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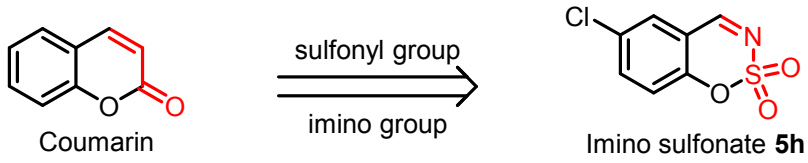
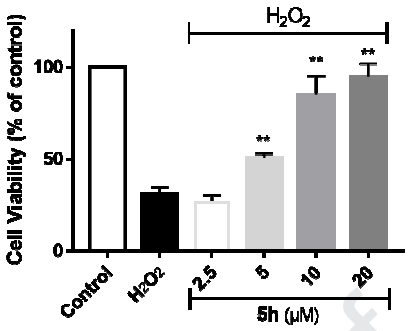


Coumarin

Imino sulfonate **5h**

5h presents potential cardioprotective activity and low cytotoxicity with the ability of attenuating oxidative stress directly by reducing intracellular ROS level via promoting Nrf2 pathway.



Discovery of coumarin-derived imino sulfonates as a novel class of potential cardioprotective agents

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Abbreviations

Con, control; CVD, cardiovascular disease; DHE, dihydroethidium; LDH, lactate dehydrogenase; LUT, luteolin; ROS, reactive oxygen species; Nuclear factor erythroid 2 related factor 2 (Nrf2); heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1).

Abstract

The burst of reactive oxygen species (ROS) contributes to and exacerbates cardiac injury. Exogenous supplementation of antioxidants or upregulation of endogenous antioxidant defense genes should be the potential therapies for cardiovascular disease. Sixteen coumarin-derived imino sulfonates compounds were synthesized with the ability of attenuating oxidative stress directly by reducing intracellular ROS level via promoting Nrf2 pathway. The cell-based assays showed that most of the compounds had significant protective activity against H_2O_2-induced oxidative injury in H9c2 cells. Compound **5h** with the highest activity and low cytotoxicity was demonstrated to remarkably remove the intracellular ROS accumulation by activating expressions of Nrf2 and its downstream antioxidant proteins (ie. HO-1 and NQO1), indicating a novel promising antioxidant and Nrf2 activator. Overall, these findings demonstrated that compound **5h** could serve as a potential cardioprotective agent. Moreover, our study features developing new antioxidants and Nrf2 activators by introducing a sulfonyl group and nitrogen atom to the α,β-unsaturated carbonyl entity in coumarin, rather than adding new functional groups or active fragments to coumarin itself.

Keywords:

Cardiovascular disease; Coumarin; Imino sulfonates; Cardioprotective agents; Oxidative stress; Nrf2

1. Introduction

Cardiovascular disease (CVD) is one of the critical diseases seriously endangering human health [1]. Accumulated evidences have demonstrated that the burst of reactive oxygen species (ROS) induced by oxidative stress extremely exacerbate structural and functional alterations in the heart tissue in cardiovascular disease, including myocardial ischemia/reperfusion injury, cardiac hypertrophy and heart failure [2-9]. Thus, exogenous supplementation of antioxidants and upregulation of endogenous antioxidant defense genes should be the potential therapies for cardiovascular diseases [10-12].

Nuclear factor erythroid 2 related factor 2 (Nrf2) is a transcription factor that regulates oxidative stress by inducing expression of antioxidant enzyme genes, such as heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase (GCL), and several members of the glutathione S-transferase family [13]. Notably, Nrf2 is closely associated with cancer, neurodegenerative diseases, especially for cardiovascular diseases [14-17]. Our previous study has also proved that activation of Nrf2 signaling could upregulate the damaged cardiac endogenous antioxidant system and decrease cardiac injury in rats [11,12,18]. Therefore, development of antioxidants and Nrf2 activators may display significant advantages against cardiovascular disease [19,20].

Kumar reported that a chalcone derivative (**1**) was a potent activator of the Nrf2 signaling pathway [21] (Fig. 1). Park found that the introducing of a vinyl sulfonyl group (**2**) to the *α,β*-unsaturated carbonyl entity of chalcone **1** led to higher activity to activate Keap1-Nrf2 signaling pathway and subsequent dose-dependent induction of Nrf2-dependent antioxidant enzymes expression (HO-1, NQO1, GCLC, and GLCM) [22,23]. In addition, nitrogen heterocycles are among the most significant structural components of pharmaceuticals, and 59% of unique small-molecule drugs in U.S. Food and Drug Administration-approved drugs contain a nitrogen heterocycle [24-29]. Optimized vinyl sulfone (**3**) by introducing nitrogen heterocycle showed superior effect on Nrf2 activation in cell-based assays compared to compound **2** [30]. These

results indicate that sulfonyl group and nitrogen heterocycle are benefit for activating Nrf2 signaling pathway.

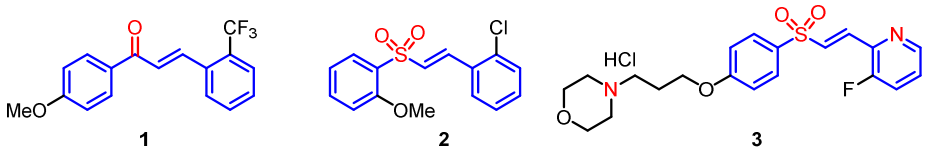
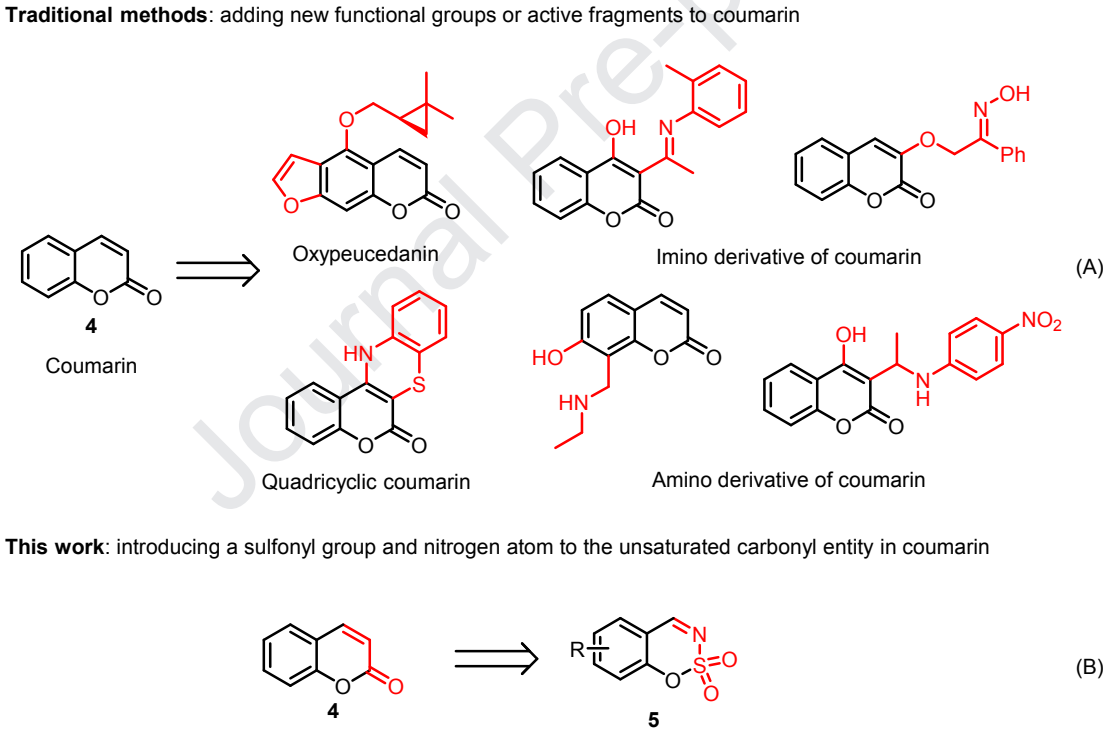


Fig. 1. Superior effect on Nrf2 activation by introducing sulfonyl group and nitrogen atom to chalcone.

Coumarins, a wide class of natural and synthetic compounds, have attracted considerable attention in recent years due to its diverse pharmacological actions, such as neuroprotective [31-35], antifungal [36] and anticancer [37] activities. Some coumarin derivatives have also been proven to be excellent antioxidants and Nrf2 activators [38-43].



Scheme 1. Previous reports and our design for developing new antioxidants or Nrf2 activators based on the structure of coumarin.

Traditional methods for developing new coumarin-derived active compounds rely on adding new functional groups or active fragments to coumarin (Scheme 1A). Inspired by Park's work, what we did innovatively in this study was that we introduced a sulfonyl group and nitrogen atom to the α,β-unsaturated carbonyl entity in coumarin to generate coumarin-derived imino sulfonates **5**, and the potential

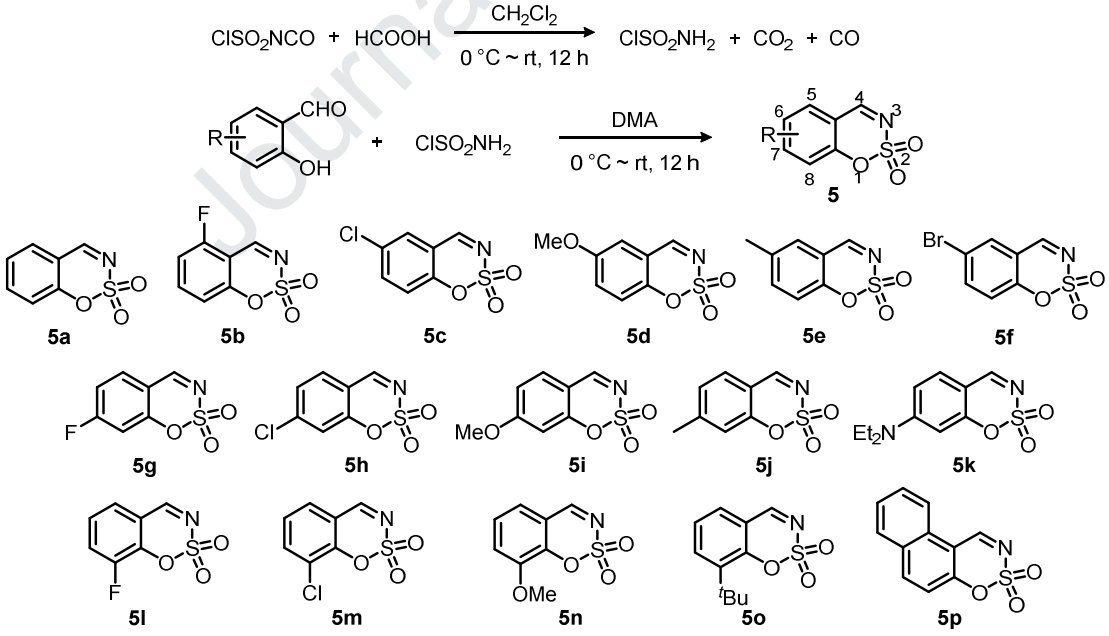
cardioprotective activities of these synthesized compounds were further evaluated (Scheme 1B).

Initially, we screened the derivatives for their potential capacity to protect H9c2 cardiomyocyte cells from oxidative stress injury induced by hydrogen peroxide (H_2O_2). We found that the introducing of a sulfonyl group and imino group indeed led to the potent activities. The compound with highest activity was demonstrated to activate Nrf2 signaling pathway, thus further inducing the expressions of Nrf2-dependent endogenous antioxidant proteins (ie. HO-1 and NQO1).

2. Results and discussion

2.1 Chemistry

As illustrated in Scheme 2, sixteen coumarin-derived imino sulfonates (**5**) were prepared based on a standard protocol [44]. Briefly, the reaction of chlorosulfonyl isocyanate with formic acid generate chlorosulfonamide. Next, target compounds **5** were synthesized by cyclization reaction of various salicylaldehydes and chlorosulfonamide.



Scheme 2. Synthesis of compounds **5**.

2.2 Biological screenings

Primarily, all target compounds **5** were screened for their protective activity against oxidative stress induced by H_2O_2 in H9c2 cells, a pivotal model for potential

cardioprotective agents screening [45].

2.2.1 Protective activity against H_2O_2-induced H9c2 cells damage and inherent toxicity

In order to explore the potential cardioprotective activity of the target compounds, sixteen coumarin-derived imino sulfonates (**5**) were evaluated against oxidative stress injury induced by H_2O_2 in H9c2 cells by MTT assay. To demonstrate the structure-activity relationship, different substituents were introduced to coumarin-derived imino sulfonates.

As depicted in Fig. 2A, most of analogues, except **5o**, were found to effectively increased the cell viability in H9c2 subjected to H_2O_2.

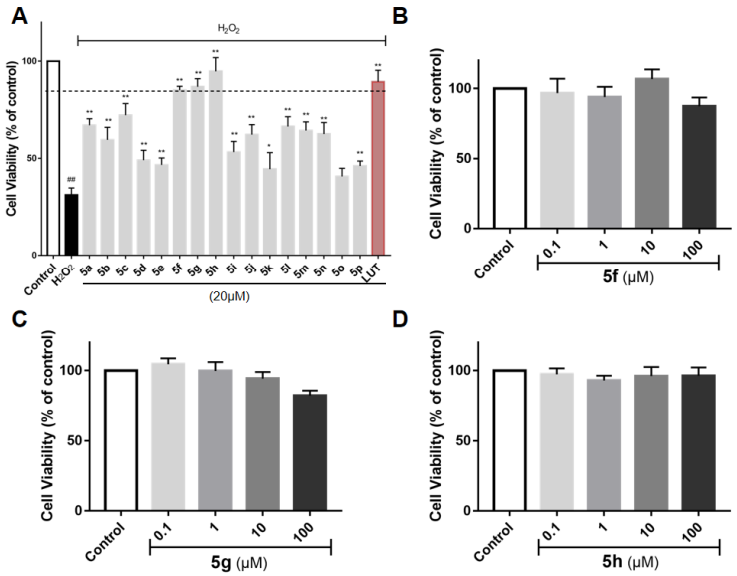


Fig. 2. Compounds' cytoprotection on H9c2 cells in H_2O_2 damage model (A) and cytotoxicity evaluation of three targeted compounds (**5f**, **5g** and **5h**) at different concentrations in H9c2 cells (B-D). H9c2 cells were pretreated for 24 h with LUT or coumarin-derived imino sulfonates **5** at 20 μM, respectively, followed by 2 h exposure in H_2O_2 (250 μM). The cytotoxicity of compounds was determined by the MTT assay. H9c2 cells were pretreated for 24 h with **5f**, **5g** or **5h** at 0.1, 1, 10, 100 μM, respectively, Three individual experiments were performed for each group. The data was expressed as the Mean ± SD. ##P < 0.01, #P < 0.05 vs Control, **P < 0.01, *P < 0.05 vs H_2O_2. one way ANOVA, followed by Tukey's multiple comparison test.

All electron-donating group substituted compounds (**5d**, **5e**, **5i**, **5j**, **5k**, **5n**, **5o**, **5p**) are unfavorable for the activity comparing to **5a**.

Comparison of fluoride substituted compounds (**5b**, **5g**, **5l**) (5, 7, 8-position on

benzene ring) demonstrated that substitutes on 5- and 8- position are unfavorable for the activity.

Comparison of chloride substituted compounds (**5c**, **5h**, **5m**) (6, 7, 8-positionon benzene ring) further demonstrated that substitute on 8-position is unfavorable for the activity.

Notably, for 5- and 8- substituted compounds, neither electron withdrawing group nor electron donating group is unfavorable for the activity.

For 6- and 7- substituted compounds, electron-withdrawing group substituted compounds (**5c**, **5f**, **5g**, **5h**) led to increased activities. However, electron-donating group substituted compounds (**5d**, **5e**, **5i**, **5j**, **5k**) led to obvious decreased activities comparing to **5a**. Moreover, substituted on 7- position is more favorable than substituted on 6- position (**5c** vs **5h**).

Among the sixteen compounds, **5f**, **5g** and **5h** showed the cardioprotective action similar or even better to that of LUT. In addition, we found that **5f**, **5g** and **5h** at up to 100 μM did not exhibit any significant cytotoxicity in H9c2 cells, suggesting the low cytotoxicity of these compounds (Fig. 2B-D).

*2.2.2 Protection of H9c2 cells from H_2O_2-induced damage by **5f**, **5g** or **5h***

To further explore the exact compound possessing cardioprotective activity, different concentrations of three targeted compounds (**5f**, **5g** and **5h**) were conducted in H9c2 suffered from H_2O_2 injury, respectively. As showed in Fig. 3A, pre-incubation with these compounds for 24 h, 2.5 μM of **5f**, **5g** and **5h** exhibited none cardioprotective action, as well as LUT. At 5 μM, only **5h** significantly increased the cell viability, as compared to the H_2O_2 group (Fig. 3B). Moreover, **5h** at 10 μM were found to have better advantageous cytoprotection, relative to that in cells preincubated with **5f**, **5g** or LUT (Fig. 3C).

Meanwhile, the release rate of LDH is an important index to monitor the cellular membrane integrity [10,46]. Our study revealed that treatment with H_2O_2 caused dramatically increased LDH release rate, compared to the control group, indicating the loss of cell membrane integrity and necrotic cell death caused by oxidative stress.

Pretreatment with compound **5h** at 10 μM could significantly decreased such index (Fig. 3D), compared to the H_2O_2 treated cells. However, administration with compounds **5f** and **5g** showed no significant difference compared to those in H_2O_2 group, respectively. Moreover, **5f**, **5g** and **5h** at 20 μM could markedly decrease the LDH release rate (Fig. 3E). Notably, **5h** (20 μM) exhibited the most excellent activity in declining LDH release rate in H9c2 against H_2O_2 induced injury.

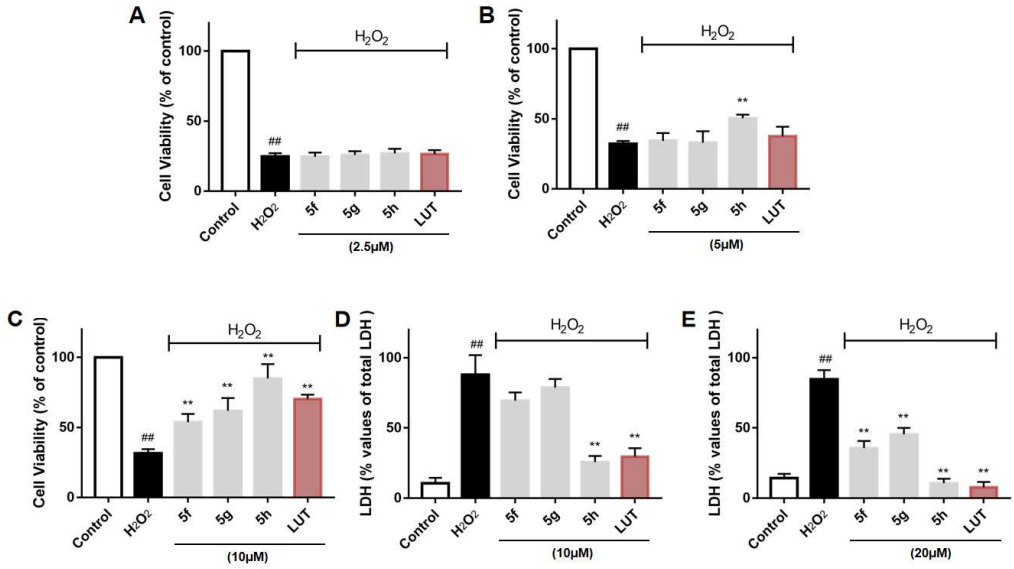


Fig. 3. Compound **5h** exhibited the excellent cytoprotective activity in H9c2 against H_2O_2-induced cell damage. H9c2 cells were pretreated with **5f**, **5g**, **5h** and LUT at different concentrations (2.5, 5, 10 μM) for 24 h, respectively, then treated with 250 μM H_2O_2 for 2 h, and determined by the MTT assay (A-C). The viability of untreated cells is defined as 100%. H9c2 cells were pre-incubated with **5f**, **5g**, **5h** or LUT at 10, 20 μM for 24 h, respectively, then treated with 250 μM H_2O_2 for 2 h, thus the LDH release rate was determined (D and E). Three individual experiments were performed for each group. The data was expressed as the Mean ± SD. ##P < 0.01, #P < 0.05 vs Control, **P < 0.01, *P < 0.05 vs H_2O_2. one way ANOVA, followed by Tukey's multiple comparison test.

From the bioactivity data of compounds **5f**, **5g** and **5h**, the results revealed that **5h** had the most valuable cellular protective activity for cardiomyocytes. Given the fact that coumarin derivatives have been demonstrated to possess positive cardiovascular effects, which are not only based on their anti-oxidant action, but also based on the anti-inflammatory effects, regulation of the intracellular calcium or nitric oxide (NO) level, vasorelaxant properties and so on [47-50]. Therefore, in order to investigate the

mechanism of this series of compounds, compound **5h** with the highest potency was further evaluated for the possibility of signaling pathways in its cardioprotective effect.

Due to the important role of oxidative stress, inflammation, and overload of calcium in cardiovascular diseases (CVD), we have conducted an experiment to analyse the possible mechanisms underlying the potential cardioprotective effect of compound **5h**. Specifically, transcription factor Nrf2 is the master regulator of the antioxidative enzyme genes expression [51], and activation of Nrf2 has been considered to be the crucial therapy against oxidative stress in CVD. NF-κB, another important transcription factor, mainly mediates the inflammatory responses, and downregulation of NF-κB could significantly inhibit the inflammation in CVD [52]. In addition, prior animal and human studies have documented that decreased expression of sarcoplasmic reticulum Ca^{2+}-ATPase 2 (SERCA2), a major cardiac calcium cycling protein ameliorating the overload of calcium, as a primary defect found in CVD [53,54]. Importantly, increasing the activity of SERCA2α in patients with moderate to severe heart failure improves their cardiac function, disease status and quality of life [55,56].

Hence, the protein expressions of Nrf2, NF-κB p65 in nucleus, along with SERCA2 in cytoplasm were measured by Western blotting, as Fig. 4 depicted. Our data showed that H_2O_2 treatment induced a robust increase in the expression of NF-κB and Nrf2 in nucleus. The levels of SERCA2 were significantly decreased in H_2O_2 group, compared to the control cells. By contrast, pretreatment with compound **5h** further stimulated protein expression of Nrf2, suggest a hyperactivation of the Nrf2 pathway. However, **5h** administration only showed a trend to reverse the abnormally increased NF-κB expression and declined SERCA2 level induced by H_2O_2, and no statistical difference was found. Collectively, these results indeed elucidated that compound **5h** reduced the myocardial injury induced by H_2O_2 mainly through its antioxidant activity.

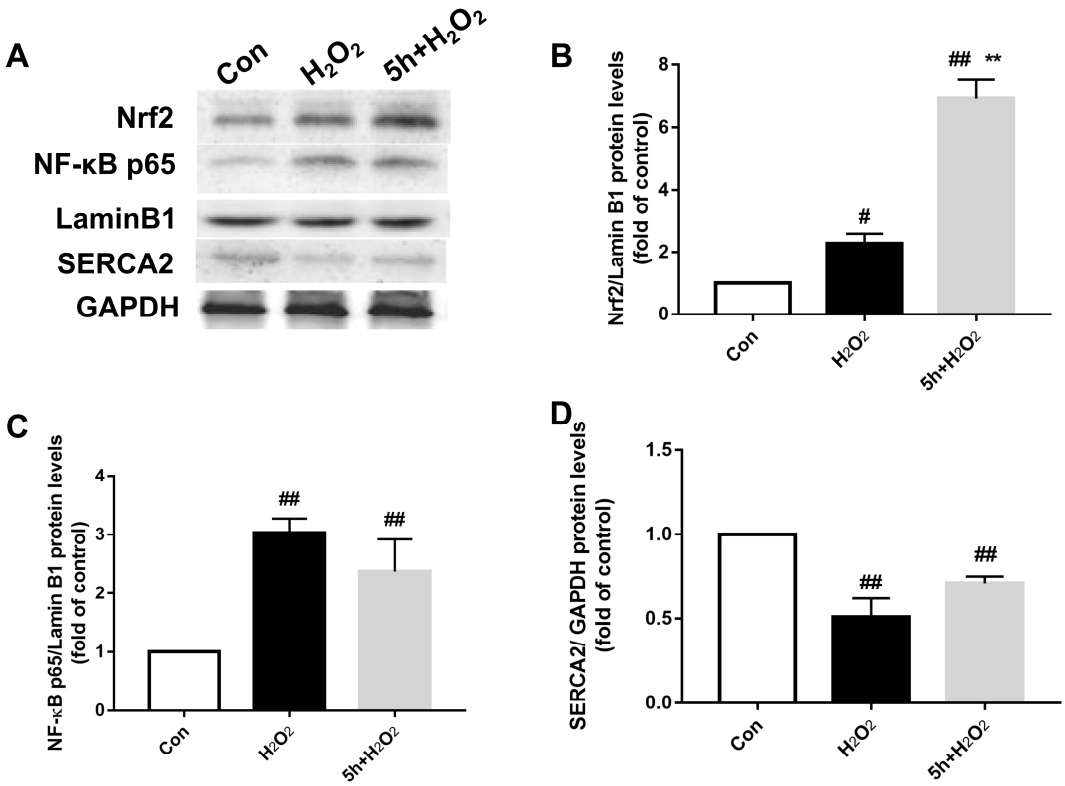


Fig. 4. Possible mechanisms underlying the protective effect of compound **5h**. H9c2 cells were pretreated with **5h** (20 μM) for 24 h, then treated with 250 μM H_2O_2 for 2 h. The protein expressions of Nrf2, NF-κB p65 and SERCA2 were determined by Western Blotting (A-D). Cells treated with none compound were served as the Control (Con). Protein expression levels were normalised against levels of Lamin B1 or GAPDH, which was used as a loading control for nuclear and cytoplasmic protein samples, respectively. The data was expressed as the Mean ± SD (n=3). ##P < 0.01, #P < 0.05 vs Con, **P < 0.01, *P < 0.05 vs H_2O_2. one way ANOVA, followed by Tukey's multiple comparison test.

*2.2.3 Compound **5h** scavenged the cellular ROS by activating the Nrf2 signaling pathway*

The burst of oxidants, such as reactive oxygen species (ROS), has been demonstrated to participate in breaking down the cell membrane integrity, which cause the cardiac injury [10,11]. Stimulation of cardiomyocytes with H_2O_2 led to the cellular ROS accumulation. Thus, we further examined whether compound **5h** could decline the ROS production, and explored the mechanisms underlying such action. As illustrated in Fig. 5A-B, pretreatment with compound **5h** (10 μM) for 24 h dramatically reduced the cellular ROS accumulation, suggesting that scanvenging ROS by **5h** may contribute to its cytoprotective activity against oxidative injury induced by H_2O_2.

The NF-E2-related factor 2 (Nrf2) transcription factor pathway has been

demonstrated to restore cellular redox homeostasis by increasing endogenous antioxidant response element-mediated expression of phase II and antioxidant enzymes, such as NAD(P)H quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 (HO-1). Importantly, Nrf2 closely correlates with cardiac protection against myocardial injury. Therefore, the Nrf2 pathway has been considered as a novel therapeutic target for cardiovascular diseases, including myocardial infarction, myocardial ischemia/reperfusion and myocardial hypertrophy [11,57]. In the present study, we discovered that **5h** could dose-dependently induce the Nrf2 translocation into the nucleus, with a corresponding upregulated Nrf2-dependent proteins, such as HO-1 and NQO1 (Fig. 5C-F). Additionally, **5h** at 10 μM showed even more stronger promoting effect on Nrf2 pathway than that of LUT.

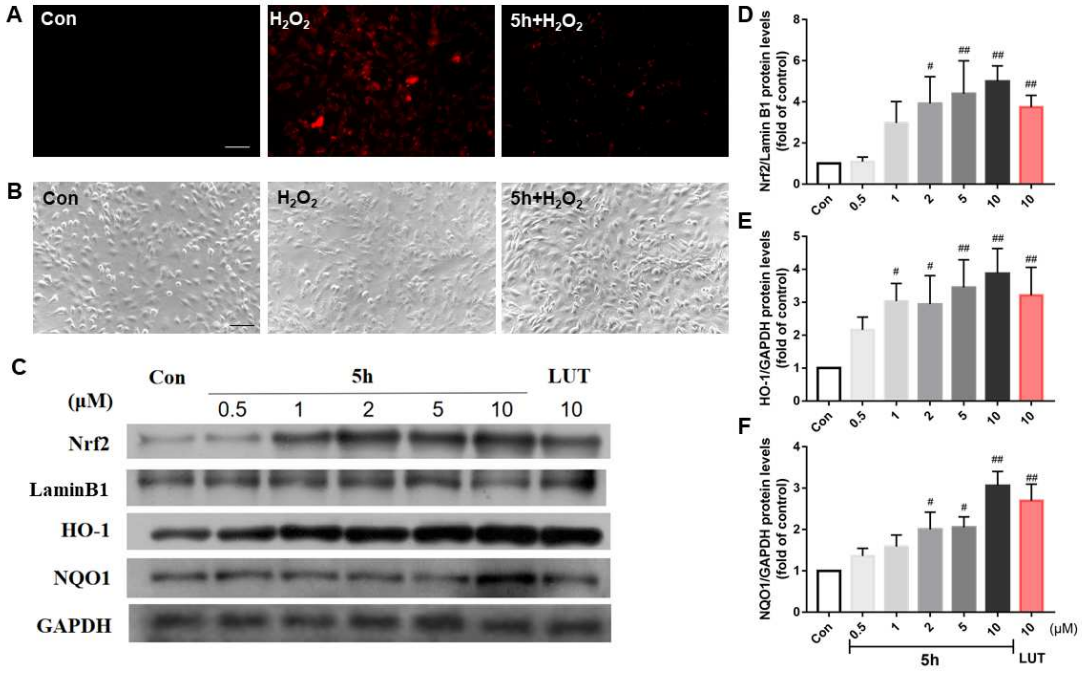


Fig. 5. Protection of compound **5h** by scavenging ROS via activating the Nrf2 signaling pathway. H9c2 cells were pretreated with **5h** (10 μM) for 24 h, then treated with 250 μM H_2O_2 for 2 h, and intracellular reactive oxygen species (ROS) generation was measured by the DHE assay (200×, bar=400μm) (A and B). H9c2 cells were treated with **5h** (0.5, 1, 2, 5, 10 μM) or LUT (10 μM) for 24 h, respectively. Then the protein expressions of Nrf2, HO-1and NQO1 were determined by Western Blotting (C-F). Cells treated with none compound were served as the Control (Con). Protein expression levels were normalised against levels of Lamin B1 or GAPDH, which was used as a loading control for nuclear and cytoplasmic protein samples, respectively. The data was expressed as the Mean ± SD (n=3). ##P < 0.01, #P < 0.05 vs Con, one way ANOVA, followed by Tukey's multiple comparison test.

3. Conclusion

In this study, a series of coumarin-derived imino sulfonates were synthesized by

introducing a sulfonyl group and nitrogen atom to the *α*,*β*-unsaturated carbonyl entity in coumarin. The cytoprotection of all these analogues was detected in H_2O_2 induced H9c2 cells injury model. Most synthesized compounds displayed potent cardioprotective activities at 20 μM. Structure-activity relationships indicated that compounds with different substituents on benzene ring have great influence on their biological activities. Notably, after pre-incubation with compounds for 24 h, compounds **5f**, **5g** and **5h** displayed valuable cytoprotection against oxidative damage in H9c2 cells. Among them, compound **5h** was screened out to exhibit low cytotoxicity and an inspirable protection against oxidative stress in myocytes in a concentration dependent manner. Consistent with its cytoprotective activity, **5h** also exhibited potent anti-oxidative activity by removing the cellular ROS accumulation, thus reducing the loss of cellular membrane integrity as demonstrated by LDH release rate. In addition, mechanism of action studies confirmed that **5h** may confer its protection for H9c2 cells against oxidative insults through inducing the nuclear translocation of Nrf2, thus upregulating the expression of endogenous antioxidant proteins HO-1 and NQO1.

In summary, our work presented a series of coumarin-derived imino sulfonates with antioxidant activity, and **5h** represents a novel therapeutic agent with potential for treating cardiovascular diseases. Our present study may prove important for the future design of coumarin-derived structurally related Nrf2 activators.

4. Experimental

4.1. Chemistry

All reactions were performed in oven-dried glassware with magnetic stirring. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All solvents were purified and dried according to standard methods prior to use. Organic solutions were concentrated under reduced pressure on a rotary evaporator or an oil pump. Reactions were monitored through thin layer chromatography (TLC) on silica gel-precoated glass plates. Subsequent to elution, plates were visualized using UV radiation at 254 nm and by staining with

aqueous potassium permanganate or ethanolic phosphomolybdic acid solution. Flash column chromatography was performed using silica gel (300-400 mesh). Nuclear Magnetic Resonance (NMR) spectras were acquired on a Varian Mercury 400 operating at 400, 100 and 376 MHz for ^{1}H, ^{13}C and ^{19}F, respectively. Chemical shifts are reported in δ ppm referenced to an internal $SiMe_4$ standard for ^{1}H NMR, chloroform-d (δ 77.16) for ^{13}C NMR. Datas for ^{1}H NMR are recorded as follows: chemical shift (δ ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad), coupling constant (Hz) and integration. Datas for ^{13}C NMR and ^{19}F NMR are reported in terms of chemical shift (δ, ppm). High-resolution mass spectra (HRMS) were recorded on a Thermo Q-Exactive Spectrometer (ESI source).

*4.1.1. General procedure for the preparation of imino sulfonates (**5**) [44]*

Anhydrous formic acid (40.0 mmol, 1.5 mL, 1 eq) was added dropwise to neat chlorosulfonyl isocyanate (40.0 mmol, 3.5 mL, 1 eq) at 0 ^{o}C with rapid stirring. Vigorous gas evolution was observed during the addition process. The resulting viscous suspension was stirred at room temperature until gas evolution ceased (12 h). The resulting colorless solid was used in the following step immediately and stored under -20 °C.

To a solution of salicylaldehyde (15.0 mmol) in DMA (100 mL) at 0 ^{o}C was carefully added freshly prepared $ClSO_2NH_2$ (4.6 g, 40.0 mmol) in small portions, and the resulting solution was stirred for 12 h at room temperature. The reaction was quenched carefully with ice-cold water (100 mL), and the mixture was transferred to a separating funnel containing CH_2Cl_2 (200 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic layers were washed with saturated $NaHCO_3$ solution (100 mL), dried ($MgSO_4$), filtered through a short pad of silica using CH_2Cl_2 as eluent and concentrated in *vacuo*. Imino sulfonates **5** were isolated by flash chromatography (PE/EtOAc) on silica gel.

*4.1.1.1 benzo[e][1,2,3]oxathiazine 2,2-dioxide (**5a**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.79-7.74 (m, 1H), 7.70-7.68 (m, 1H), 7.46-7.42 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 154.3, 137.8, 131.0, 126.3,

118.7, 115.5 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_8NO_4S$ $[M+OCH_3]^-$: 214.0180, found: 214.0160.

*4.1.1.2 5-fluorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5b**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.78-7.72 (m, 1H), 7.15-7.11 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9 (d, *J* = 6.0 Hz), 161.1 (d, *J* = 261.9 Hz), 154.7 (d, *J* = 3.0 Hz), 139.0 (d, *J* = 11.0 Hz), 114.6 (d, *J* = 4.0 Hz), 112.8 (d, *J* = 20.0 Hz), 105.9 (d, *J* = 17.0 Hz) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7FNO_4S$ $[M+OCH_3]^-$: 232.0085, found: 232.0065.

*4.1.1.3 6-chlorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5c**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.72-7.67 (m, 2H), 7.29-7.26 (m, 1H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7ClNO_4S$ $[M+OCH_3]^-$: 247.9790, found: 247.9769.

*4.1.1.4 6-methoxybenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5d**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.30-7.27 (m, 1H), 7.23 (d, *J* = 9.2 Hz, 1H), 7.09 (d, *J* = 2.8 Hz, 1H), 3.88 (s, 3H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_9H_{10}NO_5S$ $[M+OCH_3]^-$: 244.0285, found: 244.0265.

*4.1.1.5 6-methylbenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5e**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.55 (dd, *J* = 8.8 Hz, *J* = 1.8 Hz, 1H), 7.46 (d, *J* = 1.6 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 2.45 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 152.3, 138.6, 136.5, 130.8, 118.4, 115.3, 20.8 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_9H_{10}NO_4S$ $[M+OCH_3]^-$: 228.0336, found: 228.0316.

*4.1.1.6 6-bromobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5f**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.86-7.82 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 153.3, 140.4, 133.1, 120.6, 118.8, 116.6 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7BrNO_4S$ $[M+OCH_3]^-$: 291.9285, found: 291.9264.

*4.1.1.7 7-fluorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5g**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.74-7.71 (m, 1H), 7.17-7.12 (m, 1H), 7.04-7.02 (m,1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7 (d, *J* = 262.5 Hz, 1C), 166.8, 156.3 (d, *J* = 13.7 Hz, 1C), 133.4 (d, *J* = 11.3 Hz, 1C), 114.5 (d, *J* = 22.7 Hz, 1C), 112.4 (d, *J* = 2.8 Hz,

1C), 107.0 (d, *J* = 25.9 Hz, 1C) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7FNO_4S$ $[M+OCH_3]^-$: 232.0085, found: 232.0065.

*4.1.1.8 7-chlorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5h**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.41 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 7.33 (d, *J* = 1.6 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 154.8, 144.2, 131.8, 127.0, 119.3, 113.9 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7ClNO_4S$ $[M+OCH_3]^-$: 247.9790, found: 247.9769.

*4.1.1.9 7-methoxybenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5i**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 6.90 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 3.94 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 166.9, 157.0, 132.6, 113.7, 109.3, 103.0, 56.6 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_9H_{10}NO_5S$ $[M+OCH_3]^-$: 244.0285, found: 244.0265.

*4.1.1.10 7-methylbenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5j**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.10 (s, 1H), 2.51 (s, 3H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_9H_{10}NO_4S$ $[M+OCH_3]^-$: 228.0336, found: 228.0315.

*4.1.1.11 7-(diethylamino)benzo[e][1,2,3]oxathiazine 2,2-dioxide (**5k**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 6.54-6.52 (m, 1H), 6.33-6.32 (m, 1H), 3.47 (q, *J* = 7.2 Hz, 4H), 1.25 (t, *J* = 7.2 Hz, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 157.7, 154.7, 132.8, 109.1, 105.1, 98.7, 45.5, 12.5 ppm. HRMS (ESI): m/z Exact mass calcd. for $C_{11}H_{15}N_2O_3S$ $[M+H]^+$: 255.0798, found: 255.0744.

*4.1.1.12 8-fluorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5l**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 1.2 Hz, 1H), 7.59-7.54 (m, 1H), 7.51-7.48 (m, 1H),7.42-7.37 (m, 1H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7FNO_4S$ $[M+OCH_3]^-$: 232.0085, found: 232.0064.

*4.1.1.13 8-chlorobenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5m**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.82-7.79 (m, 1H), 7.62-7.60 (m, 1H), 7.41-7.37 (m, 1H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_8H_7ClNO_4S$ $[M+OCH_3]^-$: 247.9790, found:

247.9770.

*4.1.1.14 8-methoxybenzo[e][1,2,3]oxathiazine 2,2-dioxide (**5n**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.37-7.30 (m, 2H), 7.25-7.23 (m, 1H), 3.97 (s, 3H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_9H_{10}NO_5S$ $[M+OCH_3]^-$: 244.0285, found: 244.0265.

*4.1.1.15 8-(tert-butyl)benzo[e][1,2,3]oxathiazine 2,2-dioxide (**5o**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.75 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H), 7.52 (dd, *J* = 7.6 Hz, *J* = 1.6 Hz, 1H), 7.38-7.34 (m, 1H), 1.48 (s, 9H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_{12}H_{16}NO_4S$ $[M+OCH_3]^-$: 270.0806, found: 270.0785.

*4.1.1.16 naphtho[1,2-e][1,2,3]oxathiazine 3,3-dioxide (**5p**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.0 (s, 1H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.55 (d, *J* = 9.2 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.90-7.86 (m, 1H), 7.76-7.72 (m, 1H), 7.67 (d, *J* = 9.2 Hz, 1H) ppm. HRMS (ESI): m/z Exact mass calcd. for $C_{12}H_{10}NO_4S$ $[M+OCH_3]^-$: 264.0336, found: 264.0316.

4.2 Biological assays

4.2.1. Cell culture

H9c2 cell line (CRL-1446^{TM}) was provided by the American Type Culture Collection (ATCC, Shanghai, China). Cells were cultured as previously described [7]. Briefly, cells were cultured in Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F12) with 10% fetal bovine serum (FBS) containing 100 U/mL penicillin and 100 U/mL streptomycin. Cells were incubated at 37 °C with 5% CO_2, and were synchronized by serum starvation before stimulated with hydrogen peroxide (H_2O_2) or the compounds being tested.

4.2.2. Cardioprotection assay against H_2O_2 and cardiotoxicity assay in H9c2 cells

Compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich, Shanghai, China) and diluted with medium.Generally, 80% confluent cells were seeded 5×10^3 cells/well in 96-well plates and were allowed to attach overnight.

For the cardiotoxicity assay, previous medium was removed, and 0.1, 1, 10 and 100 μM of the compounds was added to the wells, respectively. 0.01% DMSO in cell

culture media served as control. Cells was incubated with compounds for 24 h.

For the cardioprotection assay, cells were subjected to 250 μM H_2O_2 for 2h to induce the myocardial injury. Different concentrations of respective compounds were added to the cells 24 h prior to hydrogen peroxide treatment. Luteolin (LUT) (Sigma Aldrich, Shanghai, China) was served as positive control [10].

4.2.3. MTT assay

MTT assay was utilized to determine H9c2 cells cytotoxicity and viability. After appropriate treatment, Cells were treated with MTT solution (4 mg/mL in PBS) for 4 h at 37 °C. The formazan crystals were dissolved in DMSO and the optical density (OD) was examined at 490 nm using a BioTek plate reader. The cell viability was given in a percentage of the OD value of the control group. *In vitro* cardiotoxicity or cardioprotection assay, the cell viability was determined after treated with the compounds being tested for 24 h.

4.2.4. Determination of intracellular H_2O_2-induced ROS production in H9c2 cells

H9c2 cells were seeded in 6-well plates (2.5×10^5 cells/well) and allowed to attach overnight. After 24 h of preincubation with compound **5h** (10 μM), H_2O_2 was added for 2 h. Then, cells were incubated with non-fluorescent dihydroethidium (DHE, 5 μM) at 37 °C for 30 min. Ethidium fluorescence (excitation at 488 nm, emission at 525 nm) was monitored by fluorescence microscopy (Nikon Eclipse Ti-S, Nikon Ltd, Japan).

4.2.5. Measurement of LDH release rate

After appropriate treatment, the LDH release rate was measured by the cytotoxicity detection kit, according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China) and quantified by absorbance at 490 nm.

4.2.6. Western blot analysis

H9c2 cells were treated with compound **5h** (0.5, 1, 2, 5, 10 μM) or LUT (10 μM) for 24 h, and were lysed with a commercial kit to extract cytoplasmic and nuclear proteins (KeyGEN BioTECH, Nanjing, China). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamidegel (SDS-PAGE) and then transferred to

Polyvinylidene Fluoride (PVDF) membranes (Millipore Corporation, MA, USA). Blots were blocked with 5% nonfat dry milk-TBS-0.1% Tween 20 for 2 h. Primary antibodies were incubated overnight at 4 °C. Thereafter, all blots were washed three times with 1×TBST and were incubated with a horseradish peroxidase-conjugated secondary anti-rabbit antibody (1:5000; Cell Signaling Technology Co., Ltd, MN, USA) for 2 h. Immunoreactivities of target proteins were detected by a gel imaging system (Protein Simple, Santa Clara, California, USA), and were analysed by Image J software. The primary antibodies used were polyclonal antibodies against Nrf2 (1:1000; Proteintech Biotech, Wuhan, China), NF-κB p65 (1:1000; Cell Signaling Technology Co., Ltd, MN, USA), SERCA2 (1:1000; Cell Signaling Technology Co., Ltd, MN, USA), HO-1 (1:1000; Cell Signaling Technology Co., Ltd, MN, USA) and NQO1 (1:1000; Cell Signaling Technology Co., Ltd, MN, USA) . Protein expression levels were normalised against levels of Lamin B1 (1:1000, Bioworld Technology, St. Louis Park, MN, USA) or GAPDH (1:1000, Bioworld Technology, St. Louis Park, MN, USA), which were used as a loading control.

4.3. Statistical analysis

Results were expressed as mean ± SD of three independent experiments. One-way ANOVA followed by Tukey's post hoc test were used for multi-group comparison (GraphPad Prizm 5.0). Significant differences were established at $P < 0.05$.

Conflicts of interest

The authors declare no competing interest.

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Appendix A. Supplementary data

Copies of the NMR spectras for all compounds.

References

[1] M. Parikh, G. N. Pierce, Dietary flaxseed: What we know and don't know about its effects on cardiovascular disease, Can. J. Physiol. Pharm. 97 (2019) 75-81.

[2] M. Zhai, B. Li, W. Duan, L. Jing, S. Yu, Melatonin ameliorates myocardial ischemia reperfusion injury through Sirt3-dependent regulation of oxidative stress and apoptosis, J. Pineal Res. 63 (2017) el2419.

[3] E. Pantazi, M. Bejaoui, E. Folch-Puy, R. Adam, J. Roselló-catafau, Advances in treatment strategies for ischemia reperfusion injury, Expert Opin. Pharmaco. 17 (2016) 169-179.

[4] X. Wang, B. Yuan, B. Cheng, Y. Liu, B. Zhang, X. Wang, X. Lin, B. Yang, G. Gong, Crocin alleviates myocardial ischemia/reperfusion-induced endoplasmic reticulum stress via regulation of miR-34a/Sirt1/Nrf2 pathway, Shock 51 (2019) 123-130.

[5] D. Bandyopadhyay, A. Chattopadhyay, G. Ghosh, A. G. Datta, Oxidative stress-induced ischemic heart disease: protection by antioxidants, Curr. Med. Chem. 11 (2004) 369-387.

[6] C. D. Ochoa, R. F. Wu, L. S. Terada, ROS signaling and er stress in cardiovascular disease, Mol. Aspects. Med. 63 (2018) 18-29.

[7] R. Wu, E. Wyatt, K. Chawla, M. Tran, M. Ghanefar, M. Laakso, C. L. Epting, H. Ardehali, Hexokinase II knockdown results in exaggerated cardiac hypertrophy via increased ros production, EMBO Mol. Med. 4 (2012) 633-646.

[8] A. D. Hafstad, A. A. Nabeebaccus, A. M. Shah, Novel aspects of ros signalling in heart failure, Basic Res. Cardiol. 108 (2013) 359.

[9] S. Dey, D. DeMazumder, A. Sidor, D. B. Foster, B. O'Rourke, Mitochondrial ros drive sudden cardiac death and chronic proteome remodeling in heart failure, Circ. Res.123 (2018) 356-371.

[10] B. Wei, Q. Lin, Y.-G. Ji, Y.-C. Zhao, L.-N. Ding, W.-J. Zhou, L.-H. Zhang, C.-Y.

Gao, W. Zhao, Luteolin ameliorates rat myocardial ischemia-reperfusion injury through peroxiredoxin II activation, Brit. J. Pharmacol. 175 (2018) 3315-3332.

[11] Q. Lin, X.-Y. Chen, J. Zhang, Y.-L. Yuan, W. Zhao, B. Wei, Upregulation of Sirt1 contributes to the cardioprotective effect of rutin against myocardial ischemia-reperfusion injury in rats, J. Funct. Foods 46 (2018) 227-236.

[12] B. Wei, W.-W. Li, J. Ji, Q.-H. Hu, H. Ji, The cardioprotective effect of sodium tanshinone IIA sulfonate and the optimizing of therapeutic time window in myocardial ischemia/reperfusion injury in rats, Atherosclerosis 235 (2014) 318-327.

[13] C.-Y. Lee, E.-H. Chew, M.-L. Go, Functionalized aurones as inducers of NAD(P)H:Quinone oxidoreductase 1 that activate AhR/XRE and Nrf2/are signaling pathways: synthesis, evaluation and SAR, Eur. J. Med. Chem. 45 (2010) 2957-2971.

[14] Z.-S. Tu, Q. Wang, D.-D. Sun, F. Dai, B. Zhou, Design, synthesis, and evaluation of curcumin derivatives as Nrf2 activators and cytoprotectors against oxidative death, Eur. J. Med. Chem. 134 (2017) 72-85.

[15] I. Buendia, P. Michalska, E. Navarro, I. Gameiro, J. Egea, R. León, Nrf2-ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases, Pharmacol. Therapeut. 157 (2016) 84-104.

[16] E. Elhalem, R. Recio, S. Werner, F. Lieder, I. Fernández, N. Khiar, Sulforaphane homologues: Enantiodivergent synthesis of both enantiomers, activation of the Nrf2 transcription factor and selective cytotoxic activity, Eur. J. Med. Chem. 87 (2014) 552-563.

[17] J. Vrba, R. Gažák, M. Kuzma, B. Papoušková, J. Vacek, M. Weiszenstein, V. Křen, J. Ulrichová, A novel semisynthetic flavonoid 7-o-galloyltaxifolin upregulates heme oxygenase-1 in RAW264.7 cells via MAPK/Nrf2 pathway, J. Med. Chem. 56 (2013) 856-866.

[18] B. Wei, M.-G. You, J.-J. Ling, L.-L. Wei, K. Wang, W.-W. Li, T. Chen, Q.-M. Du, H. Ji, Regulation of antioxidant system, lipids and fatty acid β-oxidation contributes to the cardioprotective effect of sodium tanshinone IIA sulphonate in isoproterenol-induced myocardial infarction in rats, Atherosclerosis 230 (2013)

148-156.

[19] S. Yang, G. Chou, Q. Li, Cardioprotective role of azafrin in against myocardial injury in rats via activation of the Nrf2-ARE pathway, Phytomedicine 47 (2018) 12-22.

[20] L. Huang, J. Wang, L. Chen, M. Zhu, S. Wu, S. Chu, Y. Zheng, Z. Fan, J. Zhang, W. Li, Design, synthesis, and evaluation of ndga analogues as potential anti-ischemic stroke agents, Eur. J. Med. Chem. 143 (2018) 1165-1173.

[21] V. Kumar, S. Kumar, M. Hassan, H. Wu, R. K. Thimmulappa, A. Kumar, S. K. Sharma, V. S. Parmar, S. Biswal, S. V. Malhotra, Novel chalcone derivatives as potent Nrf2 activators in mice and human lung epithelial cells, J. Med. Chem. 54 (2011) 4147-4159.

[22] 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, Discovery of vinyl sulfones as a novel class of neuroprotective agents toward Parkinson's disease therapy, J. Med. Chem. 57 (2014) 1473-1487.

[23] J. A. Lee, J. H. Kim, S. Y. Woo, H. J. Son, S. H. Han, B. K. Jang, J. W. Choi, D. J. Kim, K. D. Park, O. Hwang, A novel compound VSC2 has anti-inflammatory and antioxidant properties in microglia and in Parkinson's disease animal model, Brit. J. Pharmacol. 172 (2015) 1087-1100.

[24] M. Hönig, L. Plíhalová, L. Spíchal, J. Grúz, A. Kadlecová, J. Voller, A. R. Svobodová, J. Vostálová, J. Ulrichová, K. Doležal, M. Strnad, New cytokinin derivatives possess uva and uvb photoprotective effect on human skin cells and prevent oxidative stress, Eur. J. Med. Chem. 150 (2018) 946-957.

[25] M. Loubidi, J. Jouha, Z. Tber, M. Khouili, F. Suzenet, M. Akssira, M. A. Erdogan, F. A. Köse, T. Dagcı, G. Armagan, L. Saso, G. Guillaumet, Efficient synthesis and first regioselective C-6 direct arylation of imidazo[2,1-c][1,2,4]triazine scaffold and their evaluation in H_2O_2 -induced oxidative stress, Eur. J. Med. Chem. 145 (2017) 113-123.

[26] H. Amawi, K. Chandrabose, R. Pathak, N. Hussein, R. Christmann, R. Robey, C.

546 R. Ashby, P. Trivedi, A. Malhotra, A. K. Tiwari, Thienopyrimidine derivatives exert
547 their anticancer efficacy via apoptosis induction, oxidative stress and mitotic
548 catastrophe, Eur. J. Med. Chem. 138 (2017) 1053-1065.
549 [27] C. A. Kontogiorgis, D. J. Hadjipavlou-Litina, Synthesis and antiinflammatory
550 activity of coumarin derivatives, J. Med. Chem. 48 (2017) 6400-6408.
551 [28] B. Aneja, N. S. Khan, P. Khan, A. Queen, A. Hussain, Md. T. Rehman, M. F.
552 Alajmi, H. R. El-Seedi, S. Ali, Md. I. Hassan, M. Abid, Design and development of
553 isatin-triazole hydrazones as potential inhibitors of microtubule affinity-regulating
554 kinase 4 for the therapeutic management of cell proliferation and metastasis, Eur. J.
555 Med. Chem. 163 (2019) 840-852.
556 [29] Y. Jia, L. Si, R. Lin, H. Jin, W. Jian, Q. Yu , S. Yang, Thiophenol-formaldehyde
557 triazole causes apoptosis induction in ovary cancer cells and prevents tumor growth
558 formation in mice model, Eur. J. Med. Chem. 172 (2019) 62-70.
559 [30] J. W. Choi, S. Kim, J.-H. Park, H. J. Kim, S. J. Shin, J. W. Kim, S. Y. Woo, C.
560 Lee, S. M. Han, J. Lee, A. N. Pae, G. Han, K. D. Park, Optimization of vinyl sulfone
561 derivatives as potent nuclear factor erythroid 2-related factor 2 (Nrf2) activators for
562 Parkinson's disease therapy, J. Med. Chem. 62 (2019) 811-830.
563 [31] L. Jalili-Baleh, H. Forootanfar, T. T. Küçükkılınç, H. Nadri, Z. Abdolahi, A.
564 Ameri, M. Jafari, B. Ayazgok, M. Baeeri, M. Rahimifard, M. Khoobi, A. Foroumadi,
565 Design, synthesis and evaluation of novel multi-target-directed ligands for treatment
566 of Alzheimer's disease based on coumarin and lipoic acid scaffolds, Eur. J. Med.
567 Chem. 152 (2018) 600-614.
568 [32] H.-L. Yang, P. Cai, Q.-H. Liu, X.-L. Yang, F. Li, J. Wang, J.-J. Wu, X.-B. Wang,
569 L.-Y. Kong, Design, synthesis and evaluation of coumarin-pargyline hybrids as novel
570 dual inhibitors of monoamine oxidases and amyloid-β aggregation for the treatment of
571 Alzheimer's disease, Eur. J. Med. Chem. 138 (2017) 715-728.
572 [33] B.-S. Yun, I.-K. Lee, I.-J. Ryoo, I.-D. Yoo, Coumarins with monoamine oxidase
573 inhibitory activity and antioxidative coumarino-lignans from hibiscus syriacus, J. Nat.
574 Prod. 64 (2001) 1238-1240.

575 [34] S. Y. Kang, Y. C. Kim, Neuroprotective coumarins from the root ofangelica
576 gigas: Structure-activity relationships, Arch. Pharm. Res. 30 (2007) 1368-1373.
577 [35] M. Sun, J. Hu, X. Song, D. Wu, L. Kong, Y. Sun, D. Wang, Y. Wang, N. Chen, G.
578 Liu, Coumarin derivatives protect against ischemic brain injury in rats, Eur. J. Med.
579 Chem. 67 (2013) 39-53.
580 [36] Č. Milan, P. Valentina, M. Maja, Š. Bojan, H.-S. Elizabeta, Design and synthesis
581 of some new 1,3,4-thiadiazines with coumarin moieties and their antioxidative and
582 antifungal activity, Molecules 19 (2014) 1163-1177.
583 [37] S. Emami, S. Dadashpour, Current developments of coumarin-based anti-cancer
584 agents in medicinal chemistry, Eur. J. Med. Chem. 102 (2015) 611-630.
585 [38] G. Martelli, D. Giacomini, Antibacterial and antioxidant activities for natural and
586 synthetic dual active compounds, Eur. J. Med. Chem. 158 (2018) 91-105.
587 [39] V. T. Angelova, N. G. Vassilev, B. Nikolova-Mladenova, J. Vitas, R. Malbaša, G.
588 Momekov, M. Djukic, L. Saso, Antiproliferative and antioxidative effects of novel
589 hydrazone derivatives bearing coumarin and chromene moiety, Med. Chem. Res. 25
590 (2016) 2082-2092.
591 [40] A. M. Pisoschi, A. Pop, The role of antioxidants in the chemistry of oxidative
592 stress: A review, Eur. J. Med. Chem. 97 (2015) 55-74.
593 [41] F. Pérez-Cruz, S. Vazquez-Rodriguez, M. J. Matos, A. Herrera-Morales, F. A.
594 Villamena, A. Das, B. Gopalakrishnan, C. Olea-Azar, L. Santana, E. Uriarte,
595 Synthesis and electrochemical and biological studies of novel coumarin–chalcone
596 hybrid compounds, J. Med. Chem. 56 (2013) 6136-6145.
597 [42] A. Beillerot, J.-C. R. Domínguez, G. Kirsch, D. Bagrel, Synthesis and protective
598 effects of coumarin derivatives against oxidative stress induced by doxorubicin,
599 Bioorg. Med. Chem. Lett. 18 (2008) 1102-1105.
600 [43] G.-L. Xi, Z.-Q. Liu, Antioxidant effectiveness generated by one or two phenolic
601 hydroxyl groups in coumarin-substituted dihydropyrazoles, Eur. J. Med. Chem. 68
602 (2013) 385-393.
603 [44] J. Zhou, H. Zhang, X.-L. Chen, Y.-L. Qu, Q. Zhu, C.-G. Feng, Y.-J. Chen, Regio-

and diastereoselective access to 4-imidazolidinones *via* an aza-Mannich initiated cyclization of sulfamate-derived cyclic imines with α-halo hydroxamates. J. Org. Chem. 84 (2019) 9179-9187.

[45] Y. Ihara, Y. Urata, S. Goto, T. Kondo, Role of calreticulin in the sensitivity of myocardiac H9c2 cells to oxidative stress caused by hydrogen peroxide, Am. J. Physiol.-Cell. Ph. 290 (2006) 208-221.

[46] M. P. Calatayud, C. Riggio, V. Raffa, B. Sanz, T. E. Torres, M. R. Ibarra, C. Hoskins, A. Cuschieri, L. Wang, J. Pinkernelle, Neuronal cells loaded with PEI-coated Fe_3O_4 nanoparticles for magnetically guided nerve regeneration, J. Mater. Chem. B 1 (2013) 3607-3616.

[47] D. J. Hadjipavlou-Litina, K. E. Litinas, C. Kontogiorgis, The anti-inflammatory effect of coumarin and its derivatives, Anti-Inf. Agent. Med. Chem. 6 (2007) 293-306.

[48] I. Najmanová, M. Doseděl, R. Hrdina, P. Anzenbacher, T. Filipský, M. Říha, P. Mladěnka, Cardiovascular effects of coumarins besides their antioxidant activity, Curr. Top. Med. Chem. 15 (2015) 830-849.

[49] E. Shayganni, M. Bahmani, S. Asgary, M,. Rafieian-Kopaei, Inflammaging and cardiovascular disease: management by medicinal plants, Phytomedicine 23 (2015) 1119-1126.

[50] K. Malekmohammad, R. D. E. Robert, M. Rafieian-Kopaei, Antioxidants and atherosclerosis: mechanistic aspects, Biomole. 9 (2019) 301-319.

[51] J. Li, T. Ichikawa, J. S. Janicki, T. Cui, Targeting the Nrf2 pathway against cardiovascular disease, Expert Opin. Ther. Targets 13 (2009) 785-794.

[52] W. K. Jones, M. Brown, M. Wilhide, S. He, X. Ren, NF-κB in cardiovascular disease: diverse and specific effects of a "general" transcription factor? Cardiovasc. Toxicol. 5 (2005) 183-202.

[53] V. Prasad, J. N. Lorenz, V. M. Lasko, M. L. Nieman, W. Huang, Y. Wang, D. W. Wieczorek, G. E. Shull, SERCA2 haploinsufficiency in a mouse model of darier disease causes a selective predisposition to heart failure, Biomed. Res. Int, 2015 (2014) 251598.

633 [54] W. E. Louch, M. Aronsen, I. Sjaastad, H. K. Mørk, K. B. Andersson, K. Hougen,
634 G. Christensen, O. M. Sejersted, Progressive slowing of calcium transient decline and
635 development of diastolic heart failure in SERCA2 knockout mice, J. Mol. Cell.
636 Cardiol. 44 (2008) 776.
637 [55] R. J. Hajjar, K. Zsebo, L. Deckelbaum, C. Thompson, J. Rudy, A. Yaroshinsky,
638 H. Ly, Y. Kawase, K. Wagner, K. Borow, B. Jaski, B. London, B. Greenberg, D. F.
639 Pauly, R. Patten, R. Starling, D. Mancini, M. Jessup, Design of a phase 1/2 trial of
640 intracoronary administration of AAV1/SERCA2a in patients with heart failure, J.
641 Card. Fail. 14 (2008) 355-367.
642 [56] T. J. Samuel, R. P. Rosenberry, S. Lee, Z. Pan, Correcting calcium dysregulation
643 in chronic heart failure using SERCA2α gene therapy, Int. J. Mol. Sci. 19 (2018)
644 1086-1099.
645 [57] W. Li, M. Wu, L. Tang, Y. Pan, Z. Liu, C. Zeng, J. Wang, T. Wei, G. Liang, Novel
646 curcumin analogue 14p protects against myocardial ischemia reperfusion injury
647 through Nrf2-activating anti-oxidative activity, Toxicol. Appl. Pharm. 282 (2015)
648 175-183.
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Highlights

- Sixteen coumarin-derived imino sulfonates were synthesized and characterized.
- Most compounds displayed potent cardioprotective activities with low cytotoxicity.
- **5h** attenuated the oxidative stress via promoting Nrf2 pathway in cardiomyocytes.