Asymmetric synthesis and biological evaluation of Danshensu derivatives as anti-myocardial ischemia drug candidates

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The synthesis and bioactivities of Danshensu derivatives (R)-methyl 2-acetoxy-3-(3,4-diacetoxyphenyl)propanoate (1a), (R)-methyl 2-acetoxy-3-(3,4-methylenedioxyphenyl)propanoate (1b) and their racemates 7 and 10 were reported in this paper. These derivatives were designed to improve their chemical stability and liposolubility by protecting Danshensu’s phenolic hydroxyl groups with acetyl or methylene which could be readily hydrolyzed to release bioactive Danshensu. The asymmetric synthesis of 1a and 1b were achieved by catalytic hydrogenation of (Z)-methyl 2-acetoxy-3-(3,4-diacetoxyphenyl)-2-propenoate (6a) and (Z)-methyl 2-acetoxy-3-(3,4-methylenedioxyphenyl)-2-propenoate (6b) in excellent enantiomeric excesses (92% ee and 98% ee, respectively) and good yields (>89%). An unexpected intermediate product, (Z)-2-acetoxy-3-(3,4-dihydroxyphenyl)acrylic acid (4c) was obtained with high chemoselectivity in 86% yield by keeping the reaction temperature at 60 °C and its structure was identified by X-ray single crystal diffraction analysis. 1a, 1b and their racemates 7, 10 as well as 4c exhibited potent protective activities against hypoxia-induced cellular damage. The in vitro test showed that all these compounds could increase cell viability, and inhibit lipid peroxidation. Furthermore, 1a and 4c could inhibit apoptosis by regulating the expression of apoptosis-related molecule in gene and protein levels, up-regulating the expression of bcl-2 and down-regulating bax and caspase-3. The in vivo test indicated that 4c exhibited anti-myocardial ischemic effects featured by reducing infarction size and increasing the level of the intracellular enzymes detectable in serum. Therefore, these Danshensu derivatives may be good drug candidates for anti-myocardial ischemia therapy and merit further investigation.

1. Introduction

Danshensu is an active component of Danshen, the dried root of salvia miltiorrhiza mostly responsible for many biological activities, such as dilating coronary arteries, inhibiting platelet aggregation, myocardial cell apoptosis and anti-inflammatory property.1-3 These activities are at least partially related to its anti-oxidative activity.4 Accordingly, Danshensu itself and its scaffold are privileged and of great interest as synthetic targets and building blocks for cardiovascular active drug candidates.

Isolation and purification of Danshensu from natural resources are difficult and time consuming due to the chemical unstability of phenolic hydroxyl groups, low content in Danshen and a variety of structural analogs as impurities. Several synthetic strategies have been explored to produce (±)-Danshensu,5,6 but asymmetric synthesis of Danshensu is extremely rare. Findrik developed a mathematical model for the enzymatic kinetics of the synthesis of (R)-(±)-3,4-dihydroxyphenylacetic acid, which was catalyzed by D-lactate dehydrogenase from Lactobacillus leichmannii.7 The preparation of Danshensu using a chemical method based on catalytic asymmetric hydrogenation has never been reported. Moreover, being a hydrophilic molecule and thus poorly soluble in lipidic matrices, Danshensu uneasily enters into cells by crossing the cell membranes. Therefore, it is highly desirable to search for new Danshensu derivatives with potent pharmacological activity, good stability and liposolubility.

Danshensu has the structure of phenyllactic acid bearing two phenolic hydroxyl groups on its phenyl ring which are considered as the radical scavenging moieties. The presence of different substituents in the phenol backbone structure may modulate their antioxidant property. In general, monophenol is a less efficient...
antioxidant than polyphenol. The introduction of electron donating groups such as hydroxyl in the ortho or para position increases the antioxidant activity of phenolic acid.\textsuperscript{8,9} In addition, the presence of a carbonyl group, such as aromatic acid, ester, or lactone enhanced antioxidant activity. Steric hindrance of the phenolic hydroxyls by a neighboring inert group, such as methoxyl group, enhanced its antioxidant activity.\textsuperscript{10} According to such structure-activity relationship, Danshensu derivatives (1a, 1b) were designed with the phenolic hydroxyls esterized by acetylation or protected by methylen. Such structures could enhance the stability and liposolubility, and is beneficial to the chiral catalysts used in the asymmetric reduction step. These structural modification of Danshensu were expected to exhibit similar pharmacological activity with Danshensu due to the ester and ether linkages are easily hydrolyzed.

Moreover, it is also attractive to study the pharmacological difference between the enantiomers and racemates. Herein, we report the efficient asymmetric synthesis of 1a, 1b and an unexpected intermediate product 4c and their preliminary pharmacological assay results.

2. Chemistry

The designed compounds were synthesized efficiently according to the procedures outlined in Schemes 1 and 2. 3,4-Dihydroxybenzaldehyde (2a) and its analog piperonal (2b) were chosen as the starting material, and 3a and 3b were easily accessible by the condensation of 2a and 2b with N-acetylglycine followed by hydrolysis in hydrochloric acid to give the key intermediates 4a and 4b, respectively.\textsuperscript{16,17}

To prepare the enantiomers of Danshensu derivatives, two approaches were studied. In the first strategy, compound 4b was subjected to hydrogenation in the presence of catalytic amount of Pd–C and cinchonidine as the chiral ligand, but the enantiomeric excess of the product was very low (16% ee).\textsuperscript{18} Recently the excellent performance of monophosphites, monophosphonites, and monophosphoramidites as ligands provide tremendous interest and potential in asymmetric hydrogenation.\textsuperscript{19} Thus, to improve the enantiomeric excess of product, in the second approach, a new generation of the monodentate phosphoramidites, compound 11 developed by Ding and co-workers\textsuperscript{19} was employed as the chiral ligand and [Rh(cod)\textsubscript{2}]BF\textsubscript{4} as the catalyst precursor in asymmetric hydrogenation. First, compounds 4a and 4b were protected at the OH-groups by esterization with acetic anhydride in the presence of sodium acetate to provide the corresponding products 5a and 5b in 80% and 77% yields, respectively. Followed by the reaction with diazomethane in ice-cooled dichloromethane, the thoroughly protected compounds 6a and 6b were obtained in 81% and 85% yields, respectively. Using 11 as chiral ligand, under the modified conditions of the literature procedures,\textsuperscript{19} excellent enantioselectivities (92–98% ee) and good yields (>89%) of target compounds 1a and 1b were realized. Racemic compounds 7 and 10 were also synthesized. First, compound 8 was obtained by two methods of reduction of 4b with KBH\textsubscript{4} and catalytic hydrogenation. The condensation of compound 8 with acetic anhydride in the presence of sodium acetate gave compound 9 in 95% yield. Compound 10 was attained by reacting 9 with diazomethane in ice-cooled dichloromethane in 79% yield. Using Pd/C as catalyst, racemic compound 7 was prepared by catalytic hydrogenation of compound 6a in 79% yield.

![Scheme 1](image)

**Scheme 1.** The synthetic route of Danshensu derivatives 1a, 4c and 7. Reaction conditions: (i) N-acetylglucine, acetic anhydride, NaOAc, 100 °C, 2 h, pour into cold water; (ii) 4a: 9% HCl, 100 °C, 6 h; 4c: 9% HCl, 60 °C, 3 h; (iii) acetic anhydride, NaOAc, rt, 5.5 h; (iv) CH\textsubscript{2}N\textsubscript{2}, dichloromethane, 0 °C; (v) [Rh(cod)\textsubscript{2}]BF\textsubscript{4}, chiral ligand 11, H\textsubscript{2}, 60 atm, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h; (vi) Pd–C, EtOAc, 10 atm, 24 h.
yield. Interestingly, the hydrolyzation of 3a was found to lead to different products at different temperatures. 4a was got at 100 °C for 6 h and at a lower temperature of 60 °C for 3 h compound 4c was obtained in 86% yield. However, we were unable to identify the position of acetyl group simply on the basis of its 1H NMR, 13C NMR, and MS spectra data. The single crystals of 4c were obtained by recrystallization from water and analyzed by X-ray single crystal diffraction (Fig. 1). Unexpectedly, 2-acetamido in 3a was converted to 2-acetoxy and 3,4,0-diacetoxy groups hydrolyzed to 3,4,0-dihydroxyls to form 4c. This result can be rationalized as follows: 2-acetamido and 3,4,0-diacetoxy groups was easily hydrolyzed to hydroxyls at low temperature, however acetyl recombined to 2-dihydroxyl to get 2-acetoxy, and such bonding was so tight that could be hydrolyzed only at high temperature and lasting time.

3. Pharmacological assay results

3.1. Protective effects of compounds 1a, 1b, 7, 10, 4c on hypoxia-induced neonatal rat ventricular myocytes (NRVMs)

The protective effects of compounds 1a, 1b, 7, 10 and 4c were investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and LDH leakage assay. Cells were incubated with indicated concentrations of compounds 1a, 1b, 7, 10 and 4c for 12 h before exposure to hypoxia, then cells were subjected to hypoxia for 5 h. Cell viability was assessed by MTT reduction assay. It showed that compounds 1a, 1b, 7, 10 and 4c at 1 μmol/L significantly increased cell viability compared with hypoxia group (Fig. 2). The marker of cell damage LDH leakage was assessed after hypoxia and it was found that compounds 1a, 1b,
7, 10 and 4c at 1 μmol/L could decrease LDH leakage (Fig. 3). At the same concentration of 1 μmol/L, compounds 1a and 1b possessed higher activity than racemic compounds 7 and 10 in decreasing cellular damage. Therefore, the antioxidant property of compounds 1a, 1b and 4c was further evaluated.

### 3.2. Antioxidant activity of compounds 1a, 1b and 4c in hypoxia-induced neonatal rat ventricular myocytes

To determine the antioxidant activity of compounds 1a, 1b and 4c, malondialdehyde (MDA), a marker of oxidant-mediated lipid peroxidation in cells was quantified after hypoxia. As illustrated in Figure 4, when compounds 1a, 1b and 4c were served at 1 μmol/L, MDA content was decreased compared with the hypoxia group (P < 0.05), indicating that compounds 1a, 1b and 4c could inhibit the lipid peroxidation of cell membrane.

### 3.3. Anti-apoptotic activity of compounds 1a and 4c in hypoxia-induced neonatal rat ventricular myocytes

Apoptosis is a programmed cell death that is characterized by specific structural changes that include cell shrinkage, nuclear condensation and DNA fragmentation. To assess the anti-apoptotic activity of 1a and 4c, Hoechst staining was performed to observe the morphological changes in hypoxia-induced neonatal rat ventricular myocytes. Administration of compounds 1a and 4c resulted in less nuclei-shrunk and nuclear condensation compared with the hypoxia group (Fig. 5).

At the molecular level, apoptosis is activated by the aspartate-specific cysteine protease cascade, including caspase-3 and caspase-12. Caspase-3 is considered to be the most important executioner caspases and is activated by any of the initiator caspases. Besides caspases, members of the bcl-2 family are also critical for the regulation of apoptosis. The relative concentration of these proteins in the outer mitochondrial membrane is thought to determine the survival or death of a cell following an apoptotic stimulus.

To further elucidate the mechanism of anti-apoptotic activity of 1a and 4c, their effects on modulating the expression of various apoptosis-related biomarkers, including caspase-3, bax and bcl-2 were investigated. RT-PCR assay showed that the level of bax and capase-3 mRNA in hypoxia-induced neonatal rat ventricular myocytes declined significantly after treatment by compounds 1a and 4c, whereas the level of bcl-2 mRNA markedly increased (Fig. 6). Western blot showed consistent results with RT-PCR in protein level (Fig. 7).

### 3.4. In vivo protective effects of 4c on acute myocardial ischemia in adult rat

Considering the readily synthesis and potent pharmacological activity in vitro of 4c, its cardio-protective effects in rats of acute
myocardial infarction were further evaluated. Rats were treated with 4c at different concentrations (15, 30 and 60 mg/kg). Two days after myocardial infarction surgery, the hearts were analyzed by TTC staining to quantify infarct size. As shown in Figure 8, the infarct areas in compound 4c-treated rats were smaller than in the model rats, and only the difference between the rats treated with 4c at 60 mg/kg and model rats was of statistic significance. The level of clinical markers of cardiac infarction in serum was also measured. As expected, the levels of serum lactate dehydrogenase (LDH), creatine kinase (CK) in model rats were dramatically higher compared with those in the sham-operated rats, which indicated that myocardial infarction was successfully established. Compared with model rats, the levels of serum LDH, CK and MDA in rats treated with 4c were significantly reduced, meanwhile the activity of SOD in serum was increased in 4c treated rats (Fig. 9), which was consistent with the results of infarction area, indicating that 4c treatment apparently promoted cell integrity and reduced myocardial damage.
4. Discussion

Danshensu, the only component of Danshen that has been studied in human subjects, exhibited well-known pharmacological activity. However, it is hard to obtain either from natural Danshen due to the instability of phenolic hydroxyl or from chemical synthesis due to its chiral structure. In the present study, several novel Danshensu derivatives were designed and synthesized asymmetrically. The hydroxyl groups in these Danshensu derivatives were protected by acetyl or methylene, which not only increased their stability and liposolubility but also was beneficial to asymmetric synthesis and pharmacological assay. These structural modifications of Danshensu were expected to exhibit similar pharmacological activity with Danshensu due to both the ester and ether linkages are easily hydrolyzed and readily release bioactive Danshensu. Using a chemical approach based on asymmetric hydrogenation, (R)-Danshensu derivatives 1a and 1b as well as their racemates 7 and 10 were successfully obtained in excellent ee and high yields.

Compounds 1a, 1b, 7, 10 and 4c significantly increased cell viability and reduced LDH leakage, which signified that these compounds possessed potent protective effects against hypoxia-induced cellular damage. At the same concentration of 1 μmol/L, the LDH leakage of (R)-1a treated cells was less than that of cells treated by 7, indicating that the bioactivity may be related to the configuration of the chiral carbon and R-configuration was more favorable. However, the difference of LDH leakage between (R)-1b and 10 treated cells was not significant, suggesting that different substitutions of phenolic hydroxyl groups influence on the metabolism in vivo and further impact the pharmacological activity.

Compounds 1a, 1b and 4c possessed potent antioxidant activity featured by decreased MDA content, which may be related to the hydrolysis to expose phenolic hydroxyl groups due to the cleavage of the labile ester bond. However, there may be another way for these compounds to act as antioxidant agents, for example increasing the activity of intracellular antioxidant enzyme. Therefore, the mechanism of antioxidant activity of 1a, 1b and 4c merits further investigations.

Using Hoechst staining, compounds 1a and 4c showed potent anti-apoptotic activity. From our results, the mechanism by which 1a and 4c inhibit cell apoptosis was possibly through suppressing apoptotic signaling. Systemic levels of anti-apoptotic protein bcl-2 were significantly higher in cells treated with compounds 1a and 4c, pro-apoptotic protein bax and apoptosis executive protein caspase-3 were lower in 1a and 4c treated group compared with hypoxia group. In addition, the anti-apoptosis activity of compounds 1a and 4c may be partially related to their antioxidant property.

The results in vivo showed that compound 4c could protect cardiac myocytes from myocardial infarction injury by reducing infarct area and decreasing the level of the intracellular enzymes detectable in serum. Considering readily synthesis and purification as well as potent pharmacological activity, compound 4c was promising for further therapeutic applications.

5. Conclusion

Overall, we successfully synthesized new derivatives of Danshensu, (R)-1a, (R)-1b, (±)-7, (±)-10 and 4c, which are more stable and liposoluble than Danshensu. Their structures were confirmed through NMR, MS and X-ray single crystal diffraction analysis. Pharmacological evaluation showed that these Danshensu derivatives possessed potent cardio-protective activities that occur through blocking oxidative stress and apoptotic pathways. Further structural modification, biological screening and the mechanism study are in progress as these promising pharmacological results demonstrate that these Danshensu derivatives merit attention as potential anti-myocardial ischemia drugs.

6. Experimental

Starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points were determined in open capillary tubes and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Brucker-DPX 400 MHz spectrometer, Mass spectral data was collected on a HP5973 N analytical mass spectrometer. HRMS data were determined on an IonSpec 4.7 Tesla FTMS instrument. X-ray single crystal diffraction was carried out on a Bruker Smart CCD diffractometer.

Figure 9. Effects of compound 4c at concentrations of 15, 30, 60 mg/kg on the levels of CK (A), LDH (B), MDA (C) and SOD (D) in serum. Values are expressed as means ± SE from six individual examples. *P < 0.05 versus model.
6.3. (Z)-2-Acetamido-3-(3,4-diacytlenylphenyl)acrylic acid (3a)

3,4-Dihydroxybenzaldehyde (2a) (10.8 g, 0.078 mol), N-acetyl-
glycine (9.2 g, 0.0789 mol), sodium acetate (6.4 g, 0.078 mol) and
acetic anhydride (30 mL, 32.4 g, 0.319 mol) were mixed together
and stirred at 140 °C for 2 h. After cooling, the dark-coloured
viscous solution was poured into cold water (400 mL). The sticky
yellow gum which separated hardened on keeping overnight.
It was collected and crystallized from EtOAc to give 3a as a yellow
solid (12.1 g, 48%): mp 183–185 °C (lit.18: 183.4 °C). 1H NMR
(400 MHz, CDCl3) δ 2.08 (s, 3H, CH3), 3.37 (m, 2H, 3-H), 3.78 (s,
1H, 4-H), 7.14 (m, 5H, 5'-H, 6'-H), 8.01 (s, 1H, 2-NHCO2H).

6.2. (Z)-2-Acetamido-3-(3,4-methyleneoxyphenyl)acrylic acid (3b)

3-Methylenedioxybenzylaldehyde (2b) (5.1 g, 0.034 mol), N-
acetylglucine (5.20 g, 0.044 mol), sodium acetate (4.0 g, 0.049 mol)
and acetic anhydride (20 mL, 21.6 g, 0.213 mol) were mixed to-
gether and stirred at 100 °C for 3 h. After completion of the reac-
tion, the mixture was allowed to cool to rt. Then water (100 mL)
was added, and stirred at 120 °C for 0.5 h. After cooling, the mix-
ture was filtered and the filtrate was extracted with EtOAc. The or-
ganic extract was evaporated and the product was dried under
vacuum (0.8 g, 55%): mp 215–216 °C (lit.18: 216–216.5 °C).

6.4. (Z)-3-(3,4-Dihydroxyphenyl)-2-hydroxyacrylic acid (4a)

A mixture of 3a (1.0 g, 0.016 mol) in 9% HCl (40 mL) was re-
fluxed at 100 °C for 6 h. Then the resulting mixture was allowed
to cool to rt and filtered. The filtrate was extracted with EtOAc.
The organic extract was evaporated. The crude product was
dried under vacuum and washed with water to get 4a as a slightly yellow
solid (0.53 g, 87%): mp 179–181 °C (lit.17: 181 °C). 1H NMR
(400 MHz, CDCl3) δ 4.5 (br, 3H, OH), 6.44 (s, 1H, 2-H), 6.81 (d,
1H, J = 8.2 Hz, 5'-H), 7.12 (d, 1H, J = 8.2 Hz, 6'-H), 7.50 (s, 1H,
3-H), 7.61 (br, 1H, COOH).

6.5. 3-(3-Methyleneoxyphenyl)-2-hydroxypropanoic acid (8)

A mixture of 4b (1.0 g, 4.8 mmol), Pd–C (0.21 g) and anhydrous
MeOH (10 mL) was hydrogenated at 6 atm for 24 h. The mixture
was filtered and concentrated, the residue was purified by silica
gel chromatography, providing 8 as white solid (0.44 g, 43%): mp
95–96 °C (lit.17: 95–97 °C). 1H NMR (400 MHz, acetone-d6) δ 2.77–
3.01 (m, 2H, 3-H), 4.28–4.31 (m, 1H, 2-H), 5.90 (s, 2H, OCH2O),
6.69 (s, 2H, 5'-H, 6'-H), 6.78 (s, 1H, 2-H).

A mixture of 4b (2 g, 9.6 mmol) and 25% methanol (40 mL) was
adjusted pH to 8 by sodium hydroxide then was added KBr (1.5 g,
27.8 mmol). The mixture was stirred at room temperature for 7 h
and adjusted pH to 2 by 1 N HCl and then extracted with EtOAc
(3 × 30 mL). The organic extract was concentrated and purified
by silica gel chromatography providing 8 as white solid (1.41 g,
70%).

6.6. (Z)-2-Acetoxy-3-(3,4-diacytlenylphenyl)acrylic acid (5a)

A mixture of 4a (0.9 g, 4.59 mmol) and sodium acetate (0.5 g,
6.1 mmol) in acetic anhydride (6 mL, 6.48 g, 0.064 mmol) was stirred
at rt for 5.5 h. After that, the solution was added water (15 mL),
and was extracted with EtOAc (3 × 30 mL). The combined organic layers
were dried over anhydrous MgSO4. Concentration of the dried
extracts yielded a residue which was purified by crystallization
from acetic acid to give 5a as yellow solid (1.2 g, 80%): mp 184–
186 °C (lit.19: 189–192 °C). 1H NMR (400 MHz, acetone-d6) δ
2.28–2.29 (m, 9H, 2-OCHO, 3'-OCHO, 4'-OCHO), 7.31 (d,
1H, J = 8.3 Hz, 5'-H), 7.34 (s, 1H, 3-H), 7.63 (d, 1H, J = 8.5 Hz,
6'-H), 7.64 (s, 1H, 2-H).

6.7. (Z)-2-Acetoxy-3-(3,4-methyleneoxyphenyl)acrylic acid (5b)

A mixture of 4b (1.0 g, 4.8 mmol) and sodium acetate (0.6 g,
7.3 mmol) in acetic anhydride (8 mL, 8.64 g) was stirred at rt for
5.5 h. The formed solid was filtered off, washed with water, air-
dried, and crystallized from EtOAc to give 5b as yellow solid
(0.93 g, 77%): mp 164–166 °C; IR (KBr, cm–1) 2960, 2853, 1760,
2522, 2516, 1691, 1639, 1500, 1444, 1429, 1265, 1245, 1199,
1116, 1039; 1H NMR (400 MHz, acetone-d6) δ 2.32 (s, 3H, 2-
OCHO), 6.08 (s, 2H, 3'-OCHO), 6.92 (d, 1H, J = 7.83 Hz, 3-H),
7.24–7.28 (m, 3H, 2'-H, 6'-H, 5'-H), MS (EI) m/z (%): 250 (M+,
6.44), 208 (39.85), 194 (9.29), 162 (100.00), 150 (6.67), 134
(39.25), 104(15.41), 76(27.58). HRMS calcd mass for C12H10O6
250.0477, found 250.0481.

6.8. 2-Acetoxy-3-(3,4-methyleneoxyphenyl)propanoic acid (9)

To a solution of 8 (0.4 g, 2.4 mmol) in acetic anhydride (2 mL,
2.16 mg, 0.021 mmol) was added acetic acid (1 mL) and the mix-
ture was stirred at rt for 24 h. After that, the solution was added water
(5 mL), and was extracted with EtOAc (3 × 10 mL). The com-
bined organic layers were dried over anhydrous MgSO4. Concentra-
tion of the dried extracts yielded a residue which was purified by
crystallization from acetic acid to give 9 as white solid (0.45 g,
95%): mp 151–153 °C; IR (KBr, cm–1) 2960, 2853, 2547, 1769,
1723, 1710, 1505, 1498, 1450, 1259, 1226, 1070, 1041; 1H NMR
(400 MHz, CDCl3) δ 2.09 (s, 3H, 2-OCHO), 3.00–3.15 (m, 2H, 3-
H), 5.15–5.18 (m, 1H, 2-H), 5.93 (s, 2H, 3'-OCHO), 6.59 (d,
1H, J = 8.24 Hz, 6'-H), 6.73 (s, 1H, 2'-H), 6.74 (d, 1H, J = 7.74 Hz,
5'-H), 7.86 (br, 1H, COOH). MS (EI) m/z (%): 252 (M+*, 0.32),
192 (100.00), 175 (6.00), 135 (99.68), 105(6.57), 77(20.23). HRMS calcd mass for C12H10O6
250.0634, found 250.0644.

6.9. General procedure for the synthesis of 6a, 6b and 10

To an ice-cooled solution of 5a or 5b or 9 (0.02 mol) in dry
chloromethane (20 mL) was added a solution of diazomethane
in ether dropwise with stirring. Drop adding was stopped until
the solution was clear, and the solution was stirred overnight.
After that, the mixture was concentrated and the residue was purified by
silica gel chromatography to give 6a, 6b, 10.
6.10. General procedure for the synthesis of 1a and 1b

[Rh(cod)][BF₄] (2.4 mg, 0.006 mmol), ligand 11 (7.7 mg, 0.0132 mmol) were dissolved in CH₂Cl₂ (1 mL) under nitrogen and the solution was stirred at room temperature for 10 min. The substrate (200 mg, 0.6 mmol) in CH₂Cl₂ (1.5 mL) was added to the above catalyst solution, the mixture was then transferred to a stainless steel autoclave under nitrogen atmosphere, and then sealed. After purging with hydrogen for three times, final H₂ pressure was adjusted to 60 atm. After stirring at room temperature for 24 h, H₂ was released, the mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography to give compounds 1a and 1b.

6.10.1. (R)-Methyl 2-acetoxy-3-(3,4-diacetoxyphenyl)propanoate (1a)

White solid, yield 89%, m.p 81–82 °C; IR (KBr, cm⁻¹) 2963, 1771, 1735, 1504, 1377, 1205, 1118; ¹H NMR (400 MHz, CDCl₃) δ 2.07 (3H, 2-OCOCH₃), 2.10 (s, 3H, 2-OCOCH₃), 2.97–3.10 (m, 2H, 3-H), 3.72 (s, 3H, COOCH₃), 5.17–5.20 (m, 1H, 2-H), 7.07 (s, 1H, 2-H), 7.12 (s, 2H, 5'-H, 6'-H). MS (EI) m/z (%): 264 (M⁺, 0.04), 222 (50.29), 162 (100.00), 134 (33.75), 134 (39.25), 104 (6.30), 76 (16.06). HRMS calcd. mass for C₁₁H₁₀O₆ 221.0442, found 221.0440.

6.10.2. (R)-Methyl 2-acetoxy-3-(3,4-methylenedioxyphenyl)propanoate (1b)

White solid, yield 85%, mp 84–85 °C; IR (KBr, cm⁻¹) 2963, 1771, 1754, 1736, 1503, 1377, 1205, 1118; ¹H NMR (400 MHz, CDCl₃) δ 2.09 (3H, 2-OCOCH₃), 2.10 (s, 3H, 2-OCOCH₃), 2.97–3.10 (m, 2H, 3-H), 3.72 (s, 3H, COOCH₃), 5.14–5.17 (m, 1H, 2-H), 5.94 (s, 2H, 3',4'-OCH₂O), 6.66 (d, 1H, J = 7.82 Hz, 5'-H), 7.06 (d, 1H, J = 8.22 Hz, 6'-H), 7.16 (s, 1H, 3-H), 7.23 (s, 1H, 2'-H). MS (EI) m/z (%): 264 (M⁺, 0.04), 222 (50.29), 162 (100.00), 134 (33.75), 134 (39.25), 104 (6.30), 76 (16.06). HRMS calcd. mass for C₁₁H₁₂O₆ 236.0849, found 236.0849.

6.11. Methyl 2-acetoxy-3-(3,4-diacetoxyphenyl)propanoate (7)

A mixture of 6a (0.2 g, 0.012 mol) in 9% HCl (50 mL) was heated at 60 °C for 3 h. The formed solid was filtered off, washed with water, air-dried, and crystallized from water to give 4c as a colorless slice (0.25 g, 88% yield). m.p 266–267 °C; IR (KBr, cm⁻¹) 3258, 3047, 1676, 1637, 1529, 1490, 1368, 1088; ¹H NMR (400 MHz, CDCl₃) δ 2.10 (3H, 2-OCOCH₃), 2.28 (s, 3H, 3-OCOCH₃), 2.95–3.08 (m, 2H, 3-H), 3.75 (s, 3H, COOCH₃), 6.66 (d, 1H, J = 7.82 Hz, 5'-H), 7.06 (d, 1H, J = 8.21 Hz, 6'-H), 7.30 (s, 1H, 3-H), 7.58 (s, 1H, 2-H). MS (EI) m/z (%): 236 (M⁺, 0.04), 222 (50.29), 162 (100.00), 134 (33.75), 134 (39.25), 104 (6.30), 76 (16.06). HRMS calcd. mass for C₁₁H₁₀O₆ 221.0442, found 221.0440.

6.12. (Z)-Acetoxy-3-(3,4-dihydroxyphenyl)acryl acid (4c)

A mixture of 3a (2 g, 0.012 mol) in 9% HCl (50 mL) was heated at 60 °C for 3 h. The formed solid was filtered off, washed with water, air-dried, and crystallized from water to give 4c as a colorless slice (1.27 g, 86%): mp 229–230 °C; IR (KBr, cm⁻¹) 3258, 3037, 1686, 1636, 1617, 1521, 1245, 1225, 1200, 1172, 1118; ¹H NMR (400 MHz, CD₂OD) δ 2.13 (s, 3H, 2-OCOCH₃), 6.77 (d, 1H, J = 8.21 Hz, 5'-H), 6.98 (d, 1H, J = 8.41 Hz, 2'-H), 7.16 (d, 1H, J = 1.95 Hz, 6'-H), 7.38 (s, 3H, 3-H). ¹³C NMR (100 MHz, CD₂OD) δ 22.58, 116.28, 117.55, 123.50, 124.89, 126.60, 137.09, 146.35, 148.81, 168.68, 173.46. MS (ESI) m/z (%): 238 (M⁺).
sured using a microplate reader at 570 nm. The viability of normal cell is presumed as 100%. Cell damage in cardiac myocytes was quantitatively assessed by the measurement of intracellular lactate dehydrogenase (LDH), it is presumed that in normal cell group there is no LDH leakage.

6.15. RNA isolation and RT-PCR

Total RNA was isolated from cardiac myocytes by using Trizol (Invitrogen, Carlbad, CA) as previously described. Reverse transcription(RT) was conducted by using PrimerScript™ 1st Strand cDNA Synthesis Kit according to the manufacturer's procedures. Primers used for amplification were synthesized as follows: bcl-2: forward 5'-CGGGAGAAACGGTGATAGA-3'; reverse 5'-CAGGGCTGAAGGA GAGAT-3'. Bax: forward 5'-CACGGAGATGGCTGGGAGA-3'; reverse 5'-TCCAGACAACGCCCCTCAG-3'. Caspase-3: forward 5'-CTGACTCCTATGGAGGAGA-3'; reverse 5'-GGTGAGGTAGAGTAAGAC-3'. GAPDH: forward 5'-TTCAAGGGAACAGTCAAGG-3'; reverse 5'-CGCGATGTGATCCACAA-3'. The PCR products were analyzed by electrophoresis in 1.5% agarose gels. The intensity of each band was photographed and quantified by using the Fluor Chem SP system (USA) as a ratio of a target gene over GAPDH.

6.16. Western blot

At the end of hypoxia, cardiac myocytes were scraped off in cell lysis buffer (Beyotime Biotechnology, Haimen, China) and centrifuged at 10,000 r/min for 10 min. The supernatant was collected, and protein concentrations were quantitated using the enhanced BCA Protein Assay Kit (Beyotime Biotechnology, Haimen, China). Thirty micrograms of total proteins were separated on SDS–PAGE gels as previously described. Separated proteins were transferred onto 0.45-μm polyvinylidene difluoride (PVDF) membrane (Millipore Corporation). PVDF membrane was blocked in Tris-buffered saline (TBS) containing 5% skim milk for 2 h at room temperature and probed with rabbit anti-rat bcl-2 polyclonal antibody (R&D, USA) diluted in TBS-T for 1 h at room temperature. Anti-rabbit IgG diluted in TBS-T was used as secondary antibody. Protein band intensities were quantified by using a Western blotting detection system (Alpha Innotech, USA).

6.17. Model of myocardial infarction

The method was used as described previously. Briefly, male Sprague-Dawley rats weighing 230–250 g, provided by the experimental animal center of fudan university were intubated and artificially ventilated with a rodent ventilator (DHX-150, China) under anesthesia with 7% choral hydrate (60 mg/kg ip). The normal electrocardiogram (II) was recorded after electrodes were placed subcutaneously and connected to an electrocardiograph. Then the chest was opened by left thoracotomy in the 3rd and 4th intercostal space and the pericardium was removed. The left anterior descending coronary artery was ligated with a 5–0 suture 1–2 mm below the left atrial appendage. Sham operated rats underwent an identical surgical procedure except that the left coronary artery was not ligation. Rats were randomly divided into five groups: MI group, sham-operated group, MI and drug-treated groups (15, 30, 60 mg/kg). The drug was injected intraperitoneally 7 days before surgery and 2 days after surgery once daily. Successful MI model was confirmed by pallor of the anterior wall of the left ventricle and ST-segment elevation.

6.18. Measurement of infarct size

The heart was excised immediately 2 days after the coronary artery was ligated and the entire ventricular tissue was sliced into five sections through the transverse axis from the apex to the atrio-ventricular groove and incubated in 1% triphenyltetrazolium chloride (TTC) at 37 °C for 15 min. Viable myocardium is stained in red by TTC, whereas infarction tissue remains unstained. Slices were imaged and the area of infarcted myocardium was defined using an image analysis software (Scion Image, CA, USA). The size of the infarction area was estimated by the volume as a percentage of the left ventricle.

6.19. Measurements of lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), malondialdehyde (MDA)

The levels of LDH, CK, SOD, MDA in serum were measured using diagnostic kit (NBJ, China) according to the manufacture's protocol.

6.20. Statistic analysis

Data were presented as means ± SE and analyzed by SPSS software. Pictures were processed with Photoshop software. Differences at P < 0.05 were considered statistically significant.

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References and notes

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