the undesirable side effects of chemotherapeutic agents and emergence of drug resistance, there is urgent need for developing new chemotherapeutic agents with more potent anti-tumor activity and high therapeutic index [2].

Among the small molecules introduced to prevent or treat cancer, there is an important group of compounds structurally contains α,β-unsaturated carbonyl system [3]. This structural feature was found in many naturally occurring compounds including flavonoids, isoflavonoids, chalconoids and curcuminoids. Curcumin (**1**) is the principal curcuminoid of turmeric (dry rhizomes of *Curcumin longa*) which is commonly used as a spice and food colorant [4]. Curcumin and its derivatives have extensive biological activities, including antioxidant [5] and anticancer activities [6]. Structurally, curcumin (Fig. 1) is a symmetrical methoxyphenolic dienone with an active methylene center. Considering the biological importance of curcumin, many efforts have been devoted to curcumin analogs. These efforts focused mainly on omitting the active methylene group and one carbonyl group of curcumin leading to mono-carbonyl derivatives **2**–**4** (Fig. 1) namely diaryl-1,4-pentadiene-3-ones (diarylpentanoids)

* Corresponding author. Tel.: +98 21 66406757; fax: +98 21 66461178.
*E-mail address:* ashafiee@ams.ac.ir (A. Shafiee).

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http://dx.doi.org/10.1016/j.ejmech.2013.07.014

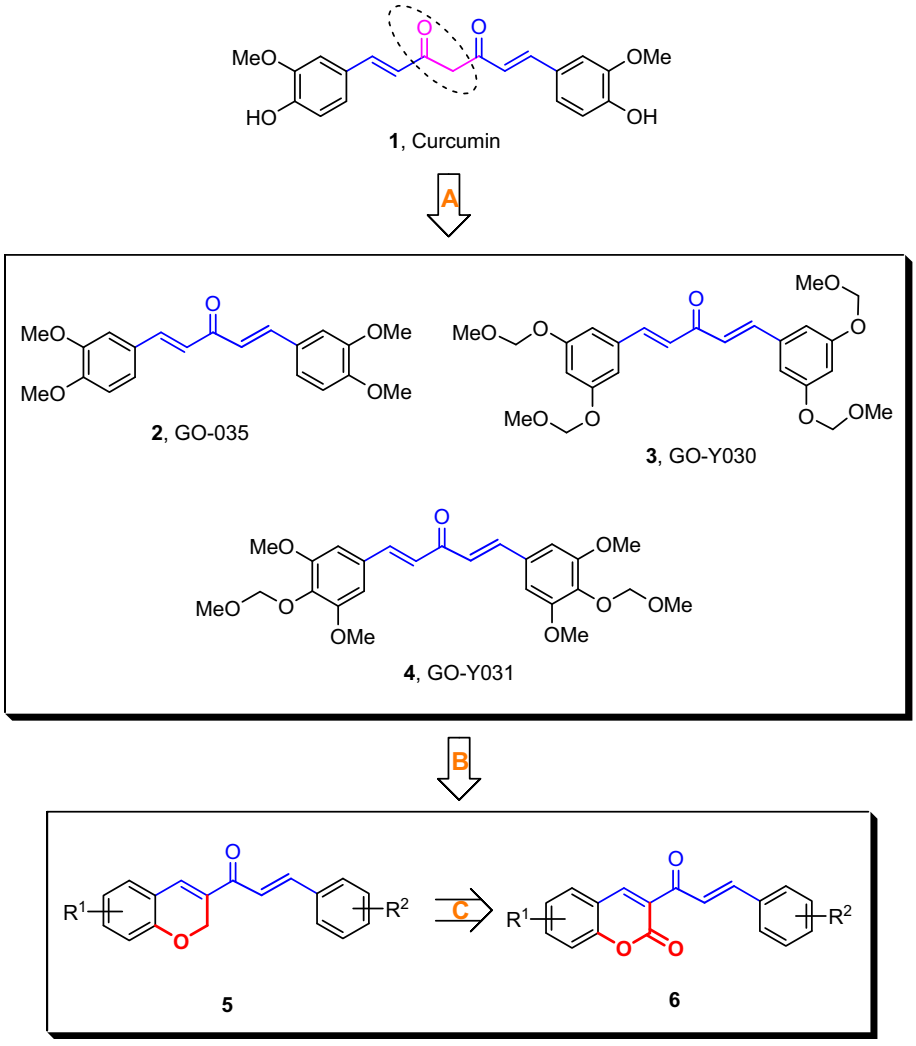



**Fig. 1.** The strategy for design of cinnamoylcoumarins **6**: (A) deletion of $CH_2CO$ from curcumin (**1**) to generate diarylpentanoids **2**–**4**; (B) conformational restriction of diarylpentanoids to chromene derivatives **5**; (C) introduction of 2-oxo functionality to yield coumarin-diarylpentanoid hybrids **6**.

and changing aryl substitution pattern of the molecule [7,8]. Ohori et al. have been reported several diarylpentanoids GO-035 (**2**), GO-Y030 (**3**) and GO-Y031 (**4**), which have a high capacity for growth suppression in many cancer cell lines. These curcumin-type compounds have a stronger potential to induce down-regulation of oncoproteins, including β-catenin, ErbB-2, c-Myc, cyclin D1, and Ki-ras, than curcumin [9].

Recently, we have described some potential cytotoxic agents namely 1-(2*H*-chromen-3-yl)-3-phenylprop-2-en-1-ones (**5**) as chromene-chalcone hybrids (Fig. 1) [10]. Indeed, these compounds are conformationally restricted analogs of diarylpentanoids.

On the other hand, coumarins belong to the flavonoid class of plant secondary metabolite, which have been found to exhibit a variety of biological activities, including antioxidant [11], and cytotoxic effects [12,13].

Encouraged by the aforementioned findings and in continuation of our research program to find novel anticancer agents [10,14], it was of our interest to design and synthesize cinnamoylcoumarins **6**. Indeed, these compounds could be considered as coumarin–diarylpentanoid hybrids, in which ester bond of the coumarin nucleus partially restricts the conformation of the diarylpentanoid system. Thus, we describe here, the synthesis and biological evaluation of polyoxygenated cinnamoylcoumarins as new antiproliferative and antioxidant agents.

## 2. Chemistry

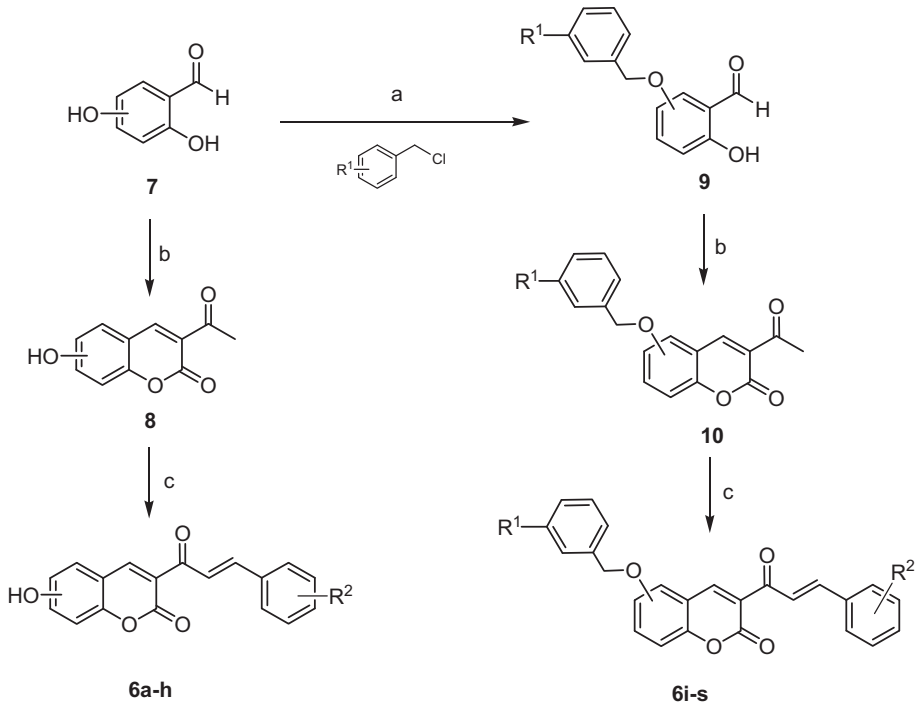
The target compounds **6a**–**s** were synthesized via the routes illustrated in Scheme 1. Firstly, commercial hydroxysalicylaldehyde **7** was converted to 6- or 7-hydroxy-3-acetylcoumarins **8** using ethyl acetoacetate in the presence of catalytic amount of piperidine [15]. In the next step, 3-acetylcoumarins **8** were condensed with several aldehydes in refluxing ethanol and in the presence of piperidine as catalyst to afford compounds **6a**–**h**. On the other hand, *O*-benzylation of hydroxysalicylaldehyde **7** in dry acetonitrile produced benzyloxysalicylaldehydes **9**. Reaction of **9** with ethyl acetoacetate yielded corresponding 3-acetylcoumarins **10** which subsequently condensed with appropriate aldehydes to afford final compounds **6i**–**s**.

The latter aldol products **6** were only in *E*-configuration as thermodynamically favored structures. The coupling constant of 15.3–16 Hz between the olefinic protons clearly showed that the compound **6** possess the *E*-configuration.

## 3. Biology

### 3.1. Cytotoxic assay

The cytotoxic activity of synthesized compounds **6a**–**s** against K562 (human chronic myelogenous leukemia), MOLT-4 (human acute




**Scheme 1.** Synthesis of substituted cinnamoylcoumarins **6a**–**s**. *Reagents and conditions*: (a) $NaHCO_3$, KI, dry $CH_3CN$; (b) ethyl acetoacetate, EtOH, piperidine; (c) appropriate benzaldehyde, piperidine, EtOH.

lymphoblastic leukemia) and MCF-7 (human breast adenocarcinoma) cell lines was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [16]. The percent inhibition of viability for 3–4 concentrations of the compounds was calculated with respect to the control. The $IC_{50}$ values of compounds were estimated with the software CurveExpert version 1.34 for Windows. Each experiment was repeated 4–5 times. The $IC_{50}$ values of tested compounds in comparison with cisplatin as standard drug are presented in Table 1 as mean ± SD (in μM).

### 3.2. Antioxidant activity evaluation

Antioxidant activity can be considered as an index of therapeutic usefulness of new compounds [17]. Numerous methods are available for the assessment of antioxidant capacity of compounds. In this study, two commonly used methods, the DPPH (1,1-diphenyl-2-picrylhydrzyl) radical scavenging and ferric reducing antioxidant power (FRAP) assays were used to determine the antioxidant potential of the target compounds **6** in comparison with quercetin. The results are summarized in Table 2.

### 3.3. Mitochondrial total thiol assay

The effect of selected compounds **6e** and **6f** on total mitochondrial thiol content was determined using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as indicator, and the absorbance was measured at 412 nm by spectrophotometry. Corrected absorbance values were used to calculate mitochondrial total thiols using the glutathione (GSH) standard curve [18].

## 4. Results and discussion

### 4.1. Cytotoxic activity

The $IC_{50}$ values of target compounds **6** against K562, MCF-7, and MOLT-4 cells in Table 1 revealed that all 6- or 7-hydroxylated compounds **6a**–**h** (with exception of **6g**) exhibited remarkable cytotoxic activity possessing $IC_{50}$ values between 1.3 and 17.3 μM. Among the benzyloxy derivatives **6i**–**s**, compound **6o** bearing a 2,6-dichloro substituent on cinnamoyl moiety showed significant activity ($IC_{50}$ values ≤ 15.6 μM) against all cell lines while the rest of compounds exhibited weak or no activity.

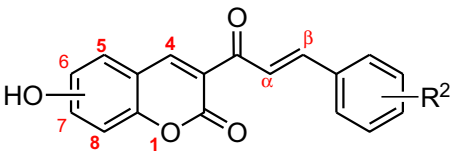
The comparison of $IC_{50}$ values against K562 cells demonstrated that carboxy methyl ester derivative **6h** had the most potent inhibitory activity against this cell line ($IC_{50}$ value = 4.4 μM). However, 3,4,5-trimethoxycinnamoyl analogs **6b** and **6f** with $IC_{50}$ values ≤8.8 μM showed potent anti-proliferative activity against K562 cells. Interestingly, carboxy methyl ester derivatives **6d** and **6h** were the most potent compounds in the term of cytotoxic activity against MCF-7 cells. The 6-hydroxy analog **6d** ($IC_{50}$ = 1.3 μM) showed better inhibition respect to the 7-hydroxy derivative **6h** ($IC_{50}$ = 5.5 μM) against the latter cell line. In the case of human acute lymphoblastic leukemia cells (MOLT-4), 3,4-methylenedioxy derivative of 6-hydroxycomarin (compound **6c**) with $IC_{50}$ value of 2.0 μM exhibited the highest activity. However, 3,4,5-trimethoxy derivatives (compounds **6b** and **6f**) as well as carboxy methyl ester analog (compound **6h**) were amongst the more potent compounds against MOLT-4 ($IC_{50}$ values = 3.7–3.9 μM).

In overall, the 7-hydroxycoumarin analog **6h** containing carboxy methyl ester group on the cinnamoyl moiety showed good anti-proliferative activity against all tested cell lines.

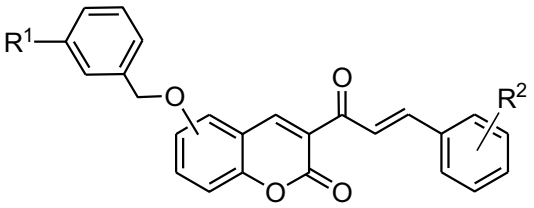
### 4.2. Reactivity towards mitochondrial thiols

A number of studies have demonstrated that conjugated enones such as diarylpentanoids act as Michael acceptor for intracellular thiol compounds [19–21]. The Michael addition of intracellular thiol compounds such as glutathione (GSH) to the olefinic double bond of enones results in the formation of corresponding conjugated adducts. In general, an initial reaction of cytotoxic agents with cellular thiols sensitizes the malignant cells to oxidative stress and modulates processes such as DNA synthesis, enzyme

**Table 1**
The $IC_{50}$ values (μM) of compounds **6a**–**s** against different cell lines.




**6a-h**




**6i-s**

| Compound | 6- or 7-substitution | $R^1$ | $R^2$ | K562 | MCF-7 | MOLT-4 |
|---|---|---|---|---|---|---|
| **6a** | 6 | – | 2,3-$(MeO)_2$ | 17.3 ± 4.8 | 8.8 ± 2.9 | 6.7 ± 1.0 |
| **6b** | 6 | – | 3,4,5-$(MeO)_3$ | 8.8 ± 4.6 | 10.9 ± 6.5 | 3.9 ± 0.7 |
| **6c** | 6 | – | 3,4-$(OCH_2O)$ | 14.6 ± 3.2 | 9.7 ± 2.3 | 2.0 ± 0.4 |
| **6d** | 6 | – | 4-(COOMe) | 15.8 ± 5.9 | 1.3 ± 0.7 | 7.1 ± 1.2 |
| **6e** | 7 | – | 2,3-$(MeO)_2$ | 14.6 ± 1.6 | 16.6 ± 6.8 | 7.0 ± 0.6 |
| **6f** | 7 | – | 3,4,5-$(MeO)_3$ | 7.3 ± 3.2 | 8.8 ± 2.8 | 3.8 ± 0.9 |
| **6g** | 7 | – | 3,4-$(OCH_2O)$ | 31.7 ± 6.3 | 38.9 ± 6.3 | 26.2 ± 10.5 |
| **6h** | 7 | – | 4-(COOMe) | 4.4 ± 0.75 | 5.5 ± 0.7 | 3.7 ± 0.4 |
| **6i** | 6 | H | 2,3-$(MeO)_2$ | 48.9 ± 23.5 | 21.3 ± 10.6 | 23.6 ± 10.5 |
| **6j** | 6 | H | 3,4,5-$(MeO)_3$ | 66.4 ± 8.0 | 26.5 ± 3.9 | 10.3 ± 4.1 |
| **6k** | 6 | H | 3,4-$(OCH_2O)$ | >50 | >50 | >50 |
| **6l** | 7 | MeO | H | >100 | 33.7 ± 9.8 | >100 |
| **6m** | 7 | MeO | 4-Me | >50 | >50 | >50 |
| **6n** | 7 | MeO | 2,4-$Cl_2$ | ND[a] | ND | ND |
| **6o** | 7 | MeO | 2,6-$Cl_2$ | 15.6 ± 7.5 | 10.1 ± 4.3 | 12.5 ± 2.0 |
| **6p** | 7 | MeO | 4-MeO | >100 | >100 | >100 |
| **6q** | 7 | MeO | 2,3-$(MeO)_2$ | 45.5 ± 7.0 | 35.1 ± 8.7 | 22.8 ± 8.9 |
| **6r** | 7 | MeO | 2,5-$(MeO)_2$ | >100 | >100 | 13.8 ± 0.4 |
| **6s** | 7 | MeO | 3,4-$(OCH_2O)$ | >100 | >100 | >100 |
| **Cisplatin** | | | | 12.2 ± 2.0 | 10.9 ± 3.7 | 3.1 ± 0.4 |

[a] Not determined.

activation, selective gene expression, and regulation of the cell cycle [22]. Physiological cell death (apoptosis) can be initiated from mitochondria via cytochrome *c* release following mitochondrial permeability transition (MPT) pores opening. It has been shown GSH have an essential role in MPT pores and oxidation of thiol groups in the inner mitochondrial membrane cause conformational changes in the pore complex that leading to MPT pores opening and imitation of apoptosis signaling [23,24].

Accordingly, we investigated the effect of selected compounds **6e** and **6f** on the total thiol content of mitochondria after 1 h exposure. As shown in Fig. 2, the tested compounds **6e** and **6f** decreased the mitochondrial thiol content in a concentration dependent manner. Therefore, thiol alkylation and induction of mitochondrial pathway of apoptosis cab be considered as cytotoxic mechanism of designed compounds. Although, we have shown that cinnamoylcoumarins such as **6e** and **6f** prototypes act as Michael acceptor for mitochondrial thiol compounds, but we cannot rebut other mechanisms such as tubulin polymerization inhibition which are responsible for cytotoxic activity.

### 4.3. Antioxidant activity

As shown in Table 2, all hydroxycoumarin derivatives **6a**–**h** exhibited better DPPH radical scavenging activity with $IC_{50}$ values less than 0.55 mM. The 2,3-dimethoxy analogs **6a** and **6e** were the most potent compounds in the scavenging of DPPH radicals ($IC_{50} \approx 0.15$ mM). Furthermore, compounds **6b** and **6f** bearing 3,4,5-trimetoxycinnamoyl showed significant radical scavenging activity with 50% inhibitory concentrations of about 0.18 mM.

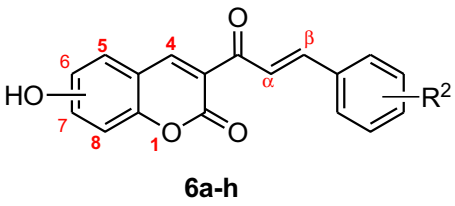
The FRAP assay shows the capability of compound to reduce the ferric 2,4,6-tripyridyl-*s*-triazine complex to the colored ferrous complex [25]. The results of FRAP assay in Table 2 demonstrated that 2,3-dimethoxy analogs of 6- or 7-hydroxycoumarins (compounds **6a** and **6e**) had superior antioxidant power in FRAP assay. The relative capability of these compounds to reduce the ferric ions respect to quercetin was about 0.3. Among the benzyloxycoumarins, compound **6p** exhibited the highest antioxidant power to reducing ferric ions.

In general, the 2,3-dimethoxycinnamoyl-hydroxycoumarins **6a** and **6e** exhibited the most potent antioxidant activity in both DPPH and FRAP methods. Although compounds **6a** and **6e** were not as potent as reference drug quercetin, but their antioxidant activity could be considered as attendant property of the title compounds.

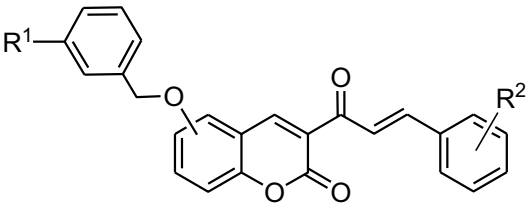
## 5. Conclusion

We described a series of polyoxygenated cinnamoylcoumarins as conformationally constrained analogs of cytotoxic diarylpentanoids. Two set of compounds bearing hydroxyl or benzyloxy moiety on coumarin ring were synthesized and tested against the viability of three different cell lines. Among them, all 6- or 7-hydroxylated compounds **6a**–**h** exhibited remarkable cytotoxic activity. Particularly, 7-hydroxycoumarin analog **6h** containing carboxy methyl ester group on cinnamoyl moiety showed good antiproliferative activity against all tested cell lines ($IC_{50}$ values $\leq$ 5.5 μM). Besides carboxy methyl ester, 3,4,5-trimethoxyphenyl scaffold was found to be a good functionality for exhibiting the cytotoxic potential of cinnamoylcoumarins. The effect of selected compounds **6e** and **6f** on total mitochondrial thiol content demonstrated that cinnamoylcoumarins act as Michael acceptor for mitochondrial thiol compounds. Furthermore, the antioxidant activity evaluation of synthesized compounds showed that hydroxylated compounds had antioxidative potential at higher concentrations.

**Table 2**
Antioxidant properties of compounds **6** in comparison with quercetin.




**6a-h**




**6i-s**

| Compound | 6- or 7-substitution | $R^1$ | $R^2$ | DPPH radical scavenging activity ($IC_{50}$, mM) | FRAP value (mM $Fe^{2+}$) (100 μg) |
|---|---|---|---|---|---|
| **6a** | 6 | – | 2,3-$(MeO)_2$ | 0.16 ± 0.01 | 25.7 ± 0.24 |
| **6b** | 6 | – | 3,4,5-$(MeO)_3$ | 0.18 ± 0.01 | 9.6 ± 0.08 |
| **6c** | 6 | – | 3,4-($OCH_2O$) | 0.48 ± 0.01 | 15.3 ± 0.07 |
| **6d** | 6 | – | 4-(COOMe) | ND | ND |
| **6e** | 7 | – | 2,3-$(MeO)_2$ | 0.15 ± 0.01 | 25.2 ± 0.10 |
| **6f** | 7 | – | 3,4,5-$(MeO)_3$ | 0.19 ± 0.01 | 8.7 ± 0.10 |
| **6g** | 7 | – | 3,4-($OCH_2O$) | 0.54 ± 0.01 | 13.7 ± 0.26 |
| **6h** | 7 | – | 4-(COOMe) | ND | ND |
| **6i** | 6 | H | 2,3-$(MeO)_2$ | 1.27 ± 0.02 | 4.4 ± 0.01 |
| **6j** | 6 | H | 3,4,5-$(MeO)_3$ | 1.15 ± 0.03 | 8.1 ± 0.07 |
| **6k** | 6 | H | 3,4-($OCH_2O$) | 0.57 ± 0.01 | 12.6 ± 0.11 |
| **6l** | 7 | MeO | H | 0.89 ± 0.01 | 10.4 ± 0.09 |
| **6m** | 7 | MeO | 4-Me | 2.63 ± 0.04 | 6.6 ± 0.02 |
| **6n** | 7 | MeO | 2,4-$Cl_2$ | 0.48 ± 0.01 | 7.1 ± 0.09 |
| **6o** | 7 | MeO | 2,6-$Cl_2$ | 0.94 ± 0.02 | 5.3 ± 0.02 |
| **6p** | 7 | MeO | 4-MeO | 0.93 ± 0.02 | 14.3 ± 0.10 |
| **6q** | 7 | MeO | 2,3-$(MeO)_2$ | 0.72 ± 0.01 | 7.6 ± 0.11 |
| **6r** | 7 | MeO | 2,5-$(MeO)_2$ | 0.75 ± 0.01 | 9.5 ± 0.07 |
| **6s** | 7 | MeO | 3,4-($OCH_2O$) | 0.46 ± 0.01 | 7.5 ± 0.06 |
| **Quercetin** | | | | 8.12 ± 0.13[a] | 87.1 ± 1.4 |

[a] $IC_{50}$ in μM.

## 6. Experimental

### 6.1. Chemistry

#### 6.1.1. General methods

All starting material and reagents were purchased from Acros Organics and Merck, and used without further purification. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrometer (KBr disks). The $^1H$ NMR spectra were recorded on a NMR instrument Bruker 500 MHz. The chemical shifts ($\delta$) and coupling constants ($J$) are expressed in parts per million (ppm) and Hertz (Hz), respectively. An HP (Agilent technologies) 5937 Mass Selective Detector was used to obtain Mass spectra of the compounds. Elemental analyses were carried out with a Perkin–Elmer model 240-C apparatus. The results of elemental analyses (C, H, N) were within ±0.4% of the calculated values.

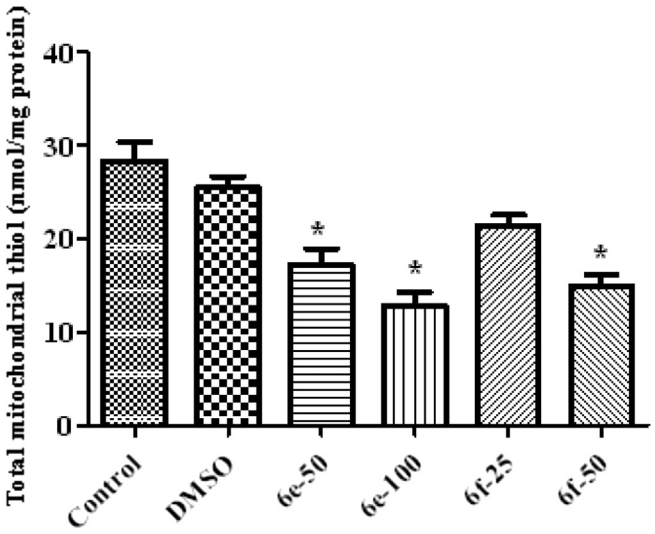



**Fig. 2.** Effect of compounds **6e** and **6f** on mitochondrial total thiol content. Isolated liver mitochondria (1 mg/mL) were incubated for 1 h with **6f** (25 and 50 μM) and **6e** (50 and 100 μM). Then, total thiol content was measured using DTNB method. Values represented as mean ± SD ($n = 3$). *$P < 0.05$ compared with control group.

#### 6.1.2. General procedure for the synthesis of benzyloxysalicylaldehyde derivatives ***9***

A suspension of hydroxysalicylaldehyde (1.0 mmol), $NaHCO_3$ (1.5 mmol), and catalytic amounts of KI in dry acetonitrile (3.0 mL) was stirred at room temperature for 5 min. Then, appropriate benzyl halide (1.2 mmol) was added to the mixture and the mixture was allowed to warm to 90 °C under argon atmosphere. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was mixed with ethyl acetate (5 mL) and the insoluble solids was filtered and washed with ethyl acetate (3 × 5 mL). The organic phase was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using petroleum ether–ethyl acetate (8:2) as eluent to give the pure product **9**.

#### 6.1.3. General procedure for the synthesis of 3-acetylcoumarin derivatives ***8*** or ***10***

A cold mixture of salicylaldehyde derivative **7** or **9** (0.2 mol) and ethyl acetoacetate (0.2 mol) in absolute ethanol (25 mL) was treated with piperidine (0.2 mL). The mixture was allowed to warm to 50 °C. After completion of the reaction, the precipitated

solid was filtered off and subsequently washed with ethanol and recrystallized from water–ethanol (30:70) to give pure product.

*6.1.4. General procedure for the synthesis of cinnamoylcoumarins* ***6***

To a mixture of 3-acetylcoumarin derivative **8** or **10** (1 mmol) and appropriate aldehyde (1 mmol) in absolute ethanol (3 mL) were added catalytic amount of piperidine (3 drops). The mixture was heated under reflux for 12–24 h. After completion of the reaction, water was added and the mixture was extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using petroleum ether–ethyl acetate (8:2) as eluent to give the pure product **6**.

*6.1.4.1. (E)-3-(3-(2,3-Dimethoxyphenyl)acryloyl)-6-hydroxy-2H-chromen-2-one (**6a**).* Yellow solid; yield 85%; mp: >250 °C; IR (KBr, $cm^{-1}$): 3262 (OH), 1684 (C=O), 1589 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 10.45 (br s, 1H, OH), 8.25 (s, 1H, $H_4$ coumarin), 7.49 (d, 1H, $J = 15.4$ Hz, $H_\beta$), 7.41 (d, 1H, $J = 15.4$ Hz, $H_\alpha$), 7.24 (d, 1H, $J = 8.2$ Hz, $H_7$ coumarin), 6.89 (s, 1H, $H_5$ coumarin), 6.84 (d, $J = 8.2$ Hz, 1H, $H_8$ coumarin), 6.55–6.52 (m, 3H phenyl), 3.01 (s, 6H, 2 × $OCH_3$). Anal. Calcd for $C_{20}H_{16}O_6$ (352.33): C, 68.18; H, 4.58. Found: C, 68.36; H, 4.49.

*6.1.4.2. (E)-6-Hydroxy-3-(3-(3,4,5-trimethoxyphenyl)acryloyl)-2H-chromen-2-one (**6b**).* Yellow solid; yield 82%; mp: >250 °C; IR (KBr, $cm^{-1}$): 3337 (OH), 1731 (C=O), 1648 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.42 (s, 1H, $H_4$ coumarin), 7.74 (d, 1H, $J = 15.7$ Hz, $H_\beta$), 7.59 (d, 1H, $J = 15.7$ Hz, $H_\alpha$), 7.38 (d, 1H, $J = 8.4$ Hz, $H_7$ coumarin), 6.75–6.70 (m, 4H, $H_{5,8}$ coumarin and $H_{2,6}$ phenyl), 3.77 (s, 6H, 2 × $OCH_3$), 3.74 (s, 3H, $OCH_3$). Anal. Calcd for $C_{21}H_{18}O_7$ (382.36): C, 65.96; H, 4.74. Found: C, 65.76; H, 4.49.

*6.1.4.3. (E)-3-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)-6-hydroxy-2H-chromen-2-one (**6c**).* Yellow solid; yield 90%; mp: >250 °C; IR (KBr, $cm^{-1}$): 3337 (OH), 1607 (C=O), 1731 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 9.49 (br s, 1H, OH), 8.47 (s, 1H, $H_4$ coumarin), 7.75 (m, 2H, $H_{\alpha,\beta}$), 7.52 (s, 1H, $H_5$ coumarin), 7.24–7.11 (m, 4H, $H_{7,8}$ coumarin and $H_{2,6}$ phenyl), 6.88 (d, $J = 7.6$ Hz, $H_5$ phenyl), 6.07 (s, 2H, $OCH_2O$). Anal. Calcd for $C_{19}H_{12}O_6$ (336.29): C, 67.86; H, 3.60. Found: C, 67.70; H, 3.36.

*6.1.4.4. Methyl (E)-4-(3-(6-hydroxy-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)benzoate (**6d**).* Yellowish solid; yield 60%; mp: 243–245 °C; IR (KBr, $cm^{-1}$): 3433 (OH), 1724 (C=O), 1685 (C=O). $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.93 (s, 1H, OH), 8.60 (s, 1H, $H_4$ coumarin), 8.01 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$ phenyl), 7.86 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$ phenyl), 7.76 (br s, 2H, $H_{\alpha,\beta}$), 7.33 (d, $J = 9.0$ Hz, 1H, $H_8$ coumarin), 7.24 (d, $J = 2.5$ Hz, 1H, $H_5$ coumarin), 7.16 (dd, $J = 9.0$ and 2.5 Hz, 1H, $H_7$ coumarin), 3.86 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 187.3, 165.7, 158.6, 154.0, 147.9, 147.2, 142.0, 138.9, 130.9, 129.7, 128.8, 127.1, 125.2, 122.6, 118.8, 117.1, 113.9, 52.3. Anal. Calcd for $C_{20}H_{14}O_6$ (350.08): C, 68.57; H, 4.03. Found: C, 68.28; H, 4.33.

*6.1.4.5. (E)-7-Hydroxy-3-(3-(2,3-dimethoxyphenyl)acryloyl)-2H-chromen-2-one (**6e**).* Yellow solid; yield 89%; mp: 181–183 °C; IR (KBr, $cm^{-1}$): 3416 (OH), 1703 (C=O), 1661 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 11.15 (s, 1H, OH), 8.67 (s, 1H, $H_4$ coumarin), 7.89–7.82 (m, 3H, $H_5$ coumarin and $H_{4,6}$ phenyl), 7.33 (t, $J = 7.5$ Hz, 1H, $H_5$ phenyl), 7.16–7.14 (m, 2H, $H_{\alpha,\beta}$), 6.86 (d, $J = 7.5$ Hz, 1H, $H_6$ coumarin), 6.78 (s, 1H, $H_8$ coumarin), 3.84 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$). Anal. Calcd for $C_{20}H_{16}O_6$ (352.33): C, 68.18; H, 4.58. Found: C, 68.36; H, 4.39.

*6.1.4.6. (E)-7-Hydroxy-3-(3-(3,4,5-trimethoxyphenyl)acryloyl)-2H-chromen-2-one (**6f**).* Yellow solid; yield 91%; mp: >250 °C; IR (KBr, $cm^{-1}$): 3337 (OH), 1730 (C=O), 1646 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 10.56 (s, 1H, OH), 8.56 (s, 1H, $H_4$ coumarin), 7.88 (d, $J = 15.4$ Hz, 1H, $H_\beta$), 7.74 (d, $J = 15.4$ Hz, 1H, $H_\alpha$), 7.53 (d, $J = 7.8$ Hz, 1H, $H_5$ coumarin), 7.45 (s, 1H, $H_8$ coumarin), 6.90–6.84 (m, 3H, $H_6$ coumarin and $H_{2,6}$ phenyl), 3.92 (s, 6H, 2 × $OCH_3$), 3.88 (s, 3H, $OCH_3$). Anal. Calcd for $C_{21}H_{18}O_7$ (382.36): C, 65.96; H, 4.74. Found: C, 66.01; H, 4.59.

*6.1.4.7. (E)-3-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)-7-hydroxy-2H-chromen-2-one (**6g**).* Yellow solid; yield 88%; mp: >250 °C; IR (KBr, $cm^{-1}$): 3330 (OH), 1695 (C=O), 1615 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.43 (s, 1H, $H_4$ coumarin), 7.72 (d, $J = 15.7$ Hz, 1H, $H_\beta$), 7.62 (d, $J = 15.7$ Hz, 1H, $H_\alpha$), 7.37 (d, $J = 8.5$ Hz, 1H, $H_5$ coumarin), 7.08 (s, 1H, $H_2$ phenyl), 7.02 (d, $J = 8.0$ Hz, 1H, $H_6$ phenyl), 6.75–6.70 (m, 3H, $H_{6,8}$ coumarin and $H_5$ phenyl), 5.91 (s, 2H, $OCH_2O$). Anal. Calcd for $C_{19}H_{12}O_6$ (336.29): C, 67.86; H, 3.60. Found: C, 65.61; H, 3.32.

*6.1.4.8. Methyl (E)-4-(3-(7-hydroxy-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)benzoate (**6h**).* Yellowish solid; yield 60%; mp: 243–245 °C; IR (KBr, $cm^{-1}$): 3442 (OH), 1725 (C=O), 1701 (C=O). $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 8.65 (s, 1H, $H_4$ coumarin), 8.00 (d, $J = 8$ Hz, 2H, $H_{3,5}$ phenyl), 7.88 (d, $J = 16.0$ Hz, 1H, $H_\beta$), 7.84 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$ phenyl), 7.77 (d, $J = 8.5$ Hz, 1H, $H_5$ coumarin), 7.72 (d, $J = 16.0$ Hz, 1H, $H_\alpha$), 6.84 (d, $J = 8.5$, 1H, $H_6$ coumarin), 6.74 (s, 1H, $H_8$ coumarin), 3.85 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.2, 165.7, 164.6, 159.0, 157.3, 148.5, 141.0, 139.1, 132.5, 130.7, 129.7, 128.6, 127.1, 119.4, 114.4, 110.9, 101.9, 52.27. Anal. Calcd for $C_{20}H_{14}O_6$ (350.08): C, 68.57; H, 4.03. Found: C, 68.32; H, 3.95.

*6.1.4.9. (E)-6-(Benzyloxy)-3-(3-(2,3-dimethoxyphenyl)acryloyl)-2H-chromen-2-one (**6i**).* Yellow solid; yield 93%; mp: 172–174 °C; IR (KBr, $cm^{-1}$): 1728 (C=O), 1662 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.50 (s, 1H, $H_4$ coumarin), 8.20 (d, $J = 15.9$ Hz, 1H, $H_\beta$), 7.96 (d, $J = 15.9$ Hz, 1H, $H_\alpha$), 7.46–7.31 (m, 7H, $H_{7,8}$ coumarin, $H_6$ phenyl and 5H benzyloxy), 7.14 (d, 1H, $J = 2.3$ Hz, 1H, $H_5$ coumarin), 7.10 (t, $J = 8.0$ Hz, 1H, $H_5$ phenyl), 6.99 (d, $J = 8.0$ Hz, 1H, $H_4$ phenyl), 5.14 (s, 2H, $CH_2$ benzyl), 3.92 (s, 3H, $OCH_3$), 3.90 (s, 3H, $OCH_3$). Anal. Calcd for $C_{27}H_{22}O_6$ (442.46): C, 73.29; H, 5.01. Found: C, 73.56; H, 5.36.

*6.1.4.10. (E)-6-(Benzyloxy)-3-(3-(3,4,5-trimethoxyphenyl)acryloyl)-2H-chromen-2-one (**6j**).* Yellow solid; yield 93%; mp: 178–180 °C; IR (KBr, $cm^{-1}$): 1714 (C=O), 1638 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.51 (s, 1H, $H_4$ coumarin), 7.85 (d, $J = 15.7$ Hz, 1H, $H_\beta$), 7.79 (d, $J = 15.7$ Hz, 1H, $H_\alpha$), 7.46–7.33 (m, 7H, $H_{7,8}$ coumarin and 5H benzyloxy), 7.14 (d, $J = 2.3$ Hz, 1H, $H_5$ coumarin), 6.91 (s, 2H, $H_{2,6}$ phenyl), 5.14 (s, 2H, $CH_2$ benzyl), 3.93 (s, 6H, 2 × $OCH_3$), 3.91 (s, 3H, $OCH_3$). Anal. Calcd for $C_{28}H_{24}O_7$ (472.48): C, 71.38; H, 5.12. Found: C, 71.44; H, 5.36.

*6.1.4.11. (E)-3-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)-6-(benzyloxy)-2H-chromen-2-one (**6k**).* Yellow solid; yield 94%; mp: 198–200 °C; IR (KBr, $cm^{-1}$): 1719 (C=O), 1661 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.51 (s, 1H, $H_4$ coumarin), 7.80 (br s, 2H, $H_{\alpha,\beta}$), 7.46–7.30 (m, 7H, $H_{7,8}$ coumarin and 5H benzyloxy), 7.13 (d, $J = 2.5$ Hz, 1H, $H_5$ coumarin), 7.17 (dd, $J = 8.0$ and 2.1 Hz, 1H, $H_6$ phenyl), 7.22 (d, $J = 2.1$ Hz, 1H, $H_2$ phenyl), 6.85 (d, $J = 8.1$ Hz, 1H, $H_5$ phenyl), 6.04 (s, 2H, $OCH_2O$), 5.14 (s, 2H, $CH_2$ benzyl). Anal. Calcd for $C_{26}H_{18}O_6$ (426.41): C, 73.23; H, 4.25. Found: C, 73.44; H, 4.36.

*6.1.4.12. 3-Cinnamoyl-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6l**).* Yellow solid; yield 92%; mp: 131–133 °C; IR (KBr, $cm^{-1}$): 1714 (C=O), 1650 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.59 (s, 1H, $H_4$ coumarin), 8.02 (d, $J = 15.7$ Hz, 1H, $H_\beta$), 7.87 (d, $J = 15.7$ Hz, 1H, $H_\alpha$), 7.70–7.68 (m, 2H, $H_{2,6}$ phenyl), 7.58 (d, $J = 8.7$ Hz, 1H, $H_5$ coumarin), 7.42–7.41 (m, 3H, $H_{3,4,5}$ phenyl), 7.34 (t, 1H, $J = 7.8$ Hz, $H_5$ benzyloxy), 7.03–6.98 (m, 3H, $H_{4,6}$ benzyloxy and $H_6$ coumarin), 6.92–6.90 (m, 2H, $H_2$ benzyloxy and $H_8$ coumarin), 5.16 (s, 2H, $CH_2$

benzyl), 3.84 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.3, 164.1, 159.9, 159.7, 157.5, 148.4, 144.5, 136.8, 134.9, 131.3, 130.6, 129.9, 128.8, 124.1, 121.4, 119.6, 114.4, 113.8, 113.0, 112.5, 101.3, 70.6, 55.2. Anal. Calcd for $C_{26}H_{20}O_5$ (412.43): C, 75.72; H, 4.89. Found: C, 75.90; H, 4.99.

*6.1.4.13. (E)-7-((3-Methoxybenzyl)oxy)-3-(3-(p-tolyl)acryloyl)-2H-chromen-2-one (**6m**).* Yellow solid; yield 91%; mp: 174–176 °C; IR (KBr, $cm^{-1}$): 1717 (C=O), 1652 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.58 (s, 1H, $H_4$ coumarin), 7.98 (d, $J$ = 15.7 Hz, 1H, $H_\beta$), 7.85 (d, $J$ = 15.7 Hz, 1H, $H_\alpha$), 7.59–7.57 (m, 3H, $H_{2,6}$ phenyl and $H_5$ coumarin), 7.34 (t, $J$ = 7.8 Hz, 1H, $H_5$ benzyloxy), 7.22 (d, $J$ = 7.9 Hz, 2H, $H_{3,5}$ phenyl), 7.03–6.98 (m, 3H, $H_{4,6}$ benzyloxy and $H_6$ coumarin), 6.92–6.90 (m, 2H, $H_2$ benzyloxy and $H_8$ coumarin), 5.16 (s, 2H, $CH_2$ benzyl), 3.84 (s, 3H, $OCH_3$), 2.40 (s, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.4, 164.0, 159.9, 159.7, 157.4, 148.3, 144.7, 141.2, 136.8, 132.2, 131.3, 129.9, 129.6, 128.9, 123.1, 121.6, 119.6, 114.3, 113.8, 113.0, 112.5, 101.3, 70.6, 55.2, 21.5. Anal. Calcd for $C_{27}H_{22}O_5$ (426.46): C, 76.04; H, 5.20. Found: C, 76.37; H, 5.44.

*6.1.4.14. (E)-3-(3-(2,4-Dichlorophenyl)acryloyl)-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6n**).* Yellow solid; yield 85%; mp: 177–179 °C; IR (KBr, $cm^{-1}$): 1723 (C=O), 1655 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.62 (s, 1H, $H_4$ coumarin), 8.18 (d, $J$ = 15.8 Hz, 1H, $H_\beta$), 8.00 (d, $J$ = 15.8 Hz, 1H, $H_\alpha$), 7.78 (d, $J$ = 8.5 Hz, 1H, $H_5$ coumarin), 7.60 (d, $J$ = 8.8 Hz, 1H, $H_6$ phenyl), 7.46 (s, 1H, $H_3$ phenyl), 7.34 (t, $J$ = 7.2 Hz, 1H, $H_5$ benzyloxy), 7.30 (d, $J$ = 8.8 Hz, 1H, $H_5$ phenyl), 7.01–6.90 (m, 5H, $H_{6,8}$ coumarin and $H_{2,4,6}$ benzyloxy), 5.17 (s, 2H, $CH_2$ benzyl), 3.84 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 185.8, 173.3, 164.3, 159.8, 157.6, 148.9, 138.5, 136.7, 136.5, 131.8, 131.5, 129.9, 128.8, 127.5, 126.7, 120.9, 119.6, 114.5, 113.8, 113.0, 112.5, 101.3, 70.6, 55.2. Anal. Calcd for $C_{26}H_{18}Cl_2O_5$ (481.32): C, 64.88; H, 3.77. Found: C, 65.01; H, 3.58.

*6.1.4.15. (E)-3-(3-(2,6-Dichlorophenyl)acryloyl)-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6o**).* Yellow solid; yield 87%; mp: 162–164 °C; IR (KBr, $cm^{-1}$): 1715 (C=O), 1663 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.60 (s, 1H, $H_4$ coumarin), 8.12 (d, $J$ = 16.0 Hz, 1H, $H_\beta$), 7.94 (d, $J$ = 16.0 Hz, 1H, $H_\alpha$), 7.60 (d, $J$ = 8.5 Hz, 1H, $H_5$ coumarin), 7.38 (d, $J$ = 8.0 Hz, 2H, $H_{3,5}$ phenyl), 7.34 (t, $J$ = 8.0 Hz, 1H, $H_4$ phenyl),7.20 (t, $J$ = 7.8 Hz, 1H, $H_5$ benzyloxy), 7.02–6.91 (m, 5H, $H_{6,8}$ coumarin and $H_{2,4,6}$ benzyloxy), 5.16 (s, 2H, $CH_2$ benzyl), 3.84 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.3, 164.2, 159.9, 159.5, 157.6, 148.8, 137.3, 136.7, 135.4, 132.5, 132.0, 131.4, 129.9, 128.7, 127.7, 121.1, 119.6, 114.4, 113.8, 113.0, 112.4, 101.3, 70.6, 55.2. Anal. Calcd for $C_{26}H_{18}Cl_2O_5$ (481.32): C, 64.88; H, 3.77. Found: C, 64.96; H, 3.90.

*6.1.4.16. (E)-7-((3-Methoxybenzyl)oxy)-3-(3-(4-methoxyphenyl) acryloyl)-2H-chromen-2-one (**6p**).* Yellow solid; yield 92%; mp: 167–169 °C; IR (KBr, $cm^{-1}$): 1718 (C=O), 1655 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.58 (s, 1H, $H_4$ coumarin), 7.91 (d, $J$ = 15.5 Hz, 1H, $H_\beta$), 7.85 (d, $J$ = 15.5 Hz, 1H, $H_\alpha$), 7.65 (d, $J$ = 8.2 Hz, 2H, $H_{2,6}$ phenyl), 7.57 (d, $J$ = 8.7 Hz, 1H, $H_5$ coumarin), 7.34 (t, $J$ = 7.9 Hz, 1H, $H_5$ benzyloxy), 7.03–6.92 (m, 7H, $H_{3,5}$ phenyl, $H_{6,8}$ coumarin and $H_{2,4,6}$ benzyloxy), 5.16 (s, 2H, $CH_2$ benzyl), 3.86 (s, 3H, $OCH_3$), 3.84 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.2, 163.9, 161.7, 159.9, 159.7, 157.4, 148.2, 144.5, 136.8, 131.2, 130.7, 129.9, 127.7, 121.8, 121.6, 119.6, 114.3, 113.8, 113.0, 112.6, 101.3, 70.6, 55.3, 55.2. Anal. Calcd for $C_{27}H_{22}O_6$ (442.46): C, 73.29; H, 5.01. Found: C, 73.47; H, 5.34.

*6.1.4.17. (E)-3-(3-(2,3-Dimethoxyphenyl)acryloyl)-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6q**).* Yellow solid; yield 87%; mp: 142–144 °C; IR (KBr, $cm^{-1}$): 1719 (C=O), 1654 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.71 (s, 1H, $H_4$ coumarin), 7.89–7.81 (m, 3H, $H_{\alpha,\beta}$ and $H_5$ coumarin), 7.33–6.93 (m, 9H, $H_{6,8}$ coumarin, 4H benzyloxy and 3H phenyl), 5.25 (s, 2H, $CH_2$ benzyl), 3.84 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.3, 163.7, 159.3, 158.8, 156.8, 152.8, 148.1, 137.4, 137.1, 132.0, 129.6, 128.0, 125.6, 124.4, 119.9, 119.0, 115.0, 114.0, 113.5, 113.4, 112.2, 101.1, 70.0, 60.8, 55.7, 55.0. Anal. Calcd for $C_{28}H_{24}O_7$ (472.48): C, 71.18; H, 5.12. Found: C, 71.39; H, 5.25.

*6.1.4.18. (E)-3-(3-(2,5-Dimethoxyphenyl)acryloyl)-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6r**).* Yellow solid; yield 89%; mp: 147–149 °C; IR (KBr, $cm^{-1}$): 1726 (C=O), 1655 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.56 (s, 1H, $H_4$ coumarin), 8.19 (d, $J$ = 15.8 Hz, 1H, $H_\beta$), 8.01 (d, $J$ = 15.8 Hz, 1H, $H_\alpha$), 7.58 (d, $J$ = 8.7 Hz, 1H, $H_5$ coumarin), 7.34 (t, $J$ = 7.9 Hz, 1H, $H_5$ benzyloxy), 7.23 (s, 1H, $H_2$ benzyloxy), 7.03–6.86 (m, 7H, $H_{6,8}$ coumarin, $H_{4,6}$ benzyloxy and 3H phenyl), 5.16 (s, 2H, $CH_2$ benzyl), 3.88 (s, 3H, $OCH_3$), 3.84 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.7, 163.9, 159.9, 159.7, 157.4, 153.4, 148.1, 139.6, 136.8, 131.2, 129.9, 124.6, 124.5, 121.8, 119.6, 117.9, 114.3, 113.8, 113.2, 113.0, 112.5, 112.4, 101.3, 70.6, 56.1, 55.8, 55.2. Anal. Calcd for $C_{28}H_{24}O_7$ (472.48): C, 71.18; H, 5.12. Found: C, 71.07; H, 5.34.

*6.1.4.19. (E)-3-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)-7-((3-methoxybenzyl)oxy)-2H-chromen-2-one (**6s**).* Yellow solid; yield 95%; mp: 169–171 °C; IR (KBr, $cm^{-1}$): 1725 (C=O), 1694 (C=O). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 8.56 (s, 1H, $H_4$ coumarin), 8.19 (d, $J$ = 15.8 Hz, 1H, $H_\beta$), 8.01 (d, $J$ = 15.8 Hz, 1H, $H_\alpha$), 7.58 (d, $J$ = 8.7 Hz, 1H, $H_5$ coumarin), 7.34 (t, $J$ = 7.9 Hz, 1H, $H_5$ benzyloxy), 7.23 (s, 1H, $H_2$ benzyloxy), 7.16 (d, $J$ = 8.1 Hz, 1H, $H_6$ phenyl), 7.03–6.84 (m, 6H, $H_{2,5}$ phenyl, $H_{6,8}$ coumarin and $H_{4,6}$ benzyloxy), 6.03 (s, 2H, $OCH_2O$), 5.16 (s, 2H, $CH_2$ benzyl), 3.84 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$: 186.1, 164.0, 159.8, 159.7, 157.4, 148.3, 144.5, 136.8, 131.3, 129.9, 129.5, 125.6, 122.2, 121.5, 119.6, 114.3, 113.8, 113.0, 112.5, 108.5, 107.1, 101.6, 101.3, 70.6, 55.2. Anal. Calcd for $C_{27}H_{20}O_7$ (456.44): C, 71.05; H, 4.42. Found: C, 71.07; H, 4.38.

### 6.2. Biological assays

#### 6.2.1. Cell viability assay

The human cancer cell lines including K562 (human chronic myelogenous leukemia), MOLT-4 (human acute lymphoblastic leukemia) and MCF-7 (human breast adenocarcinoma) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. Cells were maintained at 37 °C in humidified air containing 5% $CO_2$. All cell lines were maintained in RPMI 1640 supplemented with 10% FBS, and 100 units/mL penicillin-G and 100 μg/mL streptomycin. MCF-7 cells were grown in monolayer cultures, while K562 and MOLT-4 cells were grown in suspension.

Compounds were all first dissolved in DMSO and then diluted with the growth medium. The final concentration of DMSO in the wells did not exceed 0.5%. Cell viability following exposure to compounds **6a**–**s** side by side to the reference drug cisplatin was determined by using the MTT reduction assay [14e,16]. The MCF-7, K562 and MOLT-4 cells were plated in 96-well microplates at densities of 30,000, 40,000 and 40,000 cells/ml, respectively (100 μL/well). After overnight incubation at 37 °C, different concentrations of test compounds were added to the wells and the cells were further incubated for 72 h. Then, the medium was replaced with fresh medium containing 0.5 mg/mL of MTT. Plates were incubated for another 4 h at 37 °C, the media was removed and formazan crystals formed in the cells were dissolved in 200 μL of DMSO. Optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680). Blank wells of all compounds, which contained the same concentrations of test compounds but did not contain MTT were run in parallel.

*6.2.2. DPPH radical scavenging assay*

A mixture of test compounds (in the different concentrations) and methanolic solution of DPPH (150 μM) was incubated at 37 °C for 30 min. The absorbance of mixture was measured at 517 nm. The percent scavenging activity was calculated using the following formula: Inhibition (%) = 100 × ($Abs_{control} - Abs_{compound}$)/$Abs_{control}$. The $IC_{50}$ values were obtained from linear regression plot between concentrations of test compound and percent inhibitions [26,27].

*6.2.3. Ferric reducing antioxidant power (FRAP) assay*

The FRAP assay was accomplished according to the reported method [25]. Briefly, the assay mixture was prepared by adding 10 vol of acetate buffer (300 mM, pH 3.6), 1 vol of 2,4,6-tripyridyl-*s*-triazine (10 mM) prepared in HCl (40 mM) and 1 vol of $FeCl_3$ (20 mM). The mixture was diluted to 1/3 with methanol and pre-warmed at 37 °C. This reagent (3 mL) was mixed with diluted test compounds (0.1 mL). The mixture was shaken and incubated at 37 °C for 20 min and the absorbance was read at 593 nm. FRAP values for both quercetin and tested compounds were achieved by standard calibration curve obtained by using different concentrations of $FeSO_4 \cdot 7H_2O$.

*6.2.4. Determination of mitochondrial total thiol content*

Mitochondria were prepared from Wistar rat's liver using differential centrifugation [28]. Tissues were minced and homogenized with glass handheld homogenizer. The nuclei and broken cell debris were sedimented through centrifuging at 1500 × *g* for 10 min at 4 °C and the pellet was discarded. The supernatant was subjected to a further centrifugation at 10,000 × *g* for 10 min and the superior layer was carefully discarded. The liver mitochondrial pellet was washed by gently suspending in the isolation medium and centrifuged again at 10,000 × *g* for 10 min. Final mitochondrial pellets were suspended in Tris buffer containing (0.05 M Tris–HCl, 0.25 M sucrose, 20 mM KCl, 2.0 mM $MgCl_2$, and 1.0 mM $Na_2HPO_4$, pH of 7.4) at 4 °C to assess total thiol content. Protein concentrations were determined through the Coomassie blue protein-binding method as explained by Bradford [29].

The total thiol content of mitochondrial suspension was measured using DTNB solution as indicator and the absorbance was read at 412 nm spectrophotometrically. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate mitochondrial total thiols using the GSH standard curve [18].

## Acknowledgments

This work was supported by grants from the research council of Tehran University of Medical Sciences, Iran National Elite Foundation (INEF) and Iran National Science Foundation (INSF). The authors also wish to thank the financial support of Shiraz University of Medical Sciences, Vice Chancellor for Research (Grant 5712).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.07.014.

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