Novel oxime-bearing coumarin derivatives act as potent Nrf2/ARE activators in vitro and in mouse model

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.10.029

European Journal of Medicinal Chemistry 106 (2015) 60–74

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Novel oxime-bearing coumarin derivatives act as potent Nrf2/ARE activators *in vitro* and in mouse model

Ken-Ming Chang [a], Huang-Hui Chen [b, c, 1], Tai-Chi Wang [d, 1], I-Li Chen [d], Yu-Tsen Chen [b], Shyh-Chyun Yang [a, e], Yeh-Long Chen [f], Hsin-Huei Chang [b], Chih-Hsiang Huang [b], Jang-Yang Chang [g, h], Chuan Shih [b], Ching-Chuan Kuo [b, h, i, **], Cherng-Chyi Tzeng [a, f, *]

[a] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[b] Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan 350, Taiwan
[c] Department of Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei, Taiwan
[d] Department of Pharmacy, Tajen University, Pingtung 907, Taiwan
[e] Department of Fragrance and Cosmetic Science, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[f] Department of Medicinal and Applied Chemistry, College of Life Science, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[g] National Institute of Cancer Research, National Health Research Institutes, Tainan 704, Taiwan
[h] Institute of Clinical Pharmacy and Pharmaceutical Sciences, National Cheng Kung University Medical College, Tainan 704, Taiwan
[i] Graduate Program for Aging, China Medical University, Taichung 404, Taiwan

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form
18 August 2015
Accepted 15 October 2015
Available online 19 October 2015

Keywords:
Nrf2/ARE activators
Oxime-bearing coumarins
Antioxidant activity
Cytoprotection

ABSTRACT

We have designed and synthesized certain novel oxime- and amide-bearing coumarin derivatives as nuclear factor erythroid 2 p45-related factor 2 (Nrf2) activators. The potency of these compounds was measured by antioxidant responsive element (ARE)-driven luciferase activity, level of Nrf2-related cytoprotective genes and proteins, and antioxidant activity. Among them, (*Z*)-3-(2-(hydroxyimino)-2-phenylethoxy)-2*H*-chromen-2-one (**17a**) was the most active, and more potent than the positive *t*-BHQ in the induction of ARE-driven luciferase activity. Exposure of HSC-3 cells to various concentrations of **17a** strongly increased Nrf2 nuclear translocation and the expression level of Nrf2-mediated cytoprotective proteins in a concentration-dependent manner. HSC-3 cells pretreated with **17a** significantly reduced *t*-BOOH-induced oxidative stress. In the animal experiment, Nrf2-mediated cytoprotective proteins, such as aldo-keto reductase 1 subunit C-1 (AKR1C1), glutathione reductase (GR), and heme oxygenase (HO-1), were obviously elevated in the liver of **17a**-treated mice than that of control. These results suggested that novel oxime-bearing coumarin **17a** is able to activate Nrf2/ARE pathway *in vivo* and are therefore seen as a promising candidate for further investigation.

1. Introduction

The induction of the antioxidant proteins and metabolizing/detoxifying enzymes has been considered as an effective way to protect cells against acute and chronic cell injury provoked by environmental stresses [1,2]. Nuclear factor erythroid 2 p45-related factor 2 (Nrf2), a member of the Cap'n'Collar (CNC) family of transcription factors that share a highly conserved basic region-leucine zipper (bZIP) structure, mainly regulates transcriptional activation through antioxidant responsive element (ARE, 5′-(A/G) TGACNNNGC(A/G)-3′) [3], which is a *cis*-acting regulatory element in promoter regions of several cytoprotective genes. Nrf2 is ubiquitously expressed in all human organs at low transactivating levels due to tight regulation by Kelch-like ECH associating protein 1

Abbreviations: ABCC3, ATP-binding cassette, subfamily C, member 1; AKR1C1, aldo-keto reductase 1 subunit C-1; AKR1C2, aldo-keto reductase 1 subunit C-2; AKR1C3, aldo-keto reductase 1 subunit C-3; ARE, antioxidant responsive element; *t*-BHQ, *tert*-butylhydroquinone; *t*-BOOH, *tert*-butyl hydroperoxide; CAPE, caffeic acid phenethyl ester; GCLC, γ-glutamyl cysteine synthetase catalytic subunit; GCLM, γ-glutamyl cysteine synthetase modifier subunit; GR, glutathione reductase; HO-1, heme oxygenase-1; Keap 1, Kelch-like ECH associating protein 1; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2 p45-related factor 2; ROS, reactive oxygen species.

* Corresponding author. School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan.
** Corresponding author. Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan 350, Taiwan.
E-mail addresses: cckuo@nhri.org.tw (C.-C. Kuo), tzengch@kmu.edu.tw (C.-C. Tzeng).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2015.10.029
0223-5234/

(Keap 1), an actin-binding protein, which is a substrate adaptor protein for a Cullin3-based E3 ubiquitin ligase [4]. Under basal condition, Nrf2 is constantly targeted for Keap 1-mediated ubiquitination and subsequent proteasomal degradation to maintain low Nrf2 protein levels. Upon activation, the enzymatic activity of the Keap-Cullin3 E3 ubiquitin ligase is compromised, resulting in stabilization of Nrf2 and activation of Nrf2 downstream antioxidant proteins-, phase II metabolizing/detoxifying enzymes-, and phase III APT-dependent drug efflux pumps-encoded genes [5–7]. Therefore, the Nrf2/ARE pathway has been highlighted to be the most important regulators of cytoprotective responses to oxidative and/or electrophilic stresses, which is believed to play a critical role in the development of many disease, such as cancer [5,7], Alzheimer's [8], Parkinson's [8], multiple sclerosis [8], chronic kidney disease [9], chronic obstructive pulmonary disease [10], and inflammatory bowel disease [11].

The vast number of both natural and synthetic compounds able to induce Nrf2/ARE pathway were identified, including Michael reaction acceptors [12], sulforaphane [13,14], dithiolethione [15,16], curcumin [17], caffeic acid phenethyl ester (CAPE) [18], chalcones [19,20], and vinyl sufones [21]. Notably, one Nrf2 activator, dimethyl fumarate, was approved by the Food and Drug Administration (FDA) for the treatment of multiple sclerosis. In addition, bardoxolone methyl, a synthetic oleanane triterpenoid, is under clinical investigation of the treatment of pulmonary hypertension. These results encouraged us to further discover novel Nrf2 activators for clinical application.

Coumarin constitutes a class of natural phytochemicals found in many plant species. Certain coumarin derivatives such as novobiocin (an antibiotic), dicoumarol (an anticoagulant), and warfarin (a rodent poison) were found to be biologically active. Therefore, extensive studies on coumarin derivatives have continuously attracted much attention [22–28]. For the past few years, we have also synthesized a number of coumarin derivatives and evaluated for their cytotoxic and antiplatelet activities [29–34]. It is notable that coumarin bears a α,β-unsaturated ketone moiety, which is a common functional group of certain Nrf2 activators such as curcumin, CAPE, and chalcone (Fig. 1). The present report describes the synthesis of novel oxime- and amide-bearing coumarins and evaluate for their Nrf2 activating activities.

2. Chemistry

The preparation of oxime-bearing coumarin derivatives is illustrated in Scheme 1. Alkylation of 3-hydroxycoumarin (**1**) with 2-(bromoacetyl)naphthalene under basic conditions gave 3-(2-(naphthalen-2-yl)-2-oxoethoxy)-2*H*-chromen-2-one (**9e**) in a 94% yield. The same procedure was applied to convert compounds **2**, **3**, and **4** to compounds **10e**, **11e**, and **12e**, respectively. Preparation of compounds **5**–**8**, **9a**–**d**, **10a**–**d**, **11a**–**d**, and **12a**–**d** were previously reported [29–34].

Treatment of 3-(2-oxopropoxy)-2*H*-chromen-2-one (**5**) with NH_2OH afforded exclusively (*E*)-3-(2-(hydroxyimino)propoxy)-2*H*-chromen-2-one (**13**) in a 89% yield. The configuration of the oxime moiety was determined by through-space nuclear Overhauser effect spectroscopy (NOESY) which revealed a coupling connectivity to CH_3 protons. In addition, the ^{13}C NMR signal for OCH_2 of the (*E*)-form oxime derivative was shifted downfield [35,36] to approximately 70 ppm (δ = 70.5 ppm for (*E*)-**13**). Accordingly, reaction of the ketone derivatives, **6**–**8**, with NH_2OH afforded their respective *E*-form oximes, **14**–**16**. However, treatment of 3-(2-oxo-2-phenylethoxy)-2*H*-chromen-2-one (**9a**) with NH_2OH under the same reaction conditions gave exclusively (*Z*)-3-(2-(hydroxyimino)-2-phenylethoxy)-2*H*-chromen-2-one (**17a**) in a 93% yield. The ^{13}C NMR signal for OCH_2 of the (Z)-form oxime derivative was shifted upfield to approximately 60 ppm [35,36] (δ = 59.6 ppm for (*Z*)-**17a**). The same synthetic procedures were applied for the synthesis of (*Z*)-**17b**–**e** from **9b**–**e** respectively; (*Z*)-**18a**–**e** from **10a**–**e** respectively; (*Z*)-**19a**–**e** from **11a**–**e** respectively; (*Z*)-**20a**–**e** from **12a**–**e** respectively.

The preparation of amide-containing coumarins is illustrated in Scheme 2. Alkylation of 3-hydroxycoumarin (**1**) with 2-bromoacetophenone under basic conditions gave 3-(2-oxo-2-phenylethoxy)-2*H*-chromen-2-one (**9a**) which was then treated with H_2SO_4 and NaN_3 to afford 2-(2-oxo-2*H*-chromen-3-yloxy)-*N*-phenylacetamide (**21a**) in a good overall yield. The same synthetic procedures were applied for the synthesis of *N*-(4-fluorophenyl) counterpart **21b**, *N*-(4-methoxyphenyl) counterpart **21c**, *N*-(4-biphenyl-4-yl) counterpart **21d**, and *N*-(naphthalen-2-yl) counterpart **21e** from their respective ketone precursors **9b**, **9c**, **9d**, and **9e** which in turn was prepared *via* alkylation of **1**. Accordingly, compounds **22a**–**e**, **23a**–**e**, and **24a**–**e** were also prepared by the same reaction sequences from their respective ketones **10a**–**e**, **11a**–**e**, and **12a**–**e** which in turn were prepared *via* alkylation of 4-hydroxycoumarin (**2**), 6-hydroxycoumarin (**3**), and 7-hydroxycoumarin (**4**) respectively. Structures of newly synthesized compounds were confirmed by NMR spectra and elementary analysis.

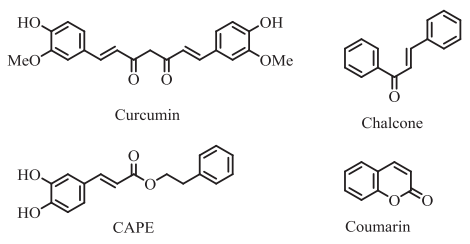
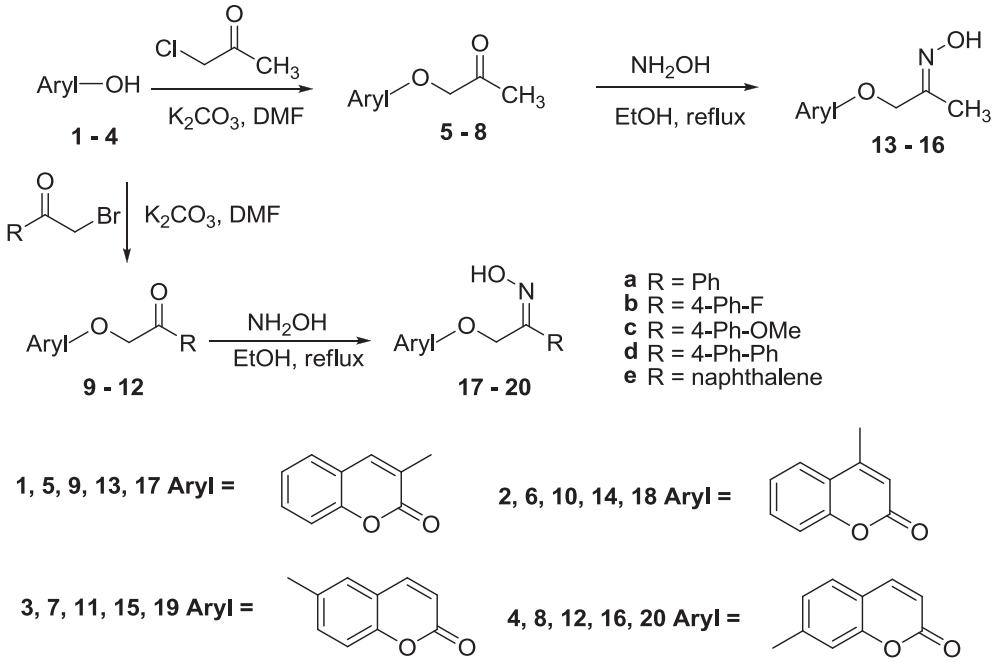


Fig. 1. Structures of curcumin, chalcone, CAPE, and coumarin.

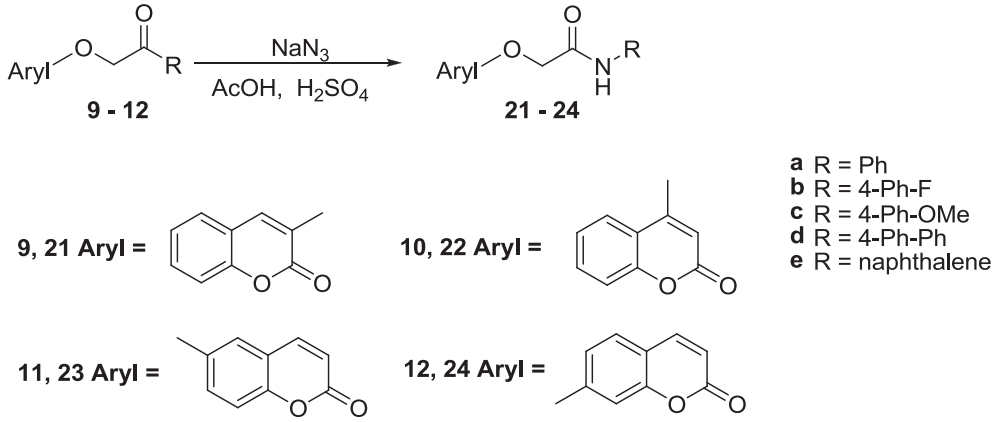
3. Results and discussion

3.1. Novel coumarin derivatives induced ARE-driven luciferase activity in HSC3-ARE9 cells

In this study, we used a stable ARE-driven reporter system to screening the potential Nrf2 activators [37]. Luciferase activities were determined after cells treated with test compounds at a concentration of 50 μM for 24 h. *tert*-Butylhydroquinone (*t*-BHQ), a well-known Nrf2 activator in ARE-controlled gene transcription, showed two-fold induction of ARE-driven luciferase activity, was used as positive control. All the hydroxycoumarins **1**–**4** and their respective 2-oxopropoxy derivatives **5**–**8** showed no induction of luciferase activity as shown in Table 1. Although 3-(2-oxopropoxy)-2*H*-chromen-2-one (**5**) was inactive, replacement of the methyl group with a phenyl ring significantly enhanced activity (1.72-fold induction for compound **9a** *vs* 0.92-fold induction for **5**). For 3-substituted coumarin derivatives, the potency decreased in an order **9a** (R = Ph; 1.72-fold) > **9b** (R = 4-F-Ph, 1.64-fold) > **9c** (R = 4-MeOPh, 1.54-fold) indicated substitution at phenyl ring is unfavorable especially the electron-donating group such as the methoxy group. The same trends were observed in 6-substituted coumarins (**11a** > **11b** > **11c**) and 7-substituted coumarins (**12a** > **12b** > **12c**). Among a total of 24 oxime-bearing coumarin derivatives tested, methyl group substituted derivatives **13**–**16** exhibited 1.37–1.68 fold induction of luciferase activity which was less active than that of *t*-BHQ (Table 1). Replacement of the methyl group with a phenyl ring significantly enhanced activity (3.45-fold induction for compound **17a** *vs* 1.63-fold induction for **13**). For 3-



Scheme 1.



Scheme 2.

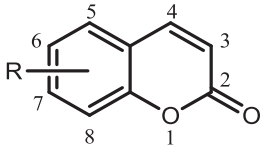
substituted coumarin derivatives, the potency decreased in an order **17a** (R = Ph; 3.45-fold) > **17b** (R = 4-F-Ph, 3.25-fold) > **17c** (R = 4-MeOPh, 1.49-fold) indicated substitution at phenyl ring is unfavorable especially the electron-donating group such as the methoxy group. The same trends were observed in 6-substituted coumarins (**19a** > **19b** > **19c**) and 7-substituted coumarins (**20a** > **20b** > **20c**). The position of substitution play an important role in which the potency decreased in an order **17a** (coumarin-3-yl) > **19a** (coumarin-6-yl) > **20a** (coumarin-7-yl) > **18a** (coumarin-4-yl). The weak activity exhibited by 4-substituted coumarins (1.35–1.76 fold induction of luciferase activity) implied C-4 position could be the site for nucleophiles and therefore, any substituent at this position will hinder the nucleophilic addition.

The induction of ARE-driven luciferase activity for amide-bearing coumarins is outlined in Table 2. Among a total of 20 compounds tested, phenyl group substituted derivatives **21a**, **23a**, and **24a** showed comparable or more potent activity to the positive *t*-BHQ. The 4-substituted derivative **22a** was inactive while the 3-substituted counterpart **21a** was the most active which exhibited a 2.51-fold induction of ARE-driven luciferase activity at 50 μM.

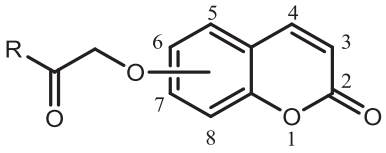
Since **17a**, **17b**, and **19a** showed the highest ARE-driven luciferase activity in HSC3-ARE9 cells and is significantly more potent than that of *t*-BHQ at equal molar concentration, we therefore examined the dose effect of these compounds. The result demonstrated that treatment of cells with compounds **17a**, **17b**, and **19a** significantly increased ARE-luciferase activity in a concentration-dependent manner. The ability for the induction of ARE-driven luciferase activity was found to follow the order **17a** > **17b** > **19a**. Notably, **17a** at the concentration of 5 μM even have greater activity in inducing luciferase activity than that of 50 μM of *t*-BHQ (Fig. 2, *left panel*). Parallel cell viability assays revealed no any cytotoxic effects of these compounds (Fig. 2, *right panel*).

We have also investigated the effects of these hydroxylated coumarins **1**–**4** and compounds **17a**, **17b**, and **19a** on scavenging 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical in order to elucidate whether these compounds exert direct free radical scavenging effect. The results indicated that 3-hydroxycoumarin (**1**) exhibited a significant DPPH scavenging activity with EC_{50} values of 65.8 μM

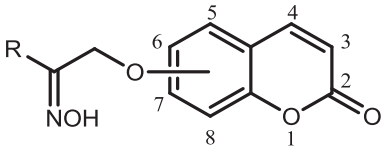
Table 1
The induction of ARE-driven luciferase activity for coumarin derivatives at the concentration of 50 μM.



1 - 4



5 - 12



13 - 20

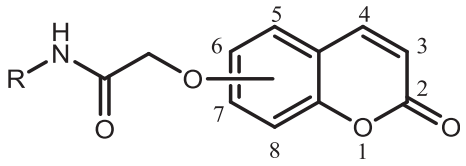
Compound	Position of substitution	R	Luciferase activity (Fold to control)
1	3	OH	0.94 ± 0.06
2	4	OH	0.84 ± 0.07
3	6	OH	0.99 ± 0.08
4	7	OH	0.98 ± 0.11
5	3	Me	0.92 ± 0.03
6	4	Me	0.79 ± 0.05
7	6	Me	1.03 ± 0.05
8	7	Me	1.09 ± 0.08
9a	3	Ph	1.72 ± 0.05
9b	3	4-F–Ph	1.64 ± 0.11
9c	3	4-MeO–Ph	1.54 ± 0.04
9d	3	4-Ph–Ph	0.98 ± 0.05
9e	3	Naphthalene	0.98 ± 0.03
10a	4	Ph	1.98 ± 0.19
10b	4	4-F–Ph	1.26 ± 0.06
10c	4	4-MeO–Ph	1.36 ± 0.10
10d	4	4-Ph–Ph	0.42 ± 0.06
10e	4	Naphthalene	1.52 ± 0.04
11a	6	Ph	2.54 ± 0.05
11b	6	4-F–Ph	1.58 ± 0.02
11c	6	4-MeO–Ph	0.82 ± 0.05
11d	6	4-Ph–Ph	0.85 ± 0.07
11e	6	Naphthalene	0.61 ± 0.01
12a	7	Ph	1.93 ± 0.03
12b	7	4-F–Ph	1.14 ± 0.06
12c	7	4-MeO–Ph	1.07 ± 0.04
12d	7	4-Ph–Ph	0.85 ± 0.07
12e	7	Naphthalene	0.17 ± 0.05
13	3	Me	1.63 ± 0.09
14	4	Me	1.37 ± 0.11
15	6	Me	1.64 ± 0.04
16	7	Me	1.68 ± 0.08
17a	3	Ph	3.45 ± 0.25
17b	3	4-F–Ph	3.25 ± 0.18
17c	3	4-MeO–Ph	1.49 ± 0.12
17d	3	4-Ph–Ph	1.44 ± 0.09
17e	3	Naphthalene	1.31 ± 0.04
18a	4	Ph	1.35 ± 0.07
18b	4	4-F–Ph	1.67 ± 0.18
18c	4	4-MeO–Ph	1.76 ± 0.11
18d	4	4-Ph–Ph	1.32 ± 0.10
18e	4	Naphthalene	1.43 ± 0.07
19a	6	Ph	3.02 ± 0.28
19b	6	4-F–Ph	2.32 ± 0.24
19c	6	4-MeO–Ph	1.33 ± 0.03
19d	6	4-Ph–Ph	1.50 ± 0.07
19e	6	Naphthalene	1.46 ± 0.04
20a	7	Ph	2.16 ± 0.04
20b	7	4-F–Ph	1.32 ± 0.06
20c	7	4-MeO–Ph	1.28 ± 0.09
20d	7	4-Ph–Ph	0.90 ± 0.10
20e	7	Naphthalene	1.04 ± 0.22
t-BHQ	(Positive control)		2.01 ± 0.08

which is approximately equal potent to the positive butylated hydroxytoluene (BHT) (EC_{50} = 57.5 μM) (Table 3). These results are consistent with the findings that published previously [38,39]. However, compounds **17a**, **17b**, and **19a** were inactive in scavenging DPPH radicals, indicated their antioxidant mechanisms are distinct from the original 3-hydroxycoumarin (**1**).

*3.2. Novel coumarin **17a** activated the Nrf2/ARE-mediated cytoprotective genes and proteins in HSC3 cells*

The abovementioned result indicated that **17a** is the most potent oxime-bearing coumarin derivative in inducing ARE-driven luciferase activity, we then measured the mRNA level of Nrf2/ARE-

Table 2
The induction of ARE-driven luciferase activity for amide-bearing coumarin derivatives at the concentration of 50 μM.



Compound	Position of substitution	R	Luciferase activity (Fold to control)
21a	3	Ph	2.51 ± 0.11
21b	3	4-F–Ph	1.34 ± 0.05
21c	3	4-OMe-Ph	0.95 ± 0.05
21d	3	4-Ph–Ph	1.22 ± 0.06
21e	3	Naphthalene	1.13 ± 0.03
22a	4	Ph	1.06 ± 0.01
22b	4	4-F-–Ph	1.11 ± 0.09
22c	4	4-OMe–Ph	1.34 ± 0.08
22d	4	4-Ph–Ph	1.17 ± 0.02
22e	4	Naphthalene	1.37 ± 0.06
23a	6	Ph	2.08 ± 0.09
23b	6	4-F–Ph	0.95 ± 0.03
23c	6	4-OMe–Ph	0.89 ± 0.02
23d	6	4-Ph–Ph	0.95 ± 0.01
23e	6	Naphthalene	0.91 ± 0.07
24a	7	Ph	2.27 ± 0.06
24b	7	4-F–Ph	1.16 ± 0.03
24c	7	4-OMe–Ph	0.98 ± 0.01
24d	7	4-Ph–Ph	1.09 ± 0.09
24e	7	Naphthalene	1.06 ± 0.07
t-BHQ	(positive control)		2.01 ± 0.08

mediated genes in **17a**-treated cells by real-time RT-PCR analysis. Exposure of HSC-3 cells to 50 μM **17a** for 24 h significantly increased the mRNA levels of ABCC3, AKR1C1, AKR1C2, AKR1C3, GCLC, GR, NQO1, and Prx1 approximately 1.3-, 1.6-, 1.4-, 1.3-, 1.7-, 1.3-, 1.2-, and 1.7-fold, respectively (Fig. 3A). Because the mRNA level of AKR1C1, GCLC, and Prx1 was highly enhanced after **17a** treatment in HSC3 cells (Fig. 3A, *purple bars* (in the web version)), therefore, we compared reference compound *t*-BHQ and compound **17a** on the regulation of AKR1C1, GCLC, and PRX1 mRNA level. As the result, compound **17**a showed greater effect on the induction of Prx1 mRNA expression than *t*-BHQ (Fig. 3B). In contrast, *t*-BHQ is more effective to induced AKR1C1 and GCLC mRNA level than that of compound **17a** (Fig. 3B), suggested that detail regulation of these two type of Nrf2 activators are differ. Since compound **17a** is more potent to induced ARE-driven luciferase activity (Table 1 and Fig. 2), we suggested that oxime-bearing coumarin derivatives may affect other Nrf2/ARE-mediated genes, and merit for further investigation.

The effect of **17a** on the levels of AKR1C1, GCLC, and Prx1 proteins was investigated using Western blot analysis. As shown in Fig. 4, the same trends were observed that compound **17a** significantly increased the protein levels of AKR1C1, GCLC, and Prx1 in a dose-dependent manner. We also investigated the subcellular localization of Nrf2. Notably, the increment of Nrf2 protein level in the nucleus was observed in **17a**-treated in a dose-dependent manner (Fig. 5). Taken together, these results suggested that **17a** is active in retaining Nrf2 protein level in the nucleus and enhancing ARE-driven gene transactivation.

3.3. Novel coumarin *17a* reduced tert-butyl hydroperoxide (t-BOOH)-induced oxidative stress in HSC-3 cells

Since compound **17a** enhanced Nrf2/ARE-driven gene transactivation, we wondered whether this compound is able to suppress oxidative stress. *tert*-Butyl hydroperoxide (*t*-BOOH), a short-chain analogue of lipid hydroperoxide mentioned to act as a mutagenic and carcinogenic agent, was used as oxidative stress generator in this study. As shown in Fig. 6, cells treated with *t*-BOOH significantly increased the intracellular ROS level. Notably, pretreatment of cells with compound **17a** for 24 h significantly reduced *t*-BOOH-induced oxidative stress (Fig. 6).

3.4. Compound *17a* activates Nrf2/ARE pathway in vivo

Several lines of evidence demonstrated that activation of Nrf2/ARE pathway leads to increase detoxification and clearance of reactive metabolites and xenobiotics. The liver participates in the detoxification process, largely by the action of two sequential steps referred to as phase I and phase II systems [40]. Since Nrf2 is a master transcriptional factor for phase II system transactivation, we therefore clarified whether compound **17a** stimulates Nrf2-mediated cytoprotection *in vivo* by determining the level of phase II enzyme in the liver in conventional mouse model. C57BL/6 mice were administrated with a single dose of vehicle (90% PEG300 + 10% 1-methyl-Z-pyrrolidone) or compound **17a** at a dose of 50 mg/kg/day by gavage for consecutive 7 days, and liver were harvested 24 h later. The body weights of mice administered vehicle or compound **17a** did not obviously differ (Data not shown). As shown in Fig. 7, Western blot analysis revealed that Nrf2-mediated cytoprotective proteins, including AKR1C1, GR, and HO-1, were obviously elevated in liver of compound **17a** treated mice as compared to vehicle control, indicating these compounds activated Nrf2/ARE pathway *in vivo*.

3.5. Compound *17a* activates Nrf2 pathway probably through the induction of PI3K and PKC-mediated Nrf2 phosphorylation in HSC-3 cells

Since compound **17a** exhibited potent Nrf2 activation property *in vitro* as well as *in vivo*, we therefore examined the underlying

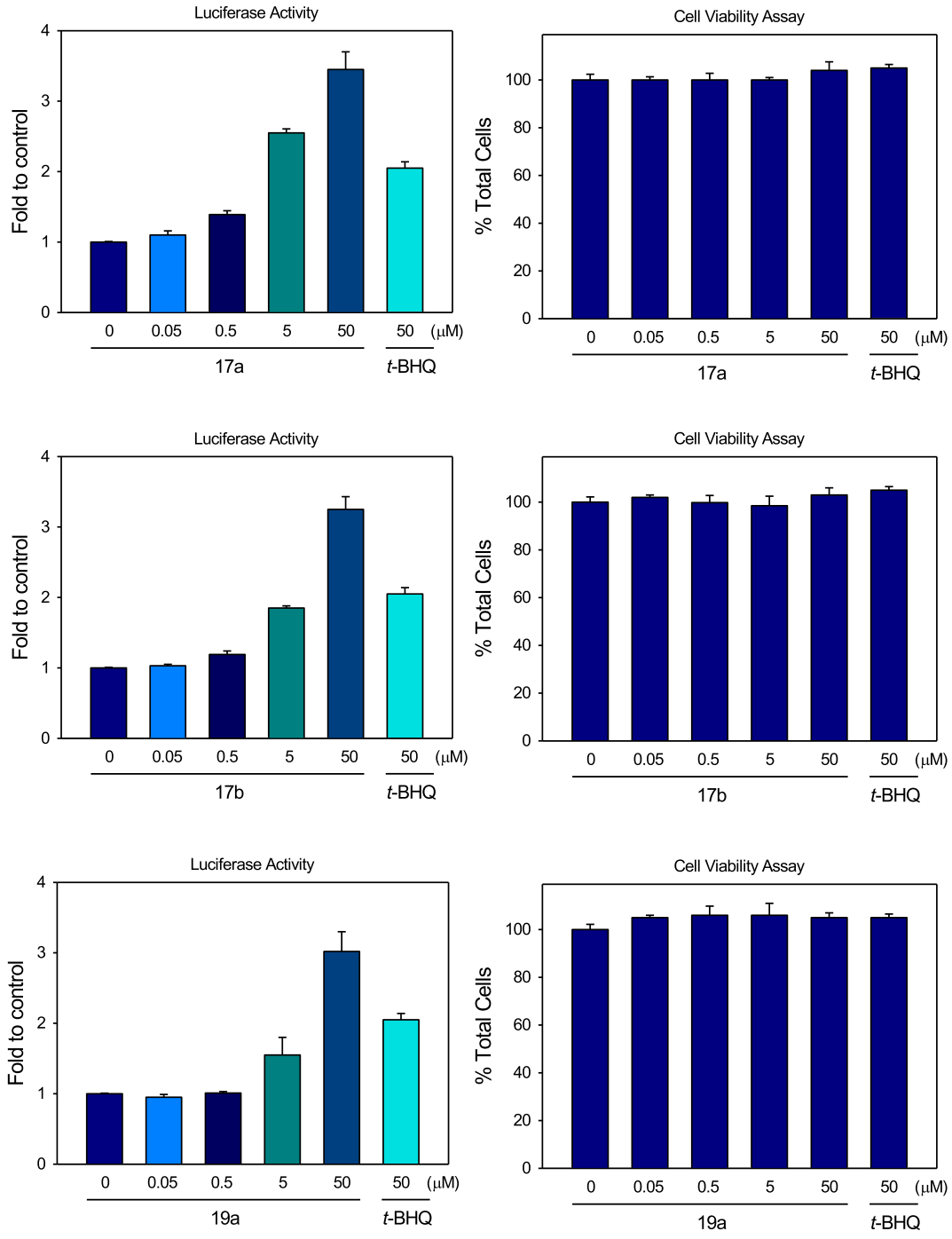


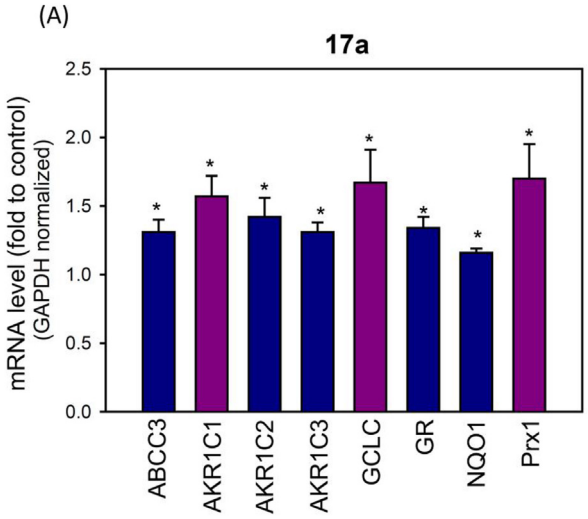
Fig. 2. Dose effects of compounds **17a** (*top*), **17b** (*middle*), and **19a** (*bottom*) on ARE-driven luciferase activity and Cell viability in HSC3-ARE9 cells. HSC3-ARE9 cells were treated with various concentrations of test compounds for 24 h. Luciferase activity and cell viability were assayed in parallel as described in *Materials and Methods*. *t*-BHQ treatment represented an internal positive control. **Luciferase activity** (*Left panels*) and **Cell proliferation index** (*Right panels*): Data are expressed as mean ± S.D. (of triplicate analyses).

mechanism in the present study. Phosphorylation of Nrf2 at Ser-40 by several kinases is a critical process in its stabilization and nuclear translocation [41]. Therefore, we examined the phosphorylation of Nrf2 in HSC-3 cells under compound **17a** treatment. As shown in Fig. 8A, Western blot analysis demonstrated that significant augmentation of Nrf2 phosphorylation was observed following **17a** treatment. The signaling pathways that regulate Nrf2 phosphorylation vary depending on the stimulus. A number of studies have identified that several signaling pathways, including phosphoinositide 3-kinase (PI3K) [37,42,43], mitogen-activated protein kinases (MAPK) [44–46], and protein kinase C (PKC) [46–48], are involved in the induction of Nrf2/ARE-driven gene expression. We used PI3K, MEK, p38, and PKC inhibitor in order to evaluate the possible regulatory mechanism of compound **17a**. In the ARE-driven luciferase system, pre-treatment of HSC3-ARE9 cells with non-toxic LY294002 (PI3K inhibitor) and Ro-31-8220 (PKC inhibitor) partially suppressed compound **17a**-induced luciferase activity, yet pre-incubation of cells with the U0126 (MEK inhibitor) and SB203580 (p38 inhibitor) did not inhibit or even stimulate luciferase activity (Fig. 8B). These results suggested that PI3K and PKC

Table 3
EC_{50} of coumarin derivatives on scavenging DPPH radicals.

Compound	Scavenging DPPH radicals (EC_{50}: μM)
1	65.8 ± 1.5
2	351.3 ± 8.9
3	>400
4	>400
17a	>400
17b	>400
19a	>400
BHT (positive control)	57.5 ± 1.5

BHT: butylated hydroxytoluene.



(B)

Gene	mRNA level (fold to control)		
	Control	**17a**	*t*-BHQ
AKR1C1	1.0	1.5 ± 0.21	2.3 ± 0.12
GCLC	1.0	1.6 ± 0.35	2.0 ± 0.22
Prx1	1.0	1.6 ± 0.24	1.1 ± 0.14

Fig. 3. Effects of compound **17a** on the mRNA levels of Nrf2-mediated cytoprotective genes. (A) HSC-3 cells were treated with 50 μM compound **17a** for 24 h, and then harvested for RNA preparation. The mRNA levels of ABCC3, AKR1C1, AKR1C2, and AKR1C3, GCLC, GR, NQO1, and Prx1 were evaluated using real-time RT-PCR, with GAPDH as an internal control. Data are expressed as means (of triplicate analyses) (column) and the standard deviation (bars). *Significantly different from vehicle control ($p < 0.05$). (B) Cells were treated with 50 μM compound **17a** or *t*-BHQ for 24 h, and then harvested for RNA preparation. The mRNA levels of AKR1C1, GCLC, and Prx1 were evaluated using real-time RT-PCR, with GAPDH as an internal control.

signaling may involve in Nrf2 activation, and merits for further investigation.

4. Conclusion

In conclusion, we identified for the first time that the novel oxime-bearing coumarin derivatives are potent Nrf2 activators. Structure features responsible for such activities have been well-characterized in the present study. Among them, compound **17a** exerts greatest activity in activation of the Nrf2-dependent cytoprotective machinery *in vitro* and *in vivo*, thereby protecting cells against the carcinogen-triggered oxidative stress. Since the activation of Nrf2/ARE pathway has been highlighted to suppress many oxidation/inflammation-related degenerative diseases, including

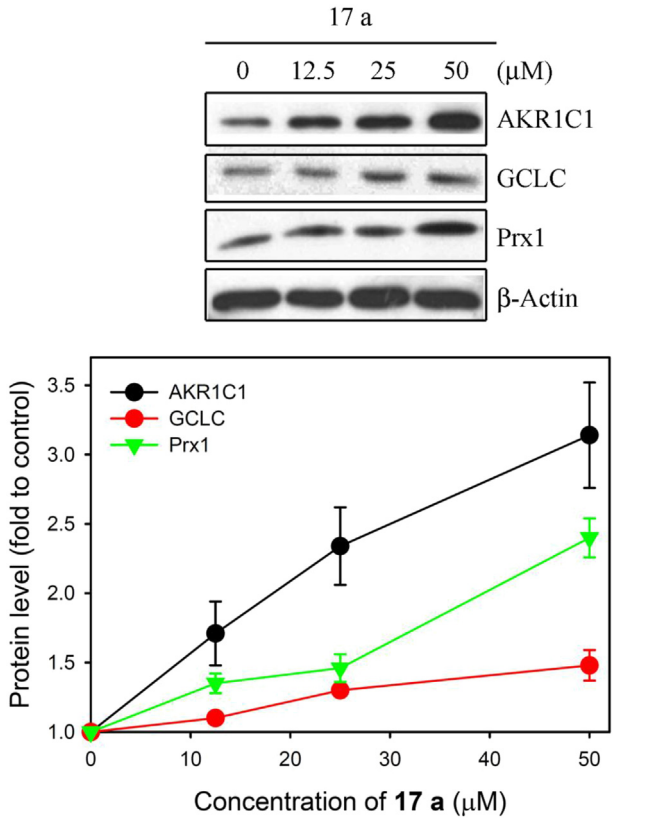


Fig. 4. Effects of compound **17a** on the level of selected Nrf2/ARE-mediated proteins. HSC-3 cells were treated with various concentration of **17a** for 24 h. The protein levels of AKR1C1, GCLC, and Prx1 in response to **17a** were determined using Western blot analyses (*top panel*). The intensities of bands were quantitated using ImageJ software (*bottom panel*). The expression of Nrf2/ARE-mediated proteins were normalized with β-Actin.

cancer, neurodegenerative diseases, chronic kidney disease, *etc.*, therefore, future studies evaluating the efficacy of **17a** as potential cytoprotective agent in various disease models are warranted.

5. Experimental section

5.1. General

TLC was done with precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories Inc.; detection was done by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). Melting points: Electrothermal IA9100 digital melting-point apparatus; uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a Varian-Unity-400 spectrometer at 400 and 100 MHz, chemical shifts δ in ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants *J* in Hz. Elemental analyses were carried out on a Heraeus CHN–O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.2. 3-[2-(Naphthalen-2-yl)-2-oxoethoxy]-2H-chromen-2-one (***9e***)

3-Hydroxycoumarin (**1**, 1.55 g, 5 mmol), K_2CO_3 (0.69 g, 5 mmol), and dry DMF (50 mL) were stirred at room temperature (r.t.) for 30 min. To this solution was added 2-(bromoacetyl)naphthalene (1.25 g, 5 mmol) in DMF (10 mL) in one portion. The resulting mixture was stirred continuously at r.t. for 24 h (TLC monitoring) and then poured into ice-water (100 mL). The white solid thus

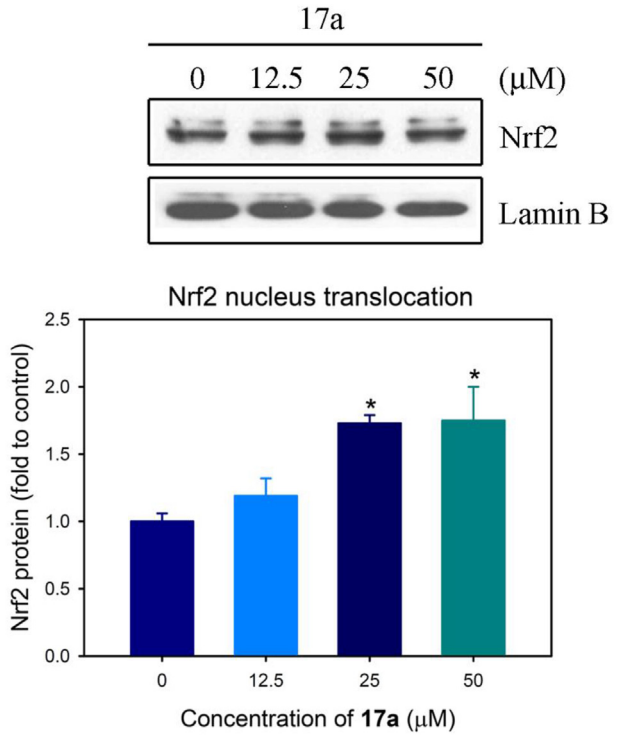


Fig. 5. Dose effect of **17a** on Nrf2 nuclear accumulation. HSC-3 cells were exposed to various concentration of **17a** for 24 h, and nuclear extracts were prepared for Western blot analysis (*top panel*). The intensities of bands were quantitated using ImageJ software (*bottom panel*). The level of Nrf2 protein in the nucleus were normalized with Lamin B. $^{*}p < 0.05$ compared to vehicle control.

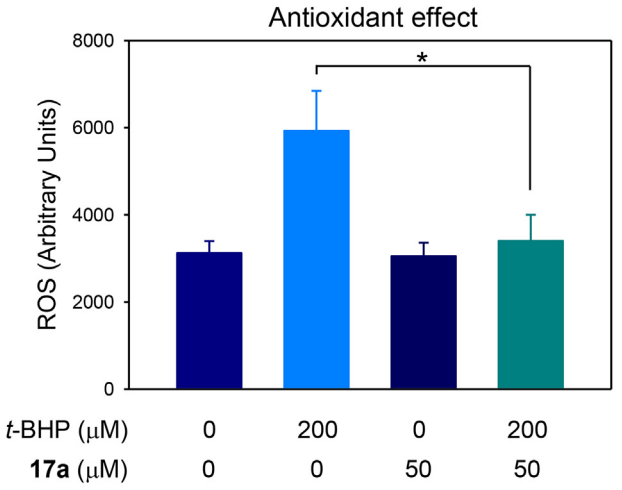


Fig. 6. Effects of compound **17a** on *t*-BOOH induced oxidative stress in HSC-3 cells. Cells were pretreated with 50 μM compound **17a** for 24 h and then exposed to 200 μM *t*-BOOH for an additional 1 h. Intracellular ROS levels were measured using DCF fluorescence. The values represent mean ± S.D. from three independent experiments. $^{*}p < 0.05$ compared to *t*-BOOH alone group.

obtained was collected and crystallized from Et_2O to give **9e** (1.55 g, 94%). Mp 233–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.86 (*s*, 2H, $-OCH_2$), 7.29–7.33 (*d*, 1H, $J = 1.2$), 7.39–7.48 (*m*, 3H, arom. H), 7.57 (*dd*, 1H, H-5, $J = 7.6$, $J = 1.2$), 7.65–7.74 (*m*, 2H, arom. H), 8.02–8.17 (*m*, 4H, arom. H), 8.78 (*s*, 1H, arom. H). ^{13}C NMR (DMSO-d_6) 71.0, 115.2, 115.7, 119.6, 123.3, 124.8, 126.9, 127.2, 127.8, 128.5, 128.7, 129.0, 129.5, 129.9, 131.4, 132.0, 135.3, 142.5, 149.2, 156.4, 192.8. Anal. Calcd for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27. Found: C, 76.18; H, 4.14.

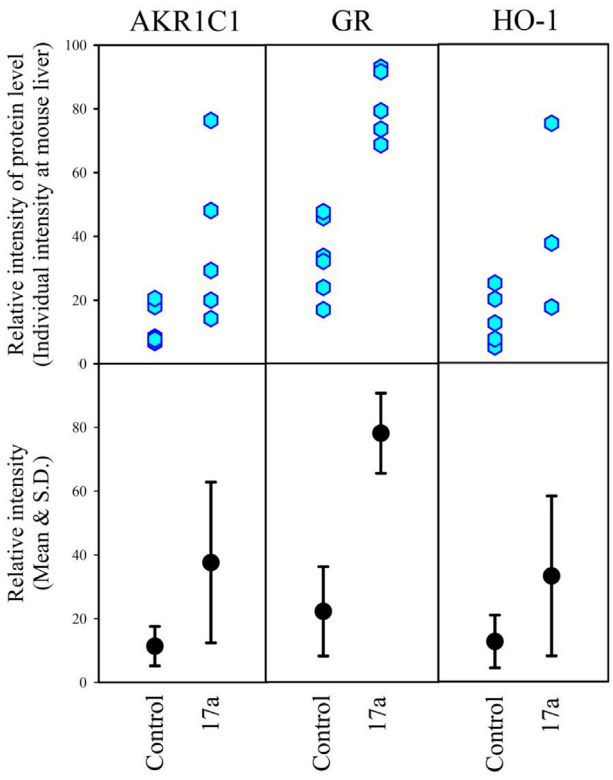


Fig. 7. Effect of **17a** on activation of Nrf2/ARE pathway *in vivo*. Male wild type C57BL/6J mice were fed with vehicle (90% PEG300 + 10% 1-methyl-Z-pyrrolidone) ($n = 6$) or compound **17a** (50 mg/kg/day) ($n = 5$) by oral gavage for consecutive 7 days, and the liver were harvested 24 h later. The expression of Nrf2-regulated proteins AKR1C1, GR, and HO-1 were analyzed in the liver tissues by Western blot analysis. β-Actin was used for normalization. *Top panel*: the relative intensity of protein level from the individual mouse liver was quantified by ImageJ software based analysis. *Bottom panel*: the relative protein amount of each group was expressed as the mean ± S.D.

The same procedure was applied to convert compounds **2**, **3**, and **4** to compounds **10e**, **11e**, and **12e**, respectively.

5.3. 4-[2-(Naphthalen-2-yl)-2-oxoethoxy]-2H-chromen-2-one (**10e**)

Yield: 84%; mp 161–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.89 (*s*, 2H, $-OCH_2$), 6.32 (*d*, 1H, H-3, $J = 9.6$), 7.08 (*d*, 1H, H-5, $J = 8.4$), 7.15 (*d*, 1H, H-7, $J = 2.4$), 7.65–7.67 (*m*, 3H, arom. H), 7.99–8.07 (*m*, 5H, arom. H), 8.79 (*s*, 1H, arom. H). ^{13}C NMR (DMSO-d_6) 70.6, 101.6, 112.6, 112.8, 123.2, 127.1, 127.8, 128.4, 128.9, 129.4, 129.5, 129.9, 131.5, 132.0, 135.3, 144.2, 155.2, 160.2, 161.2, 193.6 (One signal was not detected and is believed to overlap with nearby peaks). Anal. Calcd for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27. Found: C, 76.34; H, 4.25.

5.4. 6-[2-(Naphthalen-2-yl)-2-oxoethoxy]-2H-chromen-2-one (**11e**)

Yield: 87%; mp 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.80 (*s*, 2H, $-OCH_2$), 6.50 (*d*, 1H, H-3, $J = 9.6$), 7.31–7.38 (m, 3H, arom. H), 7.64–7.73 (*m*, 2H, arom. H), 7.79–8.16 (*m*, 5H, arom. H), 8.77 (*s*, 1H, arom. H). ^{13}C NMR (DMSO-d_6) 70.7, 111.9, 116.6, 117.3, 119.1, 119.8, 123.2, 127.1, 127.7, 128.4, 128.9, 129.5, 129.8, 131.6, 132.1, 135.3, 143.9, 148.0, 154.4, 160.1, 194.1. Anal. Calcd for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27. Found: C, 76.08; H, 4.30.

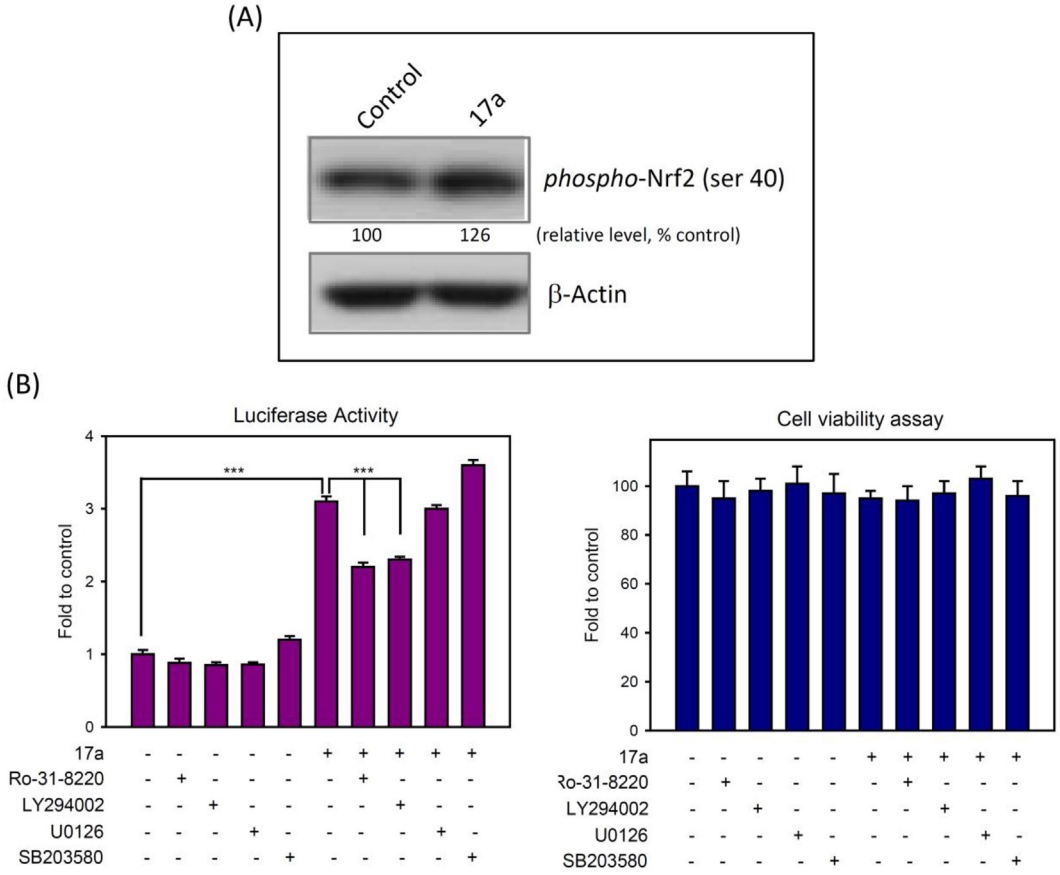


Fig. 8. Effects of compound 17a on Nrf2 phosphorylation and kinase signaling in HSC-3 cells. (A) Effect of Nrf2 phosphorylation. HSC-3 cells were treated with 50 μM compound **17a** for 24 h. Phosphorylated Nrf2 (serine 40) was determined using Western blotting and appropriate specific antibodies. β-Actin was used as internal controls. (B) Effects of signaling inhibitors on compound **17a**-induced luciferase activity. HSC3-ARE9 cells were pre-treated with various kinase inhibitors for 1 h, then treated with 50 μM compound **17a** for an additional 24 h prior to analysis of luciferase activity. The values represent the means ± S.D. in triplicate experiments. Significantly different: $^{***}p < 0.001$.

5.5. 7-[2-(Naphthalen-2-yl)-2-oxoethoxy]-2H-chromen-2-one (*12e*)

Yield: 82%; mp 237–238 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 6.04 (*s*, 2H, $-OCH_2$), 6.13 (*s*, 1H, H-3), 7.42–7.46 (*t*, 2H, H-6, H-8), 7.66–7.74 (*m*, 3H, arom. H), 7.98 (*d*, 1H, H-4, $J = 8.4$), 8.03–8.15 (*m*, 4H, arom. H), 8.81 (*s*, 1H, arom. H). ^{13}C NMR (DMSO-d_6) 71.4, 91.5, 115.1, 116.5, 123.0, 123.2, 124.3, 127.2, 127.8, 128.5, 129.0, 129.5, 130.2, 131.2, 132.0, 132.9, 135.4, 152.8, 161.6, 164.5, 192.0. Anal. Calcd for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27. Found: C, 76.00; H, 4.28.

5.6. (E)-3-[2-(Hydroxyimino)propoxy]-2H-chromen-2-one (*13*)

A solution of **5** (0.22 g, 1 mmol) in EtOH (20 mL) was added a solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) in EtOH (2 mL). The mixture was heated at reflux for 4 h (TLC monitoring) and evaporated to give a residual solid. The white solid thus obtained was collected, purified by flash column chromatography (FC; silica gel; n-hexane/EtOAc 1:1), and recrystallized from CH_2Cl_2 to give **13** (0.21 g, 89%). Mp 193–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 1.87 (*s*, 3H, $-CH_3$), 4.63 (*s*, 2H, $-OCH_2$), 7.30–7.38 (*m*, 2H, H-6, H-8), 7.40 (*s*, 1H, H-4), 7.43 (*m*, 1H, H-7), 7.56 (*dd*, 1H, H-5, $J = 7.6$, $J = 1.6$), 11.1 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 11.6, 70.5, 115.3, 115.7, 119.6, 124.8, 126.9, 128.7, 142.6, 149.2, 151.0, 156.5. Anal. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.82; H, 4.74; N, 5.95.

The same procedures were applied to convert **6**–**8** to **14**–**16**, **9a**–**e** to **17a**–**e**; **10a**–**e** to **18a**–**e**; **11a**–**e** to **19a**–**e** and **12a**–**e** to **20a**–**e**, respectively.

5.7. (E)-4-[2-(Hydroxyimino)propoxy]-2H-chromen-2-one (*14*)

Yield: 71%; mp 168–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 1.91 (*s*, 3H, $-CH_3$), 4.82 (*s*, 2H, $-OCH_2$), 5.96 (*s*, 1H, H-3), 7.34–7.41 (*m*, 2H, H-6, H-8), 7.68 (*dd*, 1H, H-7, $J = 7.2$, $J = 1.6$), 7.82 (*dd*, 1H, H-5, $J = 8.2$, $J = 1.6$), 11.20 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 11.5, 70.9, 91.2, 115.1, 116.4, 122.8, 124.3, 132.8, 150.5, 152.7, 161.5, 164.5. Anal. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.64; H, 4.76; N, 5.95.

5.8. (E)-6-[2-(Hydroxyimino)propoxy)-2H-chromen-2-one (*15*)

Yield: 73%; mp 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 1.86 (*s*, 3H, $-CH_3$), 4.61 (*s*, 1H, $-OCH_2$), 6.51 (*d*, 1H, H-3, $J = 9.6$), 7.23–7.38 (*m*, 3H, arom. H), 8.00 (*d*, 1H, H-4, $J = 9.6$), 11.0 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 11.4, 69.9, 112.1, 116.5, 117.2, 119.0, 119.9, 143.8, 147.9, 151.6, 154.3, 160.0 Anal. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.75; H, 4.70; N, 5.96.

5.9. (E)-7-[2-(Hydroxyimino)propoxy)-2H-chromen-2-one (*16*)

Yield: 69%; mp 150–151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 1.84 (*s*, 3H, $-CH_3$), 4.69 (*s*, 2H, $-OCH_2$), 6.31 (*d*, 1H, H-3, $J = 9.6$), 7.69–7.05 (*ddd*, 2H, H-6, H-8, $J = 8.8$, $J = 2.4$, $J = 2.4$), 7.64 (*d*, 1H, H-

5, J = 8.8), 8.00 (*d*, 1H, H-4, J = 9.6), 11.05 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 11.4, 70.0, 101.6, 112.6, 112.7, 112.9, 129.4, 144.2, 151.3, 155.2, 160.2, 161.2. Anal. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.52; H, 4.89; N, 5.98.

5.10. (Z)-3-[2-(Hydroxyimino)-2-phenylethoxy]-2H-chromen-2-one (**17a**)

Yield: 93%; mp 149–150 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.29 (*s*, 2H, −OCH_2), 7.31–7.42 (*m*, 5H, H-6, H-8, H-3′, H-4′, H-5′), 7.43 (*m*, 1H, H-7), 7.48 (*s*, 1H, H-4), 7.58 (*dd*, 1H, H-5, J = 7.6, J = 1.6), 7.68 (*m*, 2H, H-2′, H-6′), 12.07 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.6, 114.9, 115.7, 119.5, 124.8, 126.3, 127.0, 128.4, 128.7, 129.1, 134.0, 142.5, 149.2, 151.5, 156.3. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.23; H, 4.46; N, 4.74.

5.11. (Z)-3-[2-(4-Fluorophenyl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**17b**)

Yield: 60%; mp 162–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.29 (*s*, 2H, −OCH_2), 7.21–7.25 (*m*, 2H, H-3′, H-5′), 7.31–7.36 (*m*, 2H, H-6, H-8), 7.43 (*m*, 1H, H-7), 7.47 (*s*, 1H, H-4), 7.58 (*dd*, 1H, H-5, J = 7.6, J = 1.6), 7.71–7.75 (*m*, 2H, H-2′, H-6′), 12.09 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.7, 115.3 (*d*, J_{C-F} = 21.2 Hz, ArC–F), 115.4, 115.7, 119.4, 124.8, 127.0, 128.6 (*d*, J_{C-F} = 8.3 Hz, ArC–F), 128.7, 130.5 (*d*, J_{C-F} = 3.0 Hz, ArC–F), 142.4, 149.2, 150.8, 156.2, 162.6 (*d*, J_{C-F} = 244.9 Hz, ArC–F). Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.03; H, 3.85; N, 4.72.

5.12. (Z)-3-[2-(Hydroxyimino)-2-(4-methoxyphenyl)ethoxy]-2H-chromen-2-one (**17c**)

Yield: 70%; mp 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.76 (*s*, 3H, −OCH_3), 5.26 (*s*, 2H, −OCH_2), 6.93–6.96 (*m*, 2H, H-3′, H-5′), 7.30–7.36 (*m*, 2H, H-6, H-8), 7.43 (*m*, 1H, H-7), 7.46 (*s*, 1H, H-4), 7.57 (*dd*, 1H, H-5, J = 7.6, J = 1.6), 7.60–7.64 (*m*, 2H, H-2′, H-6′), 11.85 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 55.1, 59.6, 113.8, 114.8, 115.7, 119.5, 124.8, 126.4, 126.9, 127.6, 128.6, 142.5, 149.2, 151.0, 156.2, 159.9. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.40; H, 4.71; N, 4.25.

5.13. (Z)-3-[2-(Biphenyl-4-yl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**17d**)

Yield: 66%; mp 198–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.32 (*s*, 2H, −OCH_2), 7.31–7.51 (*m*, 8H, H-4, arom. H), 7.59 (*dd*, 1H, H-5, J = 7.6, J = 1.6), 7.68–7.72 (*m*, 3H, arom. H), 7.78 (*d*, 1H, J = 1.6), 7.80 (*d*, 1H, J = 2.0), 12.14 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.5, 115.0, 115.7, 119.5, 124.8, 126.6, 126.7, 126.8, 127.0, 127.7, 127.8, 129.0, 133.1, 139.4, 140.6, 142.6, 149.2, 151.2, 156.3. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.32; H, 4.56; N, 3.69.

5.14. (Z)-3-[2-(Hydroxyimino)-2-(naphthalen-2-yl)ethoxy]-2H-chromen-2-one (**17e**)

Yield: 71%; mp 198–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.39 (*s*, 2H, −OCH_2), 7.32–7.36 (*m*, 2H, H-6, H-8), 7.47 (*d*, 1H, J = 1.2), 7.51–7.55 (*m*, 3H, arom. H), 7.60 (*d*, 1H, J = 1.2), 7.88–7.96 (*m*, 4H, arom. H), 8.21 (*s*, 1H, arom. H), 12.18 (*s*, −NOH). ^{13}C NMR (DMSO-d_6) 59.5, 115.0, 115.7, 119.5, 123.4, 124.8, 126.1, 126.4, 126.7, 127.0, 127.5, 127.8, 128.4, 128.7, 131.4, 132.6, 133.0, 142.6, 149.2, 151.4, 156.3. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 73.05; H, 4.37; N, 4.05.

5.15. (Z)-4-[2-(Hydroxyimino)-2-phenylethoxy]-2H-chromen-2-one (**18a**)

Yield: 68%; mp 198–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.53 (*s*, 2H, −OCH_2), 6.01 (*s*, 1H, H-3), 7.30 (*t*, 1H, J = 7.6), 7.35–7.42 (*m*, 4H, arom. H), 7.55–7.63 (*m*, 2H, arom. H), 7.70–7.72 (*m*, 2H, arom. H), 12.22 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 60.2, 90.8, 114.9, 116.5, 122.6, 124.3, 126.4, 128.5, 129.3, 132.9, 133.5, 151.2, 152.7, 161.5, 164.2. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.03; H, 4.48; N, 4.72.

5.16. (Z)-4-[2-(4-Fluorophenyl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**18b**)

Yield: 64%; mp 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.52 (*s*, 2H, −OCH_2), 6.00 (*s*, 1H, H-3), 7.20–7.30 (*m*, 3H, H-6, H-8, H-3′, H-5′), 7.38 (*d*, 1H, H-7, J = 8.4), 7.56 (*dd*, 1H, H-5, J = 8.0, J = 1.6), 7.59–7.63 (*m*, 1H, arom. H), 7.73–7.78 (*m*, 2H, arom, H), 12.21 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 60.1, 90.8, 114.8, 115.5 (*d*, J_{C-F} = 21.2 Hz, ArC–F), 116.4, 122.5, 124.3, 128.6 (*d*, J_{C-F} = 8.4 Hz, ArC–F), 130.0 (*d*, J_{C-F} = 3.0 Hz, ArC–F), 132.8, 150.4, 152.6, 161.4, 162.6 (*d*, J_{C-F} = 244.7 Hz, ArC–F), 164.0. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.10; H, 3.88; N, 4.41.

5.17. (Z)-4-[2-(Hydroxyimino)-2-(4-methoxyphenyl)ethoxy]-2H-chromen-2-one (**18c**)

Yield: 68%; mp 184–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.74 (*s*, 3H, −OCH_3), 5.49 (*s*, 2H, −OCH_2), 6.00 (*s*, 1H, H-3), 6.94 (*t*, 1H, J = 2.8), 6.96 (*t*, 1H, J = 2.8), 7.31 (*t*, 1H, J = 7.2), 7.38 (*d*, 1H, J = 8.4), 7.57–7.66 (*m*, 4H, arom. H), 11.98 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 55.1, 60.0, 90.7, 113.9, 114.9, 116.5, 122.5, 124.3, 125.8, 127.7, 132.8, 150.6, 152.7, 160.0, 161.4, 164.1. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.42; H, 4.68; N, 4.24.

5.18. (Z)-4-[2-(Biphenyl-4-yl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**18d**)

Yield: 78%; mp 240–241 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.56 (*s*, 2H, −OCH_2), 6.05 (*s*, 1H, H-3), 7.26–7.30 (*m*, 1H, arom. H), 7.34–7.38 (*m*, 2H, arom. H), 7.47 (*t*, 2H, J = 8.0), 7.58–7.72 (*m*, 6H, arom. H), 7.81–7.84 (*m*, 2H, arom. H), 12.27 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 60.0, 90.8, 114.9, 116.4, 122.5, 124.2, 126.5, 126.6, 126.8, 127.7, 128.9, 132.5, 132.8, 139.2, 140.7, 150.7, 152.7, 161.4, 164.1. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.05; H, 4.64; N, 3.79.

5.19. (Z)-4-[2-(Hydroxyimino)-2-(naphthalen-2-yl)ethoxy]-2H-chromen-2-one (**18e**)

Yield: 75%; mp 224–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.64 (*s*, 2H, −OCH_2), 6.08 (*s*, 1H, H-3), 7.21–7.25 (*m*, 1H, arom. H), 7.35 (*d*, 1H, J = 7.6), 7.50–7.61 (*m*, 4H, arom. H), 7.88–7.98 (*m*, 4H, arom. H), 8.27 (*s*, 1H, arom. H), 12.33 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.9, 90.8, 114.9, 116.4, 122.5, 123.4, 124.2, 126.1, 126.5, 126.8, 127.5, 127.9, 128.4, 130.9, 132.6, 132.8, 133.0, 151.0, 152.7, 161.4, 164.2. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 72.77; H, 4.42; N, 3.99.

5.20. (Z)-6-[2-(Hydroxyimino)-2-phenylethoxy]-2H-chromen-2-one (**19a**)

Yield: 87%; mp 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.29 (*s*, 2H, −OCH_2), 6.50 (*d*, 1H, H-3, J = 9.6), 7.20 (*dd*, 1H, H-5, J = 9.2, J = 2.8), 7.32–7.40 (*m*, 5H, arom. H), 7.64–7.67 (*m*, 2H, arom. H), 7.98

(*d*, 1H, H-4, J = 9.6), 11.97 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.1, 111.7, 116.6, 117.3, 119.1, 119.3, 126.2, 128.2, 128.8, 134.0, 143.8, 147.9, 152.4, 154.1, 160.0. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.04; H, 4.47; N, 4.74.

*5.21. (Z)-6-[2-(4-Fluorophenyl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**19b**)*

Yield: 66%; mp 170–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.29 (*s*, 2H, $-OCH_2$), 6.50 (*d*, 1H, H-3, J = 9.6), 7.17–7.24 (*m*, 3H, arom. H), 7.31–7.34 (*m*, 2H, arom. H), 7.68–7.72 (*m*, 2H, arom. H), 7.98 (*d*, 1H, H-4, J = 9.6), 11.99 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.2, 111.8, 115.2 (*d*, J_{C-F} = 21.2 Hz, ArC−F), 116.6, 117.3, 119.1, 119.3, 128.5 (*d*, J_{C-F} = 8.4 Hz, ArC−F), 130.5 (*d*, J_{C-F} = 3.1 Hz, ArC−F), 143.8, 148.0, 151.6, 154.0, 160.0, 162.4 (*d*, J_{C-F} = 244.7 Hz, ArC−F). Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.08; H, 3.89; N, 4.46.

*5.22. (Z)-6-[2-(Hydroxyimino)-2-(4-methoxyphenyl)ethoxy]-2H-chromen-2-one (**19c**)*

Yield: 59%; mp 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.76 (s, 3H, $-OCH_3$), 5.25 (*s*, $-OCH_2$), 6.50 (*d*, 1H, H-3, J = 9.6), 6.92–6.95 (*dd*, 2H, J = 2.4, J = 2.0), 7.20 (*dd*, 1H, H-5, J = 9.2, J = 2.8), 7.33 (*t*, 2H, J = 4.8, arom. H), 7.58–7.60 (*dd*, 2H, J = 4.8, J = 2), 7.97 (*d*, 1H, H-4, J = 9.6), 11.72 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 55.1, 59.2, 111.8, 113.7, 116.7, 117.3, 119.2, 119.4, 126.5, 127.7, 143.9, 148.0, 152.0, 154.2, 159.8, 160.0. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.11; H, 4.70; N, 4.19.

*5.23. (Z)-6-[2-(Biphenyl-4-yl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**19d**)*

Yield: 69%; mp 200–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.32 (*s*, 2H, $-OCH_2$), 6.50 (*d*, 1H, H-3, J = 9.6), 7.23 (*dd*, 1H, H-5, J = 9.2, J = 2.8), 7.32–7.39 (*m*, 3H, arom. H), 7.44–7.48 (*m*, 2H, arom. H), 7.67–7.77 (*m*, 6H, arom. H), 7.98 (*d*, 1H, H-4, J = 9.6), 12.02 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.2, 111.9, 116.7, 117.4, 119.2, 119.4, 126.6, 126.8, 127.7, 129.0, 133.2, 139.4, 140.5, 143.9, 148.1, 152.1, 154.2, 160.0. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.16; H, 4.68; N, 3.71.

*5.24. (Z)-6-[2-(Hydroxyimino)-2-(naphthalen-2-yl)ethoxy]-2H-chromen-2-one (**19e**)*

Yield: 66%; mp 173–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.41 (s, 2H, $-OCH_2$), 6.50 (d, 1H, H-3, J = 9.2), 7.24 (dd, 1H, J = 9.2, J = 3.2), 7.32–7.37 (m, 2H, arom. H), 7.52–7.56 (m, 2H, arom, H), 7.85–7.98 (m, 5H, arom. H), 8.19 (s, 1H, arom. H), 12.10 (s, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.0, 111.8, 116.6, 117.3, 119.1, 119.4, 123.5, 125.9, 126.4, 126.6, 127.4, 127.7, 128.2, 131.5, 132.5, 132.9, 143.8, 148.0, 152.3, 154.1, 160.0. Anal. Calcd for $C_{21}H_{15}NO_4 \cdot 0.5\ H_2O$: C, 71.18; H, 4.55; N, 3.95. Found: C, 71.58; H, 4.38; N, 3.95.

*5.25. (Z)-7-[2-(Hydroxyimino)-2-phenylethoxy]-2H-chromen-2-one (**20a**)*

Yield: 63%; mp 176–177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.36 (*s*, 2H, $-OCH_2$), 6.29 (*d*, 1H, H-3, J = 9.6), 6.91 (*dd*, 1H, H-6, J = 8.8, J = 2.0), 7.03 (*d*, 1H, H-8, J = 2.0), 7.36–7.39 (*m*, 3H, arom. H), 7.59 (*d*, 1H, H-5, J = 8.8), 7.64–7.66 (*m*, 2H, arom. H), 7.97 (*d*, 1H, H-4, J = 9.6), 12.08 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.1, 101.0, 112.7, 112.8, 126.3, 128.3, 129.0, 129.5, 133.9, 144.2, 152.1, 155.2, 160.2, 160.8. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.07; H, 4.50; N, 4.70.

*5.26. (Z)-7-[2-(4-Fluorophenyl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**20b**)*

Yield: 67%; mp 172–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.36 (*s*, 2H, $-OCH_2$), 6.30 (*d*, 1H, H-3, J = 9.6), 6.90 (*dd*, 1H, H-6, J = 8.8, J = 2.4), 7.02 (*d*, 1H, H-8, J = 2.4), 7.18–7.24 (*m*, 2H, arom. H), 7.60 (*d*, 1H, H-5, J = 8.8), 7.66–7.72 (*m*, 2H, arom. H), 7.97 (*d*, 1H, H-4, J = 9.6), 12.10 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.15, 101.02, 112.7, 112.7, 112.8, 115.3 (*d*, J_{C-F} = 21.2 Hz, ArC−F), 128.5 (*d*, J_{C-F} = 8.3 Hz, ArC−F), 129.5, 130.4 (*d*, J_{C-F} = 3.0 Hz, ArC−F), 144.2, 151.4, 155.2, 160.2, 160.7, 162.6 (*d*, J_{C-F} = 244.8 Hz, ArC−F). Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.04; H, 3.95; N, 4.44.

*5.27. (Z)-7-[2-(Hydroxyimino)-2-(4-methoxyphenyl)ethoxy]-2H-chromen-2-one (**20c**)*

Yield: 68%; mp 148–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.75 (*s*, 3H, $-OCH_3$), 5.33 (*s*, 2H, $-OCH_2$), 6.29 (*d*, 1H, H-3, J = 9.6), 6.91 (*dd*, 1H, H-6, J = 8.8, J = 2.4), 6.92–6.94 (*m*, 2H, arom. H), 7.03 (*d*, 1H, H-8, J = 2.4), 7.57–7.61 (*m*, 3H, arom. H), 7.97 (*d*, 1H, H-4, J = 9.6), 11.86 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 55.1, 59.1, 100.9, 112.6, 112.7, 113.1, 113.8, 126.2, 127.6, 129.5, 144.2, 151.6, 155.2, 159.8, 160.1, 160.9. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.12; H, 4.65; N, 4.31.

*5.28. (Z)-7-[2-(Biphenyl-4-yl)-2-(hydroxyimino)ethoxy]-2H-chromen-2-one (**20d**)*

Yield: 56%; mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.39 (*s*, 2H, $-OCH_2$), 6.30 (*d*, 1H, H-3, J = 9.6), 6.94 (dd, 1H, H-6, J = 8.8, J = 2.4), 7.07 (*d*, 1H, H-8, J = 2.4), 7.34–7.47 (*m*, 3H, arom. H), 7.61 (*d*, 1H, H-5, J = 8.4), 7.66–7.76 (*m*, 6H, arom. H), 7.97 (*d*, 1H, H-4, J = 9.6), 12.16 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.1, 101.0, 112.7, 122.8, 126.6, 126.7, 126.9, 127.8, 129.0, 129.6, 133.0, 139.4, 140.6, 144.3, 151.9, 155.3, 160.3, 160.9. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.62; H, 4.72; N, 3.72.

*5.29. (Z)-7-[2-(Hydroxyimino)-2-(naphthalen-2-yl)ethoxy]-2H-chromen-2-one (**20e**)*

Yield: 66%; mp 200–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.48 (*s*, 2H, $-OCH_2$), 6.29 (*d*, 1H H-3, J = 9.6), 6.94 (*dd*, 1H, H-6, J = 8.4, J = 2.4), 7.09 (*d*, 1H, H-8, J = 2.8), 7.51–7.54 (*m*, 2H, arom. H), 7.59 (*d*, 1H, H-5, J = 8.4), 7.84–7.96 (*m*, 5H, H-4, arom. H), 8.19 (*s*, 1H, arom. H), 12.21 (*s*, 1H, −NOH). ^{13}C NMR (DMSO-d_6) 59.0, 101.0, 112.7, 112.7, 123.5, 126.0, 126.5, 126.7, 127.5, 127.9, 128.4, 129.5, 131.4, 132.6, 133.0, 144.2, 152.1, 155.3, 160.2, 160.9. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 72.91; H, 4.45; N, 3.94.

*5.30. 2-(2-Oxo-2H-chromen-3-yloxy)-N-phenylacetamide (**21a**)*

A solution of 3-(2-oxo-2-phenylethoxy)-2*H*-chromen-2-one (**9a**, 0.28 g, 1 mmol) in H_2SO_4 (5 mL) was stirred at r.t. for 10 min. To this solution, sodium azide (0.4 g, 5.80 mmol) was added in one portion. The mixture was stirred at ice-bath for 1 h (TLC monitoring) and then poured into ice-water (100 mL). The white solid thus obtained was collected and purified by flash column chromatography (FC; silica gel; CH_2Cl_2/MeOH 30:1) and recrystallized from CH_2Cl_2 to give **21a** (0.26 g, 88%). Mp 261–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.78 (*s*, 2H, $-OCH_2$), 7.08 (*t*, 1H, H-4, J = 7.2), 7.29–7.37 (*m*, 5H, arom, H), 7.47 (*dd*, 1H, H-7, J = 7.2, J = 1.6), 7.56–7.59 (m, 3H, arom. H), 10.21 (*s*, 1H, −NH). ^{13}C NMR (DMSO-d_6) 68.1, 116.1, 116.1, 119.7, 120.2, 124.5, 125.4, 127.6, 129.3, 129.5, 138.4, 142.8, 149.6, 157.3, 165.8. Anal. Calcd for $C_{17}H_{13}NO_4 \cdot 0.5\ H_2O$: C,

67.10; H, 4.63; N, 4.60. Found: C, 66.99; H, 4.73; N, 4.62.

The same procedures were applied to convert **9b**–**e** to **21b**–**e**; **10a**–**e** to **22a**–**e**; **11a**–**e** to **23a**–**e** and **12a**–**e** to **24a**–**e**, respectively.

5.31. N-(4-Fluorophenyl)-2-(2-oxo-2H-chromen-3-yloxy) acetamide (**21b**)

Yield: 71%; mp 216–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.82 (*s*, 2H, $-OCH_2$), 7.15–7.20 (*m*, 2H, arom. H), 7.30–7.49 (*m*, 4H, arom, H), 7.61–7.66 (*m*, 3H, arom. H), 10.21 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.7, 115.4 (*d*, J_{C-F} = 22.0 Hz, ArC–F), 115.6, 115.7, 119.4, 121.6 (*d*, J_{C-F} = 7.5 Hz, ArC–F), 124.8, 127.1, 128.9, 134.6, 142.6, 149.3, 156.5, 158.5 (*d*, J_{C-F} = 238.8 Hz, ArC–F), 165.3. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.18; H, 3.86; N, 4.47.

5.32. N-(4-Methoxyphenyl)-2-(2-oxo-2H-chromen-3-yloxy) acetamide (**21c**)

Yield: 80%; mp 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.72 (*s*, 3H, $-OCH_3$), 4.79 (*s*, 2H, $-OCH_2$), 6.91 (*dd*, 2H, arom. H, J = 6.8, J = 2.4), 7.30–7.54 (*m*, 6H, arom, H), 7.63 (*dd*, 1H, H-5, J = 7.6, J = 1.6), 10.01 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 55.2, 67.8, 113.9, 115.5, 115.7, 119.5, 121.3, 124.8, 127.1, 128.8, 131.3, 142.7, 149.3, 155.6, 156.5, 164.8. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.43; H, 4.69; N, 4.25.

5.33. N-(Biphenyl-4-yl)-2-(2-oxo-2H-chromen-3-yloxy)acetamide (**21d**)

Yield: 81%; mp 261–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.86 (*s*, 2H, $-OCH_2$), 7.31–7.49 (*m*, 7H, arom. H), 7.63 (*d*, 1H, H-5, J = 1.2), 7.65 (*d*, 2H, arom. H, J = 1.2), 7.67 (*t*, 2H, arom. H, J = 2.8), 7.72 (*s*, 1H, arom. H) 7.75 (*s*, 1H, arom. H), 10.28 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) Anal. Calcd for $C_{23}H_{17}NO_4 \cdot 0.1\ H_2O$: C, 74.02; H, 4.64; N, 3.75. Found: C, 73.89; H, 4.53; N, 3.73.

5.34. N-(Naphthalen-2-yl)-2-(2-oxo-2H-chromen-3-yloxy) acetamide (**21e**)

Yield: 88%; mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.85 (*s*, 2H, $-OCH_2$), 7.29–7.49 (*m*, 6H, arom. H), 7.59–7.63 (*m*, 2H, arom, H), 7.79–7.89 (*m*, 3H, arom. H), 8.26 (*d*, 1H, arom. H, J = 1.6), 10.43 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 68.1, 116.1, 116.1, 116.4, 119.7, 120.5, 125.3, 125.4, 127.0, 127.5, 127.7, 127.9, 128.9, 129.4, 130.4, 133.6, 136.0, 142.8, 149.6, 157.2, 166.0. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 72.60; H, 4.45; N, 3.98.

5.35. 2-(2-Oxo-2H-chromen-4-yloxy)-N-phenylacetamide (**22a**)

Yield: 71%; mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.02 (*s*, 2H, $-OCH_2$), 5.89 (*s*, 1H, H-3), 7.08 (*t*, 1H, arom. H, J = 7.2), 7.32–7.44 (*m*, 4H, arom, H), 7.60–7.62 (*m*, 2H, arom. H), 7.71 (*dd*, 1H, H-7, J = 7.6, J = 1.2), 7.96 (*dd*, 1H, H-5, J = 8.0, J = 1.2), 10.28 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.6, 91.2, 115.0, 116.4, 119.6, 123.3, 123.9, 124.3, 128.9, 133.0, 138.2, 152.8, 161.5, 164.5, 164.6. Anal. Calcd for $C_{17}H_{13}NO_4 \cdot 0.2\ H_2O$: C, 68.31; H, 4.52; N, 4.69. Found: C, 68.09; H, 4.38; N, 4.76.

5.36. N-(4-Fluorophenyl)-2-(2-oxo-2H-chromen-4-yloxy) acetamide (**22b**)

Yield: 78%; mp 237–238 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.02 (*s*, 2H, $-OCH_2$), 5.90 (*s*, 1H, H-3), 7.17–7.22 (*m*, 2H, arom. H), 7.39–7.45 (*m*, 2H, arom, H), 7.61–7.72 (*m*, 3H, arom. H), 7.98 (*dd*, 1H, H-5, J = 8.0, J = 1.2), 10.33 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.6, 91.2, 115.0, 115.4 (*d*, J_{C-F} = 22.7 Hz, ArC–F), 116.4, 121.6 (*d*, J_{C-F} = 7.5 Hz, ArC–F), 123.3, 124.2, 132.9, 134.5, 152.7, 158.3 (*d*, J_{C-F} = 238.8 Hz, ArC–F), 161.4, 164.4, 164.6. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.15; H, 3.90; N, 4.40.

5.37. N-(4-Methoxyphenyl)-2-(2-oxo-2H-chromen-4-yloxy) acetamide (**22c**)

Yield: 70%; mp 199–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.72 (*s*, 3H, $-OCH_3$), 4.98 (*s*, 2H, $-OCH_2$), 5.88 (*s*, 1H, H-3), 6.89–6.93 (*dd*, 2H, arom. H, J = 5.6, J = 2.0), 7.38–7.43 (*m*, 2H, arom, H), 7.49–7.53 (*dd*, 2H, arom. H, J = 5.6, J = 2.0), 7.71 (*dd*, 1H, H-7, J = 7.6, J = 1.2), 7.97 (*dd*, 1H, H-5, J = 8.0, J = 1.2), 10.28 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 55.2, 67.7, 91.2, 113.9, 115.0, 116.4, 121.4, 123.3, 124.3, 131.2, 132.9, 152.7, 155.7, 161.5, 164.0, 164.6. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.35; H, 4.69; N, 4.20.

5.38. N-(Biphenyl-4-yl)-2-(2-oxo-2H-chromen-4-yloxy)acetamide (**22d**)

Yield: 67%; mp 253–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.06 (*s*, 2H, $-OCH_2$), 5.92 (*s*, 1H, H-3), 7.32–7.36 (*m*, 1H, arom. H), 7.40–7.47 (*m*, 4H, arom, H), 7.64–7.74 (*m*, 7H, arom. H), 7.98 (*dd*, 1H, H-5, J = 8.0, J = 1.2), 10.39 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.7, 91.2, 115.0, 116.4, 120.0, 123.3, 124.2, 126.3, 127.0, 127.1, 128.9, 132.9, 135.5, 137.6, 139.5, 152.7, 161.5, 164.5, 164.6. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.38; H, 4.60; N, 3.68.

5.39. N-(Naphthalen-2-yl)-2-(2-oxo-2H-chromen-4-yloxy) acetamide (**22e**)

Yield: 69%; mp 247–248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 5.10 (*s*, 2H, $-OCH_2$), 5.95 (*s*, 1H, H-3), 7.41–7.51 (*m*, 4H, arom. H), 7.65 (*dd*, 1H, arom, H, J = 8.8, J = 2.0), 7.73 (*ddt*, 1H, arom. H, J = 8.8, J = 2.0, J = 1.6), 7.83–7.92 (*m*, 3H, arom. H), 8.00 (*dd*, 1H, H-5, J = 8.0, J = 1.6), 8.32 (*d*, 1H, arom. H, J = 1.6), 10.50 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.7, 91.3, 115.0, 116.0, 116.4, 120.1, 123.3, 124.3, 124.9, 126.5, 127.3, 127.4, 128.5, 130.0, 132.9, 133.3, 135.7, 152.7, 161.5, 164.6, 164.7. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 73.02; H, 4.46; N, 4.06.

5.40. 2-(2-Oxo-2H-chromen-6-yloxy)-N-phenylacetamide (**23a**)

Yield: 70%; mp 188–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.76 (*s*, 2H, $-OCH_2$), 6.50 (*d*, 1H, H-3, J = 9.2), 7.10 (t, 1H, H-5, J = 7.6, J = 1.2) 7.29–7.39 (*m*, 5H, arom. H), 7.64 (*d*, 2H, arom, H, J = 8.8), 8.03 (*d*, 1H, H-4, J = 9.6), 8.32 (*d*, 1H, arom. H, J = 1.6), 10.10 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.5, 112.0, 116.7, 117.4, 119.1, 119.7, 119.9, 123.7, 128.7, 138.3, 144.0, 148.2, 154.1, 160.0, 166.2. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.15; H, 4.44; N, 4.74.

5.41. N-(4-Fluorophenyl)-2-(2-oxo-2H-chromen-6-yloxy) acetamide (**23b**)

Yield: 61%; mp 212–213 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.75 (*s*, 2H, $-OCH_2$), 6.50 (*d* 1H, H-3, J = 9.6), 7.13–7.19 (*m*, 2H, arom. H), 7.29–7.39 (*m*, 3H, arom), 7.68 (*m*, 2H, arom. H), 8.03 (*d*, 1H, H-4, J = 9.6), 10.17 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.5, 112.0, 115.3 (*d*, J_{C-F} = 22.0 Hz, ArC–F), 116.7, 117.4, 119.1, 119.9, 121.6 (*d*, J_{C-F} = 7.6 Hz, ArC–F), 134.7 (*d*, J_{C-F} = 2.3 Hz, ArC–F), 144.0, 148.2, 154.1, 158.3 (*d*, J_{C-F} = 238.7 Hz, ArC–F), 160.1, 166.2. Anal. Calcd for

$C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.01; H, 3.95; N, 4.40.

5.42. N-(4-Methoxyphenyl)-2-(2-oxo-2H-chromen-6-yloxy) acetamide (**23c**)

Yield: 62%; mp 211–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.72 (*s*, 3H, $-OCH_3$)4.72 (*s*, 2H, $-OCH_2$), 6.50 (*d* 1H, H-3, $J = 9.6$), 6.91 (*dd*, 2H, arom. H, $J = 5.6$, $J = 2.4$), 7.29–7.39 (*m*, 3H, arom), 7.55 (*dd*, 2H, arom. H, $J = 5.6$, $J = 2.0$), 8.03 (*d*, 1H, H-4, $J = 9.6$), 9.96 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 55.1, 67.6, 112.0, 113.8, 116.7, 117.4, 119.1, 119.9, 121.4, 131.3, 144.0, 148.2, 154.1, 155.5, 160.0, 165.7. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.22; H, 4.66; N, 4.22.

5.43. N-(Biphenyl-4-yl)-2-(2-oxo-2H-chromen-6-yloxy)acetamide (**23d**)

Yield: 66%; mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.79 (*s*, 2H, $-OCH_2$), 6.51 (*d* 1H, H-3, $J = 9.6$), 6.91 (*dd*, 2H, arom. H, $J = 5.6$, $J = 2.4$), 7.30–7.46 (*m*, 6H, arom), 7.63–7.66 (*m*, 4H, arom. H, $J = 5.6$, $J = 2.0$), 7.73–7.76 (dt, 2H, $J = 8.8$, $J = 2.0$), 8.04 (*d*, 1H, H-4, $J = 9.6$), 10.22 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.6, 112.0, 116.7, 117.4, 119.1, 119.9, 120.0, 126.2, 126.9, 127.1, 128.9, 135.3, 137.8, 139.6, 144.0, 148.2, 154.1, 160.0, 166.3. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.20; H, 4.59; N, 3.67.

5.44. N-(Naphthalen-2-yl)-2-(2-oxo-2H-chromen-6-yloxy) acetamide (**23e**)

Yield: 64%; mp 211–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.81 (*s*, 2H, $-OCH_2$), 6.49 (*d* 1H, H-3, $J = 9.6$), 7.32–7.49 (*m*, 5H, arom. H), 7.66 (*dd*, 1H, H-7, $J = 8.8$, $J = 2.0$), 7.79–7.88 (*m*, 3H, arom. H), 8.02 (*d*, 1H, H-4, $J = 10$), 8.29 (*s*, 1H, arom. H), 10.34 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.7, 112.1, 116.2, 116.8, 117.6, 119.3, 120.1, 120.4, 125.0, 126.7, 127.5, 127.6, 128.6, 130.1, 133.4, 136.0, 144.2, 148.3, 154.3, 160.3, 166.7. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 73.22; H, 4.56; N, 3.92.

5.45. 2-(2-Oxo-2H-chromen-7-yloxy)-N-phenylacetamide (**24a**)

Yield: 88%; mp 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.80 (*s*, 2H, $-OCH_2$), 6.29 (*d*, 1H, H-3, $J = 9.2$), 6.98–7.10 (*m*, 3H, arom. H), 7.29–7.33 (*m*, 2H, arom. H), 7.56–7.64 (*m*, 3H, arom. H), 7.97 (*d*, 1H, H-4, $J = 9.2$) 10.20 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.5, 101.9, 113.2, 113.3, 120.2, 124.5, 129.3, 129.3, 130.0, 138.4, 144.8, 155.5, 160.9, 161.2, 166.4. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.07; H, 4.40; N, 4.67.

5.46. N-(4-Fluorophenyl)-2-(2-oxo-2H-chromen-7-yloxy) acetamide (**24b**)

Yield: 73%; mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.81 (*s*, 2H, $-OCH_2$), 6.31 (*d*, 1H, H-3, $J = 9.6$), 6.99–7.05 (*m*, 2H, arom. H), 7.12–7.18 (*m*, 2H, arom. H), 7.60–7.66 (*m*, 3H, arom. H), 7.99 (*d*, 1H, $J = 9.6$), 10.24 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.3, 101.8, 112.9, 113.0, 115.5 (*d*, $J_{C-F} = 22.8$ Hz, ArC–F), 121.9 (*d*, $J_{C-F} = 7.6$ Hz, ArC–F), 129.8, 134.7, 144.5, 155.3, 158.5 (*d*, $J_{C-F} = 238.8$ Hz, ArC–F), 160.5, 161.0, 166.0. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 64.98; H, 3.89; N, 4.47.

5.47. N-(4-Methoxyphenyl)-2-(2-oxo-2H-chromen-7-yloxy) acetamide (**24c**)

Yield: 66%; mp 198–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.71 (*s*, 3H, $-OCH_3$), 4.79 (*s*, 2H, $-OCH_2$), 6.31 (*d*, 1H, H-3, $J = 9.6$), 6.87–6.91 (*m*, 2H, arom. H), 7.02 (*s*, 1H, H-8), 7.04 (*d*, 1H, H-6, $J = 2.4$), 7.50–7.53 (*m*, 2H, arom. H), 7.66 (*d*, 1H, $J = 2.4$), 8.00 (*d*, 1H, H-4, $J = 9.6$), 10.03 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 55.27, 67.4, 101.7, 112.8, 112.9, 113.9, 121.5, 121.5, 129.6, 131.3, 144.3, 155.2, 155.7, 160.3, 161.0, 165.4. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.34; H, 4.65; N, 4.24.

5.48. N-(Biphenyl-4-yl)-2-(2-oxo-2H-chromen-7-yloxy)acetamide (**24d**)

Yield: 65%; mp 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.86 (*s*, 2H, $-OCH_2$), 6.31 (*d*, 1H, H-3, $J = 9.6$), 7.03 (*s*, 1H, H-8), 7.06 (*d*, 1H, H-6, $J = 2.4$), 7.30–7.34 (*m*, 1H, arom. H), 7.41–7.45 (*m*, 2H, arom. H), 7.62–7.73 (*m*, 7H, arom. H), 8.00 (*d*, 1H, H-4, $J = 9.6$), 10.28 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.4, 101.7, 112.9, 113.0, 120.2, 126.4, 127.1, 127.3, 129.0, 129.7, 135.6, 137.8, 139.7, 144.4, 155.3, 160.4, 161.0, 166.0. Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.25; H, 4.59; N, 3.69.

5.49. N-(Naphthalen-2-yl)-2-(2-oxo-2H-chromen-7-yloxy) acetamide (**24e**)

Yield: 74%; mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 4.87 (*s*, 2H, $-OCH_2$), 6.29 (*d*, 1H, H-3, $J = 9.6$), 7.02–7.06 (*m*, 2H, arom. H), 7.38–7.48 (*m*, 2H, arom. H), 7.60–7.65 (*m*, 2H, arom. H), 7.78–7.88 (*m*, 3H, arom. H), 7.97 (*d*, 1H, H-4, $J = 9.6$), 8.24 (*d*, 1H, arom. H, $J = 1.6$), 10.28 (*s*, 1H, –NH). ^{13}C NMR (DMSO-d_6) 67.6, 102.0, 113.3, 113.3, 116.6, 120.6, 125.5, 127.1, 127.7, 127.9, 128.9, 130.0, 133.6, 136.0, 144.8, 155.5, 161.0, 161.3, 166.7. Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 72.98; H, 4.36; N, 3.98.

5.50. Biological evaluations

5.50.1. Cell culture

HSC-3 cells were obtained from JCR Bank and were cultured in MEM (GibcoBRL, Grand Island, NY), supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, penicillin (100 U/mL), streptomycin (100 μg/mL), and 2 mM L-glutamine, at 37 °C in a humidified incubator containing 5% CO_2.

5.50.2. Luciferase reporter activity assay

A stable ARE-driven luciferase reporter cell line, HSC3-ARE9, was previously established [37]. For luciferase activity assay, HSC3-ARE9 cells at a density of 2.5×10^4 per well were seeded in 96-well plates and incubated at 37 °C for 16 h. Cells were treated with various concentrations of test compounds for 24 h, and then cell lysates were prepared for assessment of luciferase activity.

5.50.3. Determination of the scavenging effect on 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals

The method to determine the scavenging effect was described previously [49]. Briefly, test compound was mixed with 300 μM DPPH radicals for 90 min in the dark. The absorbency of the samples was measured using an Optimax automated microplate reader (Molecular Devices, Sunnyvale, CA) at 517 nm against methanol without DPPH as the blank reference. Each sample was quadruplicated in the test, and the values were averaged.

5.50.4. Cell viability assay

2.5×10^4 cells in 100 μL of culturing medium were seeded onto 96-well plates for 24 h before treatments. Cells were then treated with appropriated concentrations of test compounds for 24 h. 25 μL of MTT (5 mg/mL) was added into each sample and incubated for 4 h, under 5% CO_2 and 37 °C. 100 μL of lysis buffer (20% SDS, 50%

DMF) was subsequently added into each sample and further reacted for 16 h. Absorbance was measured at 570 nm using a Spectramax M5 microplate reader (Molecular Devices, UK) [37].

5.50.5. Real-time reverse transcription-polymerase chain reaction (RT-qPCR)

Total RNA was isolated from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA) and treated with DNase I (Fermentas, Hanover, MD) before cDNA synthesis to remove DNA contamination. First-strand cDNA was generated using the SuperScript III first-strand synthesis system according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Real-time PCR was performed using the LightCycler 2.0 system. Primers and TaqMan probes were designed by Probe Finder™ (Roche, Indianapolis, IN; http://www.universalprobelibrary.com). TaqMan probes were from the Universal Probe Library: ABCC3 (89), AKR1C1 (49), AKR1C2 (82), AKR1C3 (27), GCLC (80), GR (14), NQO-1 (87), Prx-1 (20), and GAPDH (60). Primers used for the reactions were purchased from Invitrogen (Carlsbad, CA), and the sequences are listed in our previous report [37]. The PCR conditions were as follows: 1 cycle of initial denaturation at 94 °C for 10 min and 45 cycles of amplification at 94 °C for 10 s, 68 °C for 45 s, and 72 °C for 1 s, with a single fluorescence acquisition. The human GAPDH gene was used as an internal control. GAPDH-normalized data are presented as the fold change in gene expression of the treated samples compared with untreated samples.

5.50.6. SDS-PAGE and Western blot analysis

Cells treated with appropriated concentration of drugs were collected and lysed with CelLytic™ M cell lysis reagent (Sigma–Aldrich, St. Louis, MO) containing 1 mM DTT, 1 mM PMSF and 1× protease inhibitor (Roche, Indianapolis, IN) for whole-cell lysate preparation. Equal amounts of total cell lysates and fractions of nuclear extracts were resolved on 10% polyacrylamide SDS gels under reducing conditions. The resolved proteins were electrophoretically transferred to PVDF membranes (Amersham Life Science, Amersham, UK), conjugated with various specific primary antibodies, and then probed with appropriate secondary antibodies. Immunoreactivity was detected by Enhanced Chemiluminescence (Amersham International, Buckingham, UK) and visualized on Kodak Bio-MAX MR film [37].

5.50.7. Determination of reactive oxygen species (ROS)

The intracellular accumulation of ROS was detected using DCFH-DA fluorescence assay as previously described [50]. Briefly, cells were seeded onto 96-well plates. After treatment, cells were incubated with a serum free medium containing 25 μM DCFH-DA at 37 °C for 30 min. ROS generation was measured by the fluorescence intensity of dichlorofluorescein (DCF)-fluorescence at 535 nm after excitation at 485 nm on a spectramax M5 microplate reader (Molecular Devices, UK) [37].

5.50.8. Animal cares and drug treatment

Male wild type C57BL/6J mice (6-weeks-old) were purchased from the National Laboratory Animal Center (Taiwan). The Institutional Animal Care and Use Committees for Biotechnology and the National Health Research Institutes approved uses of animals in these studies. The mice were housed with free access to water and food under 12 h light/dark cycles. All test compounds were dissolved in 90% PEG300 + 10% 1-methyl-*Z*-pyrrolidone. Animals were divided into two groups: (1) control ($n = 6$); and (2) 50 mg/kg compound **17a** ($n = 5$). The control and treatment groups were administered either vehicle (50% PEG 400) or 50 mg/kg compound **17a** by oral gavage (0.2 mL) for consecutive 7 days. Animals were sacrificed at the time indicated, and liver samples were obtained and frozen in liquid nitrogen before storage in −80 °C until further analysis.

Acknowledgment

The study was supported by grants from the Ministry of Science and Technology (NSC102-2320-B-400-002; NSC102-2320-B-037-005-MY2; NSC99-2628-B-037-005-MY3; MOST103-2321-B-400-013), and the Department of Health (DOH102-TD-M-111-102001; MOHW103-TDU-212-114005), Taiwan, R.O.C.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.10.029.

References

[1] F.P. Guengerich, Carcinogenesis 21 (2000) 345–351.
[2] T.W. Kensler, N. Wakabayashi, S. Biswal, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[3] H.C. Huang, T. Nguyen, C.B. Pickett, J. Biol. Chem. 277 (2002) 42769–42774.
[4] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J.D. Engel, M. Yamamoto, Genes Dev. 13 (1999) 79–86.
[5] A. Giudice, M. Montella, Bioessays 28 (2006) 169–181.
[6] J.M. Maher, M.Z. Dieter, L.M. Aleksunes, A.L. Slitt, G. Guo, Y. Tanaka, G.L. Scheffer, J.Y. Chan, J.E. Manautou, Y. Chen, T.P. Dalton, M. Yamamoto, C.D. Klaassen, Hepatology 46 (2007) 1597–1610.
[7] Y. Zhang, G.B. Gordon, Mol. Cancer Ther. 3 (2004) 885–893.
[8] Y. Yang, S. Jiang, J. Yan, Y. Li, Z. Xin, Y. Lin, Y. Qu, Cytokine Growth Factor Rev. 26 (2015) 47–57.
[9] B.H. Choi, K.S. Kang, M.K. Kwak, Molecules 19 (2014) 12727–12759.
[10] J.M. Marzec, J.D. Christie, S.P. Reddy, A.E. Jedlicka, H. Vuong, P.N. Lanken, R. Aplenc, T. Yamamoto, M. Yamamoto, H.Y. Cho, S.R. Kleeberger, FASEB J. 21 (2007) 2237–2246.
[11] A.L. Theiss, M. Vijay-Kumar, T.S. Obertone, D.P. Jones, J.M. Hansen, A.T. Gewirtz, D. Merlin, S.V. Sitaraman, Gastroenterology 137 (199–208) (2009), 208 e1–6.
[12] M.Y. Xi, J.M. Jia, H.P. Sun, Z.Y. Sun, J.W. Jiang, Y.J. Wang, M.Y. Zhang, J.F. Zhu, L.L. Xu, Z.Y. Jiang, X. Xue, M. Ye, X. Yang, Y. Gao, L. Tao, X.K. Guo, X.L. Xu, Q.L. Guo, X.J. Zhang, R. Hu, Q.D. You, J. Med. Chem. 56 (2013) 7925–7938.
[13] H.J. Lin, H. Zhou, A. Dai, H.F. Huang, J.H. Lin, H.D. Frankl, E.R. Lee, R.W. Haile, Pharmacogenetics 12 (2002) 175–179.
[14] R.K. Thimmulappa, K.H. Mai, S. Srisuma, T.W. Kensler, M. Yamamoto, S. Biswal, Cancer Res. 62 (2002) 5196–5203.
[15] M.K. Kwak, P.A. Egner, P.M. Dolan, M. Ramos-Gomez, J.D. Groopman, K. Itoh, M. Yamamoto, T.W. Kensler, Mutat. Res. 480–481 (2001) 305–315.
[16] R. Munday, Y. Zhang, J.D. Paonessa, C.M. Munday, A.L. Wilkins, J. Babu, J. Med. Chem. 53 (2010) 4761–4767.
[17] E. Balogun, M. Hoque, P. Gong, E. Killeen, C.J. Green, R. Foresti, J. Alam, R. Motterlini, Biochem. J. 371 (2003) 887–895.
[18] Y. Lee, D.H. Shin, J.H. Kim, S. Hong, D. Choi, Y.J. Kim, M.K. Kwak, Y. Jung, Eur. J. Pharmacol. 643 (2010) 21–28.
[19] R. Kachadourian, B.J. Day, S. Pugazhenti, C.C. Franklin, E. Genoux-Bastide, G. Mahaffey, C. Gauthier, A. Di Pietro, A. Boumendjel, J. Med. Chem. 55 (2012) 1382–1388.
[20] V. Kumar, S. Kumar, M. Hassan, H. Wu, R.K. Thimmulappa, A. Kumar, S.K. Sharma, V.S. Parmar, S. Biswal, S.V. Malhotra, J. Med. Chem. 54 (2011) 4147–4159.
[21] S.Y. Woo, J.H. Kim, M.K. Moon, S.H. Han, S.K. Yeon, J.W. Choi, B.K. Jang, H.J. Song, Y.G. Kang, J.W. Kim, J. Lee, D.J. Kim, O. Hwang, K.D. Park, J. Med. Chem. 57 (2014) 1473–1487.
[22] K.M. Amin, F.M. Awadalla, A.A. Eissa, S.M. Abou-Seri, G.S. Hassan, Bioorg. Med. Chem. 19 (2011) 6087–6097.
[23] F. Belluti, G. Fontana, L. Dal Bo, N. Carenini, C. Giommarelli, F. Zunino, Bioorg. Med. Chem. 18 (2010) 3543–3550.
[24] X. Cai, J. Yang, J. Zhou, W. Lu, C. Hu, Z. Gu, J. Huo, X. Wang, P. Cao, Bioorg. Med. Chem. 21 (2013) 84–92.
[25] F. Carta, A. Maresca, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. 20 (2012) 2266–2273.
[26] A. Kathuria, N. Priya, K. Chand, P. Singh, A. Gupta, S. Jalal, S. Gupta, H.G. Raj, S.K. Sharma, Bioorg. Med. Chem. 20 (2012) 1624–1638.
[27] S. Marumoto, M. Miyazawa, Bioorg. Med. Chem. 20 (2012) 784–788.
[28] M.J. Matos, C. Teran, Y. Perez-Castillo, E. Uriarte, L. Santana, D. Vina, J. Med. Chem. 54 (2011) 7127–7137.
[29] Y.L. Chen, T.C. Wang, K.H. Lee, Y.L. Cheng, C.M. Teng, C.C. Tzeng, Helv. Chim. Acta 79 (1996) 651–657.
[30] Y.L. Chen, T.C. Wang, N.C. Chang, C.C. Tzeng, Helv. Chim. Acta 82 (1999) 191–197.

[31] Y.L. Chen, I.L. Chen, C.C. Tzeng, T.C. Wang, Helv. Chim. Acta 83 (2000) 989–994.
[32] Y.L. Chen, C.M. Teng, C.C. Tzeng, Chin. Pharm. J. 53 (2001) 85–95.
[33] Y.L. Chen, C.M. Lu, S.J. Lee, D.H. Kuo, I.L. Chen, T.C. Wang, C.C. Tzeng, Bioorg. Med. Chem. 13 (2005) 5710–5716.
[34] Y.L. Chen, T.C. Wang, S.C. Liang, C.M. Teng, C.C. Tzeng, Chem. Pharm. Bull. (Tokyo) 44 (1996) 1591–1595.
[35] K.C. Fang, Y.L. Chen, J.Y. Sheu, T.C. Wang, C.C. Tzeng, J. Med. Chem. 43 (2000) 3809–3812.
[36] R.M. Silverstein, F.X. Webster, ^{13}C NMR spectrometry, in: N. Rose (Ed.), Spectrometric Identification of Organic Compounds, sixth ed., John Wiley and Sons, New York, 1998, pp. 217–249.
[37] H.H. Chen, Y.T. Chen, Y.W. Huang, H.J. Tsai, C.C. Kuo, Free Radic. Biol. Med. 52 (2012) 1054–1066.
[38] A.T. Dinkova-Kostova, C. Abeygunawardana, P. Talalay, J. Med. Chem. 41 (1998) 5287–5296.
[39] J. Rattanapan, J. Sichaem, S. Tip-pyang, Rec. Nat. Prod. 6 (2012) 288–291.
[40] D.M. Grant, J. Inherit. Metab. Dis. 14 (1991) 421–430.
[41] J. Pi, Y. Bai, J.M. Reece, J. Williams, D. Liu, M.L. Freeman, W.E. Fahl, D. Shugar, J. Liu, W. Qu, S. Collins, M.P. Waalkes, Free Radic. Biol. Med. 42 (2007) 1797–1806.
[42] S.S. Hamdulay, B. Wang, G.M. Birdsey, F. Ali, O. Dumont, P.C. Evans, D.O. Haskard, C.P. Wheeler-Jones, J.C. Mason, Free Radic. Biol. Med. 48 (2010) 1013–1023.
[43] D. Martin, A.I. Rojo, M. Salinas, R. Diaz, G. Gallardo, J. Alam, C.M. De Galarreta, A. Cuadrado, J. Biol. Chem. 279 (2004) 8919–8929.
[44] M.H. Kweon, Y. In Park, H.C. Sung, H. Mukhtar, Free Radic. Biol. Med. 40 (2006) 1349–1361.
[45] C. Xu, X. Yuan, Z. Pan, G. Shen, J.H. Kim, S. Yu, T.O. Khor, W. Li, J. Ma, A.N. Kong, Mol. Cancer Ther. 5 (2006) 1918–1926.
[46] K.M. Lee, K. Kang, S.B. Lee, C.W. Nho, Cancer Lett. 330 (2013) 225–232.
[47] S.E. Lee, S.I. Jeong, H. Yang, C.S. Park, Y.H. Jin, Y.S. Park, J. Cell. Biochem. 112 (2011) 2352–2360.
[48] M. Buelna-Chontal, J.G. Guevara-Chávez, A. Silva-Palacios, O.N. Medina-Campos, J. Pedraza-Chaverri, C. Zazueta, Free Radic. Biol. Med. 74 (2014) 145–156.
[49] C.C. Kuo, W. Chiang, G.P. Liu, Y.L. Chien, J.Y. Chang, C.K. Lee, J.M. Lo, S.L. Huang, M.C. Shih, Y.H. Kuo, J. Agric. Food Chem. 50 (2002) 5850–5855.
[50] H. Wang, J.A. Joseph, Free Radic. Biol. Med. 27 (1999) 612–616.