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Synthesis and *in vivo* screening of isosteviol derivatives as new cardioprotective agents

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ABSTRACT

Isosteviol, an ent-beyerane diterpenoid, has been repeatedly reported to possess potent cardioprotective activity. With the aim of discovering new cardioprotective derivatives from isosteviol, 47 compounds, including 40 new ones, were synthesized and evaluated *in vivo* using the easy-handling and efficient zebrafish model. The structure-activity relationship of this type of compounds was thus discussed. Of these compounds, new derivative **15d** exhibited the most pronounced efficacy *in vivo*. Our results indicated that **15d** could effectively prevent the doxorubicin-induced morphological distortions and cardiac dysfunction in zebrafish. Its cardioprotective activity is much better than that of isosteviol, and Levosimendan in zebrafish model. The molecular mechanism underlying in H9c2 cells indicated that **15d** protected cardiomyocyte death and damage through inhibiting the reactive oxygen species overproduction, restoring the mitochondrial membrane potential and maintaining morphology of mitochondrial. Thus, **15d** merits further development as a potential cardioprotective clinical trial candidate. The present study is a successful example to combine synthesis, structure-activity relationship study and *in vivo* screening to effectively discover new cardioprotective agents from isosteviol.

1. Introduction

Cardiovascular disease (CVDs) is the leading cause of death and disability globally. It accounts for around 17.9 million death, 31% of all death worldwide, each year [1]. People with CVDs or who are at high cardiovascular risk need early detection and treatment. Cardioprotective drugs, which can preserve the metabolism, structure and function of the heart and vasculature and limit their damage either in primary or secondary prevention, are extremely important in the treatment of patients with CVDs or at risk for CVDs. Although several groups of cardioprotective drugs including statins, angiotensin converting enzyme inhibitors (ACEIs), beta-blockers (BBs), and blockers or receptors for angiotensin II type one (ARBs) have been proven to be effective in achieving the hard endpoints, the mortality reduction with treatment of these drugs does not exceed 30% and the residual cardiovascular risks are still rather high [2]. Therefore, discovery of novel cardioprotective agents continues to be unabated.

Natural products, the vital source of pharmaceutical agents, have long been used to treat CVDs [3]. Stevioside (**1**, Fig. 1), a diterpenoid triglycoside, is a natural sweetener. Stevioside (**1**) is the principal constituent of the leaves of Stevia rebaudiana, which is a perennial herb shrub, originated from Brazil and now cultivated worldwide for producing natural sweeter [4]. Acid hydrolysis of the glycoside of **1** following by a Wagner-Meerwein rearrangement generated isosteviol (**2**, Fig. 1), an ent-beyerane diterpenoid, in a rather high yield [5]. Both **1** and **2** exhibit broad spectrum pharmacological effects including antidiabetic, anticancer, anti-inflammatory, anti-hyperglycaemic, anti-hypertensive, anti-diarrheal, diuretic and immunomodulator activity, and cardiovascular protection effects [4,6,7]. The cardioprotective effect of **2** and its sodium form have been widely evaluated in various *in vivo* models [4]. For example, isosteviol (**2**) was reported to reduce the damage due to a rat heart ischaemia-reperfusion (IR) injury at 0.5, 1.0 and 2.0 mg/kg [8]. And it was also reported to significantly attenuate the myocardium IR injury induced by IR in the isolated guinea pig heart [9], and attenuated the IR-induced prolongation of the QTc interval and inhibited I_{kr} [10].

Because of their potent pharmacological activities, novel natural skeleton, good synthetic accessibility, and significant abundance in

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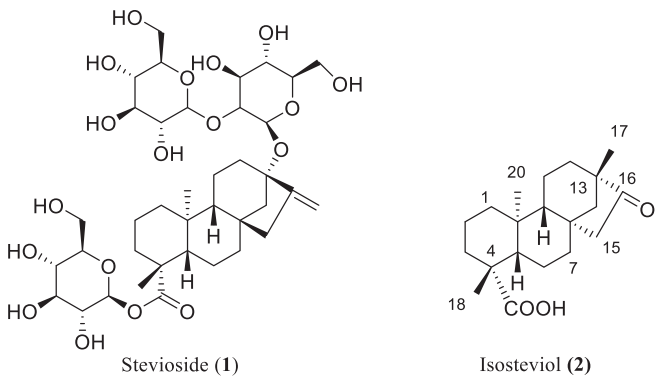


Fig. 1. The structure of stevioside (**1**) and isosteviol (**2**).

the plant, extensive modifications on the **1** and **2** for new derivatives with anticancer activity were accomplished by several research groups [11–23]. However, research on the development of new cardioprotective derivatives of **2** is rather limited. Only one paper reported the synthesis of six triphenylphosphonium-conjugated isosteviol derivatives and their cardioprotective effects [24]. And another paper reported the cardioprotective effect of one derivative of **2** in H9c2 cells [25].

In the past two decades, the zebrafish has become increasingly popular animal model which enable efficient large-scale *in vivo* compounds screening in the early phase of drug discovery [26]. As compared with the cell-based *in vitro* system, *in vivo* screening provides several advantages, such as the ability to evaluate the compounds in an intact animal and discover compounds with the ability to be effective in the complex context of the disease interest. Zebrafish screening have discovered several compounds which have been advanced in preclinical and clinical trials [27]. The cardiovascular physiology between human and zebrafish is highly conserved at anatomical, cellar and membrane-biology levels. Several cardiovascular drugs were found to exhibit identical effects on zebrafish physiology [27,28]. Zebrafish as a tool for cardiovascular drug screen combined the advantages of *in vitro* screening, such as easy handling, cost-effectiveness, and living organism evaluation which can easily access important cardiovascular parameters including heartbeats, ventricular systolic pump function, and so on [26,29].

Doxorubicin (DOX), a type of anthracycline antibiotic, is widely used to treat many types of cancers [30]. However, DOX was found to have serious cardiotoxicity which led to acute inflammation, irreversible degenerative cardiomyopathy, and congestive heart failure in humans and laboratory animals [31]. The detailed studies and characterization of the DOX-treated animals such as mice, zebrafish, indicated that their DOX-induced cardiomyopathy is comparable to that in humans and thus is an excellent model for the study of cardiomyopathy progression and cardiovascular protective drug screening [32–34].

With the aim of discovering new cardioprotective agents from **2**, we initiated the study to synthesize and *in vivo* assay the derivatives using the easy-handling DOX-treated zebrafish model. Firstly, modification of the functional groups presented in the structure of **2**, C-19 carboxylic acid and cyclopentanone in the ring D, were performed to explore their structure-activity relationship (SAR). Subsequently, based on the constructed SAR, a diversity-oriented modification of **2** was conducted. Forty-seven compounds thus synthesized were firstly evaluated for their ability to protect zebrafish against DOX-induced mortality. Subsequently, five derivatives with potent protective activity were selected for further evaluation of their cardioprotective effect in the zebrafish model. Finally, **15d** with the most pronounced cardioprotective effect was identified. And its molecular mechanism underlying in H9c2 cells was also investigated.

2. Chemistry

Our initial efforts were focused on the modification of C-19 carboxylic acid and cyclopentanone in the ring D of **2**. Compounds **3**–**10** were thus synthesized (Scheme 1). Isosteviol (**2**), the starting material, was obtained via the acid hydrolysis of **1** with HBr following the reported method [5]. Esterification of 19-COOH of **2** with benzyl bromide (BnBr) or 4-methoxybenzyl chloride (PMBCl) and K_2CO_3 in acetone afforded **3** and **4**, respectively. Methyl esterification of 19-COOH of **2** with methyl iodide (CH_3I) and tetrabutylammonium fluoride (TBAF) produced **5**. Conversion of 19-COOH of **2** into amine via the Curtius rearrangement with diphenyl phosphoryl azide (DPPA) and Et_3N in t-BuOH yielded **6**. Hydroxylation of **2** via aldol/Cannizzaro reaction with excessive paraformaldehyde and NaOH in ethanol produced the 16α-hydroxy-15β-hydroxymethyl derivative **7** in high yield [35,36]. The stereochemistry of **7**, determined with 2D NMR spectroscopy (Fig. S1, Supporting Information), is consistent with literature [35,36]. The reaction of 7 with *p*-methyl phenylsulfonyl chloride (TsCl) in pyridine gave **8**. Oxidation of the 16-OH of **8** generated the 16-keto **9** which was further converted to the 16-keto-15-ene **10** via an elimination reaction.

The synthetic routes employed to modify 16α-hydroxy-15β-hydroxymethyl moiety of **7** was depicted in Scheme 2. Selective acylation of the 15β-hydroxymethyl of **7** with acetic anhydride (Ac_2O), cyclohexanecarboxylic acid, nicotinic acid, isonicotinic acid, benzoic acid and its analogs, 3,5-dimethoxycinnamic acid, 4-methoxyphenylacetic acid and 3,4,5-trimethoxyphenylacetic acid gave **11a**–**11n**, respectively. Further oxidizing 16-OH of **11a**–**11n** produced their 16-keto derivatives **12a**–**12n**, respectively.

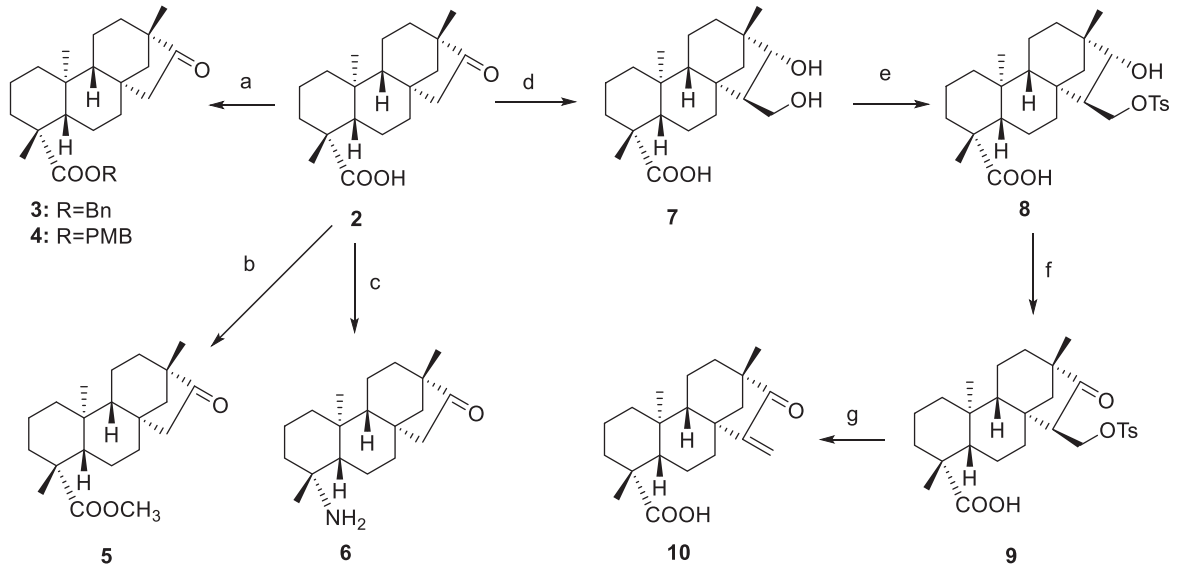
Starting from **7**, modification of 19-COOH and 16α-hydroxy-15β-hydroxymethyl moiety was carried out following the routes in Scheme 3. Conversion of 19-COOH of **7** to the primary NH_2 group via Curtius rearrangement produced **13**. Esterification of 15β-hydroxymethyl of **13** with nicotinic acid, 4-methoxybenzoic acid, 2,3-dimethoxybenzoic acid, 2,3,4-trimethoxybenzoic acid, and 3,4,5-trimethoxybenzoic acid gave **14a**–**14e**, respectively. Finally, the corresponding 16-keto products **15a**–**15e** were obtained from **14a**–**14e** via oxidation reaction with PDC reagents.

3. Results and discussion

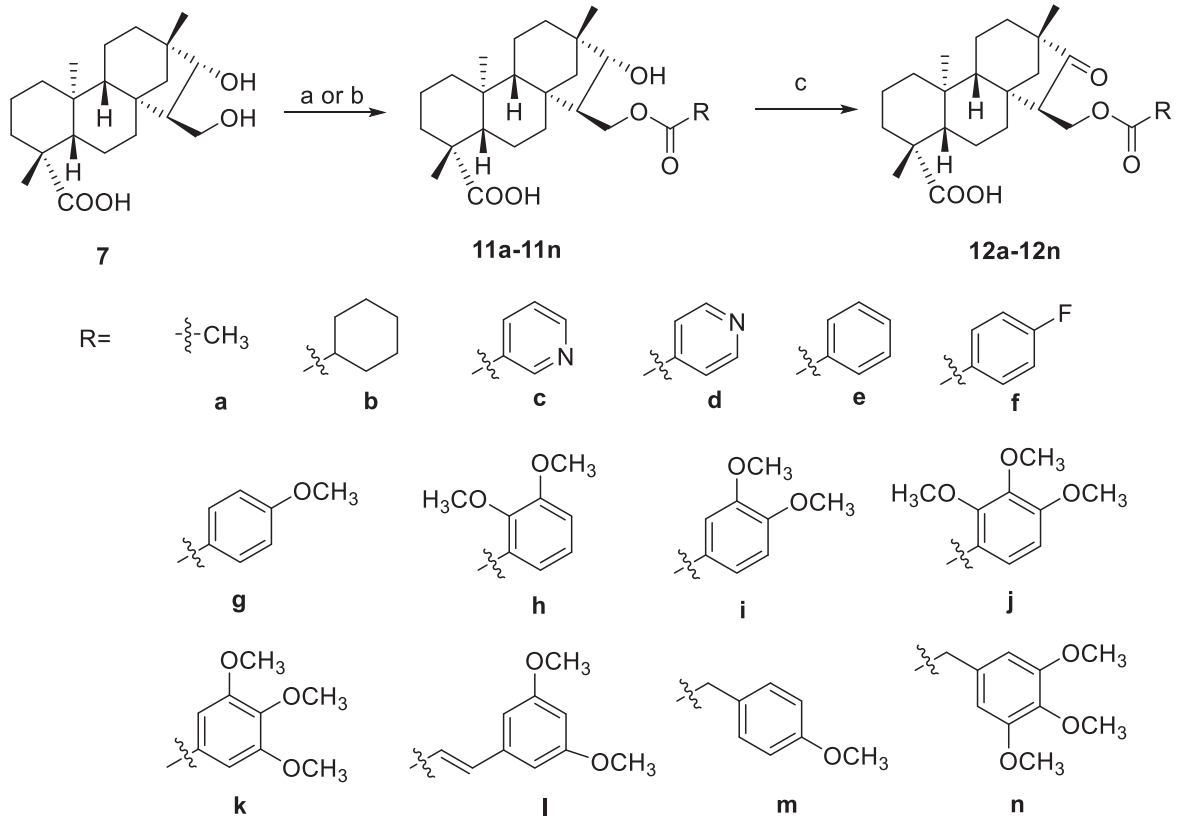
3.1. Preliminary screening of *2*–*15* in DOX-treated zebrafish embryos and SAR analysis

All synthesized derivatives, including 40 new (**4**, **11a**–**11n**, **12a**–**12n**, **13**, **14a**–**14e** and **15a**–**15e**) and 7 known compounds (**3**, **5**–**10**), accompanying with the starting material **2**, were subjected to an easy-handling and efficient zebrafish assay system for their cardioprotective effect evaluation. Levosimendan (LSD), the drug used in the treatment of heart failure and for cardioprotection, was used as the positive control [37,38]. Firstly, the survival of the DOX-treated zebrafish embryos in the absence or presence of each test compound was measured. The compounds with potent protective activity, indicating by their ability to protect zebrafish against the DOX-induced mortality, were then selected for further evaluation, including dose-response study, toxicity assay and cardioprotective effect assay.

As shown in Fig. 2, the embryos were treated with DOX (model group), or DOX with each of test compounds (**2**–**10**, **11a**–**11n**,

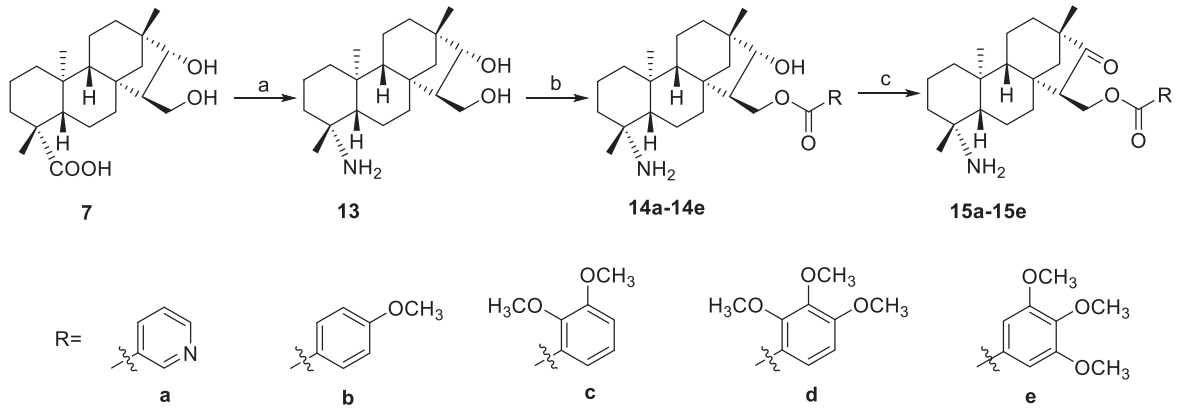


Scheme 1. Reagents and conditions: (a) BnBr for **3** or PMBCl for **4**, K_2CO_3, acetone, 50 °C; (b) CH_3I, TBAF, THF; (c) DPPA, Et_3N, t-BuOH, reflux; (d) NaOH, (HCHO)n, EtOH, reflux; (e) TsCl, pyridine, DMAP, reflux; (f) PDC, DMF; (g) Pyridine, DMAP, reflux.



Scheme 2. Reagents and conditions (a) Ac_2O, Pyridine, DMAP for **11a**; (b) acid, DMAP, EDCI, DCM for **11b**–**11n**; (c) PDC, DMF.

12a–**12n**, **13**, **14a**–**14e**, **15a**–**15e**, and LSD) at 5, 15, and 40 μM, respectively. In comparison with the control group (100% survival rate), the survival rate of the model group dramatically dropped to 52%. When treated with LSD, the positive drug, at 5, 15, and 40 μM, the survival rate has dose-dependently risen to 58%, 69% and 74%, respectively. Isosteviol (**2**), at 5 μM, increased the survival rate from 52% to 57%. While treatment of **2** at 15 and 40 μM, respectively, has no evident effect on the survival rate. Among the 47 derivatives, 37 ones, at each concentration or a certain concentration, reduced the DOX-induced mortality. In contrast, the other 10 derivatives including **10**, **11n**, **12a**, **12b**, **12e**, **12g**, **12l**, **12n**, **13**, and **15b**, at each concentration, further increased the DOX-induced mortality.



Scheme 3. Reagents and conditions: (a) DPPA, Et_3N, ^tBuOH, reflux; (b) Acid, DMAP, EDCI, DCM; (c) PDC, DMF.

Based on the result (Fig. 2), SAR was deduced (Fig. 3). Firstly, we explored the effect of modification of C-19 on the activity. Conversion the 19-COOH of **2** to its esters (**3**–**5**), led to some positive effect on the survival rate (around 60%, with **3** at 5 μM, **4** at 15 μM, and **5** at 40 μM, respectively). Replacement of the 19-COOH with the primary amine group (**6**) significantly reduced the DOX-induced mortality. The survival rate of zebrafish embryos treated with **6** (85% at 5 μM) is much higher than **2** (57% at 5 μM) and LSD (74% at 40 μM). These results suggested that 19-COOH of **2** can be modified for good protective activity. The activity of 19-COOH modified derivatives was ranked as amine derivate (**6**) > ester derivatives (**3**, **4**, and **5**) > isosteviol (**2**).

Next, we explored how the modification of cyclopentanone in the ring D affected the protective activity. Hydroxylation of **2** (**7**) and further modification of the 16α-hydroxy-15β-hydroxymethyl moiety of **7** via esterification and oxidation (**8** and **9**) led to improved activity. Derivatives **7**, **8**, and **9**, at 5, 15, 40 μM, risen the survival rate from 52% to 60%–67%. In contrast, derivative **10** with the *exo*-methylene cyclopentanone moiety in the structure significantly increased the DOX-induced mortality in a dose-dependent manner. The survival rate treated with **10** at 5, 15, 40 μM is 50%, 38%, 26%, respectively. It has been repeatedly reported that exo-methylene cyclopentanone moiety is an important pharmacophore for generating significant anticancer derivatives of isosteviol (**2**) [4]. However, in the present study, exo-methylene cyclopentanone moiety is detrimental.

Derivative **7** contained two hydroxyl groups which are readily available for further modification. The above results also suggested that good activity can be obtained by altering the 16α-hydroxy-15β-hydroxymethyl moiety. Therefore, we further performed the diversity-oriented modification on 16α-hydroxy-15β-hydroxymethyl to produce 28 derivatives containing 16α-hydroxy-15β-methyl-*O*-acyl unit (**11a**–**11n**) or 16-keto-15β-methyl-*O*-acyl fragment (**12a**–**12n**). The preliminary screening results indicated that most derivatives (21 out of 28) showed protective activity. Seven derivatives (**11g**, **11i**, **11m**, **12h**, **12i**, **12j**, and **12k**), at the test concentrations, increased the survival rate from 52% to more than 70%. Among them, **12j** containing 16-keto-15β-methyl-*O*-2,3,4-trimethyl benzoyl unit and **12k** with 16-keto-15β-methyl-*O*-3,4,5-trimethyl benzyl moiety in the structure are most potent ones, which significantly risen the survival rate to 77%–82% (**12j**) and 68%–80% (**12k**) at 5,15, and 40 μM, respectively.

In this series of compounds, the hydroxyl group or the ketone group presented at C-16 is not determinant for the activity, because both positive and negative effect on the survival rate can be observed when converting 16α-hydroxy derivatives (**11a**–**11r**) to their appropriate 16-keto derivatives (**12a**–**12r**). Among the various ester groups presented at the 15β-methyl-*O*-acyl moiety, the ethyl group (**11a**, **12a**), and cyclohexane (**11b**, **12b**), which could not decrease or even increased the DOX-induced mortality, are not conducive for the good activity. While mono-, di-, and tri-methoxy substituted benzyl group (**11g**, **11i**, **12h**, **12i**, **12j**, and **12k**), and nicotinoyl group (**12c**) benefit the activity.

Next, we further synthesized another series of derivatives via simultaneously replacing the 19-COOH with amine and acylating the mono-, di-, and tri-methoxy substituted benzoic acid and isonicotinic acid to 15β-hydroxymethyl of **7**. Derivatives **13**, **14a**–**14e** and **15a**–**15e** were thus obtained. Hydroxylation of **6** led to **13**, which greatly increased the DOX-induced mortality. Acylation of **13** with various acids led to derivatives **14a**–**14e** with moderate activity. Further oxidization of the 16-OH of **14a**–**14e** gave **15a**–**15e**, among which, **15d** and **15e**, the most potent ones, at 40 μM, were found to significantly increase the survival rate to around 98% and 87%, respectively.

3.2. Dose-response and toxicity evaluation

Based on the above results, derivatives **6**, **12j**, **12k**, **15d**, and **15e**, which showed significant activity and possesses different structural features, accompanying with starting material **2** and positive drug LSD, were selected for further study. Firstly, the dose-response of these compounds was explored. As shown in Fig. 4, all derivatives can dose-dependently improve the survival rate at a range of concentrations. The optimum concentration of **2**, **6**, **12j**, **12k**, **15d**, **15e** and LSD are 10, 0.8, 70, 60, 40, 60, and 50 μM, respectively (Fig. 4). Furthermore, to test whether these compounds will cause any toxicity, the embryos were treated with the tested compounds alone at different concentrations. The results demonstrated that none of the tested compounds caused mortality in zebrafish even at a much higher concentration than their optimum concentration (Fig. 4).

3.3. Cardioprotective effects of derivatives on DOX-induced cardiomyopathy in zebrafish embryos

Subsequently, we further assayed the cardioprotective effect of **2**, **6**, **12j**, **12k**, **15d**, **15e**, and LSD using the transgenic zebrafish Tg(cmlc2:GFP), which specifically expressed the green fluorescent protein (GFP) in the myocardium. Four cardiovascular parameters, including heartbeat, fractional shortening, stroke volume, and cardiac output, were measured to evaluate the cardiac function. As shown in Fig. 5, the zebrafish treated with DOX alone (model

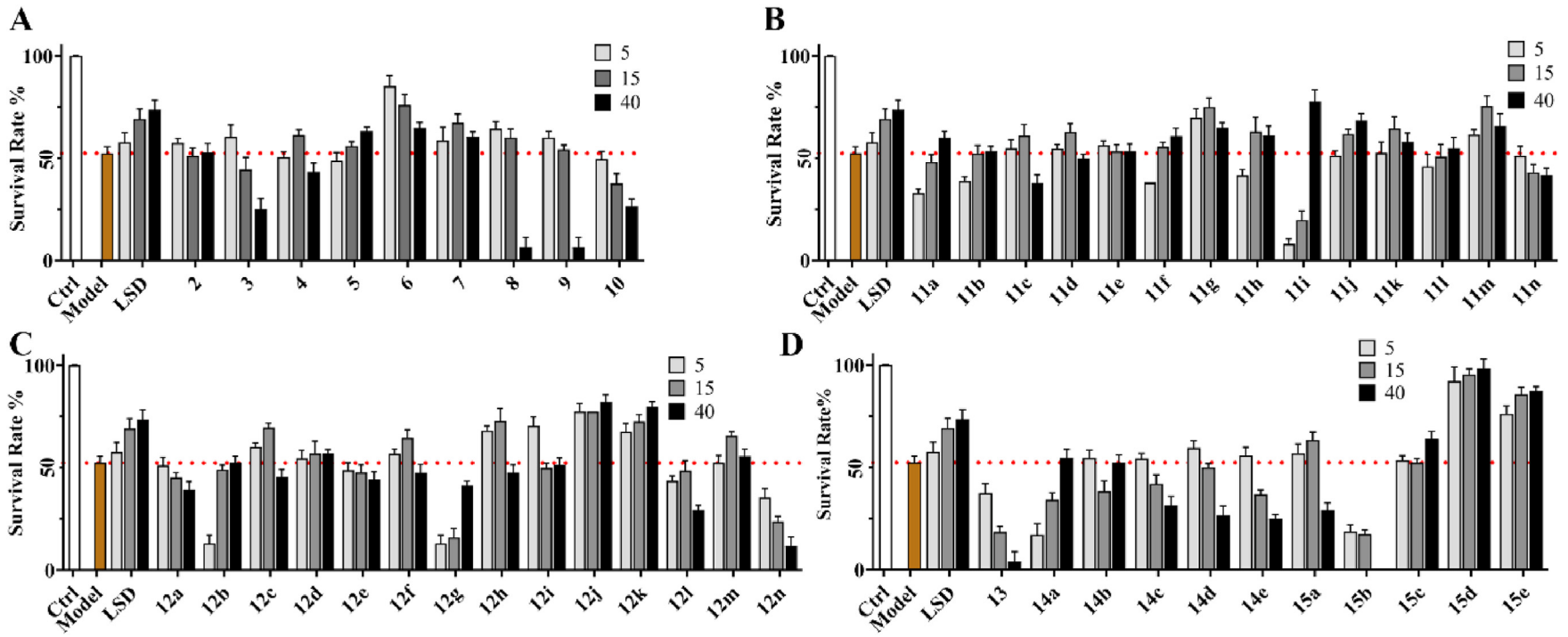


Fig. 2. The ability of **2**–**15** to protect zebrafish against the DOX-induced mortality. The 24 h post-fertilization (hpf) embryos were treated with 100 μM DOX (model), or 100 μM DOX with each test compound at 5, 15, and 40 μM. The survival of zebrafish was assessed at 96 hpf. The survival rate of DOX treated zebrafish in the presence of compound 2–10 (A), compound 11a-11n (B), compound 12a-12n (C), compound 13, 14a-14e and 15a-15e (D). Data are presented as mean ± S.D. (n = 4).

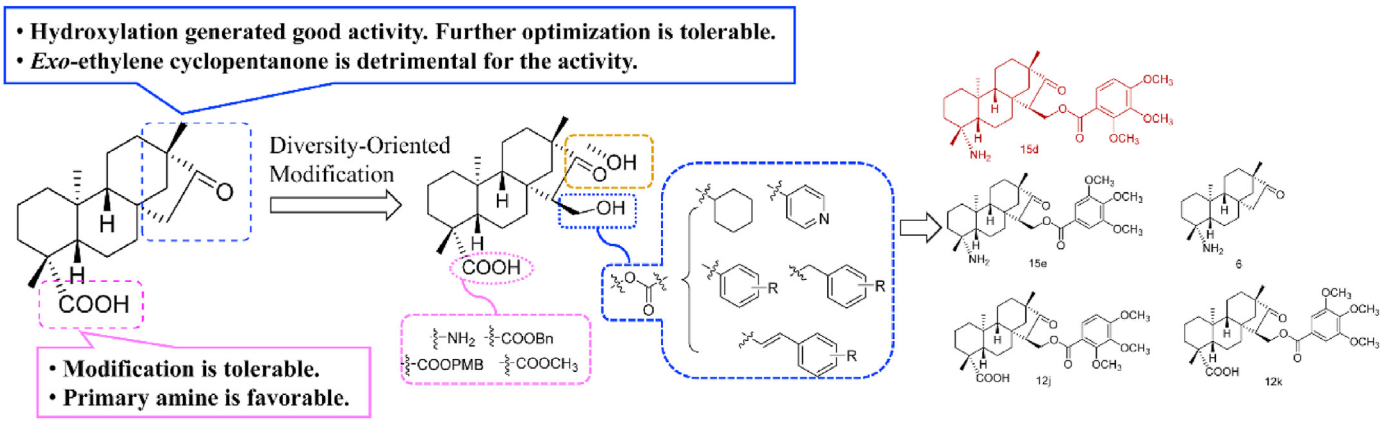


Fig. 3. The SAR analysis, diversity-oriented modification and the structure of the derivatives selected for further study.

group) exhibited severe cardiac edema and its heart was distorted into an elongated shape with compact ventricle and atrium. Significant reductions in the fractional shortening, stroke volume, and cardiac output, and moderate decrease in heart rate were observed. These cardiovascular parameters and the morphological distortions clearly indicated the abnormal ventricular filling and systolic dysfunction. Cotreatment of DOX and the test compound (**2**, **6**, **12j**, **12k**, **15d**, **15e**, and LSD) at their optimum concentration greatly reduced the cardiac edema, maintained the normal heart shape, and improved the cardiac performance impaired by DOX. Among all the tested compounds, **15d** exhibited the most significant efficiency. The cardioprotective activity was ranked as **15d** > **15e** > LSD >**6** > **12j** > **2** > **12k**.

Furthermore, the cardioprotective effect of **15d** was evaluated at 20, 40, and 60 μM. Our results indicated that **15d** (20−40 μM) dose-dependently protected the heart and improve cardiac function. While **15d** at 60 μM was unable to rescue the heart so effectively as it did at 40 μM, which confirmed our dose-response result that **15d** exerted its best efficacy at 40 μM (Fig. 6).

*3.4. Derivative **15d** decreased the elevated mRNA level of the cardiomyopathy biomarkers cTnT and ANP in DOX-treated zebrafish embryos*

Cardiac troponin T (cTnT) and atrial natriuretic peptide (ANP) are two specific diagnostic biomarkers for cardiomyopathy disease. To further confirm the cardioprotective effects of **15d** at the gene level, we assayed the mRNA level of cTnT and ANP of zebrafish by qPCR. As shown in Fig. 7, DOX treatment led to a significant increase in mRNA level of CTnT and ANP. Derivative **15d** markedly decreased the cTnT and ANP level in a dose-dependent manner. These results presented strong molecular evidence for the cardioprotective potency of **15d**.

*3.5. Derivative **15d** inhibited reactive oxygen species overaccumulation, restored mitochondrial membrane potential, and maintained the mitochondrial morphology in H9c2 cells*

After identifying **15d** as the most potent derivative, we further investigated its molecular mechanisms underlying in H9c2 cells. Firstly, the protective effect of **15d** on DOX-induced cytotoxicity in H9c2 cells was examined. The viability of H9c2 cells treated with DOX (10 μM) was markedly decreased by around 50%. Derivative **15d** (0.5−12.5 μM) dose-dependently protected the cells from damage (Fig. 8). Oxidative stress, resulting from the over-accumulation of the reactive oxygen species (ROS), plays an important role in cardiac and vascular abnormalities in various cardiovascular diseases [39]. Therefore, we then explored if **15d** can affect the DOX-induced ROS overaccumulation. As shown in Fig. 8, comparing with the control group, the higher fluorescence intensity of H9c2 cells treated with DOX was observed, which indicated that DOX induced ROS overproduction. Co-treatment of **15d** and DOX significantly decreased the fluorescence intensity in dose-dependent manner. These results clearly indicated that **15d** can reduce the overproduction of ROS thus protecting the cell from oxygen stress damage. Mitochondrial plays a pivotal role in cardioprotection and is regarded as the promising target for the new cardioprotective agents [40]. Mitochondrial membrane potential (Δψm) is commonly used as the indication of the mitochondrial status. A loss of Δψm represented the mitochondrial dysfunction. Subsequently, we, therefore, examined the influence of **15d** on the Δψm of H9c2 cells treated with DOX. JC-1, the membrane sensitive dye, was used to stain cells to detect the changes of the Δψm. Our result showed that when treated with DOX, the Δψm of the cells was markedly reduced. Cotreatment of **15d** dose-dependently recovered the Δψm (Fig. 9). The morphology changes of mitochondrial were also explored. Mitochondria treated with DOX became fragmental and the length of mitochondrial appeared short. Cotreatment of **15d** can ameliorate such morphology changes (Fig. 9). Taken together, these results indicated that **15d** could effectively attenuate the DOX-induced oxidative stress, restore the Δψm, maintain the mitochondrial morphology and thus protect the cell from damage.

4. Conclusion

With an aim of discovering new derivatives with promising cardioprotective activity from isosteviol (**2**), a diversity-oriented modification based on the structural feature of **2** was performed. Forty-seven derivatives, including 40 new ones, were thus synthesized. The cardiovascular protective activity of the derivatives was evaluated *in vivo*, with the employment of an easy-handing and effective zebrafish model. The SAR study of isosteviol derivatives was discussed. Among the 47 derivatives synthesized, **15d** exhibited the most pronounced efficiency *in vivo*. The new derivative **15d** could effectively reduce the cardiac edema, maintain the normal heart shape and prevent the cardiac dysfunction impaired by DOX in zebrafish. And its cardioprotective effect is superior to that of **2** and LSD. The molecular mechanism underlying in DOX-treated H9c2 cells indicated that **15d** protected cardiomyocyte death and damage via inhibiting the ROS overaccumulation and restoring the Δψm and morphology. Therefore, **15d** merits further

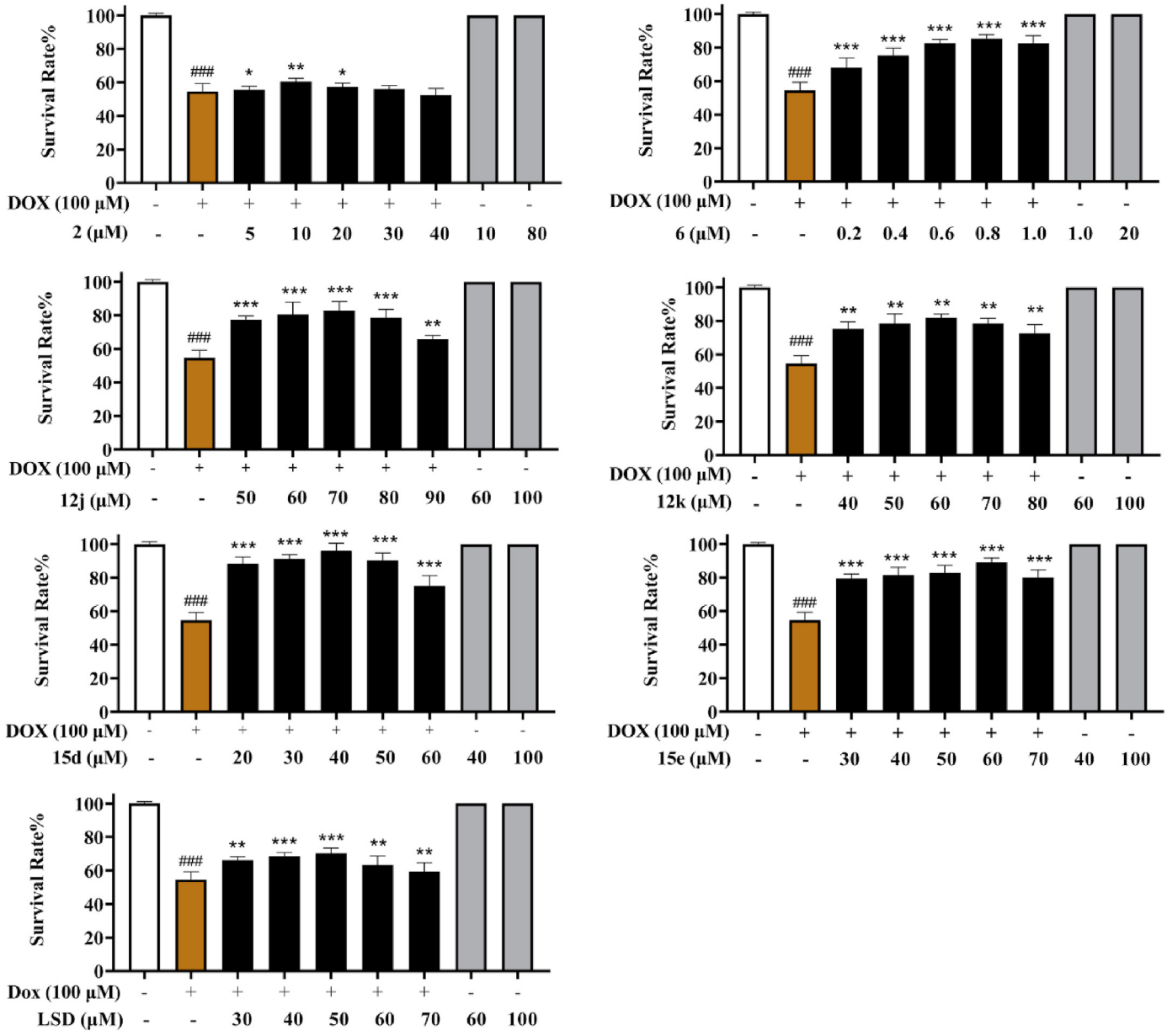


Fig. 4. Dose-response and toxicity assay of **2**, **6**, **12j**, **12k**, **15d**, **15e** and LSD. The 24 hpf embryos were treated with 100 μM DOX with or without each test compound or solely treated with each test compound (toxicity assay). Survival of the zebrafish was examined at 96 hpf. Data are presented as mean ± S.D. (n = 4). ###P < 0.001 vs. the control group, *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the DOX treated only group.

development as a potential cardioprotective clinical trial candidate. To the best of our knowledge, this is the first example to combine synthesis, SAR study, and *in vivo* screening to discover new cardioprotective derivatives from isosteviol.

5. Experiment section

5.1. Chemistry

All reagents and solvents were purchased from Energy Chemical or other commercial source and used without further purification. Thin-layer chromatography (TLC) was performed on 0.20mm silica gel 60 F-254 plates (Qingdao Ocean Chemical Factory, Shandong, China). Silica gel chromatography was carried out on Biotage CombiFlash Rf flash chromatography system. Solid compounds were not recrystallized. All final compounds were purified to ≥95% as determined by HPLC, conducted on Waters Alliance e2695 series system with a Grace Alltima 2.1 mm × 150 mm HP C18 5 μm column. ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian Unity Inova 400 MHz NMR spectrometer with Me_4Si (TMS) as an internal standard. High-resolution mass spectra were obtained on a Bruker Maxis II ETD with ESI interface.

5.1.1. General procedures for the synthesis of compounds **3** and **4**

Isosteviol (**2**) (1 eq) and K_2CO_3 (1.5 eq) were dissolved in acetone. The mixture was stirred at 50 °C for 30 min. Then BnBr (1.2 eq for synthesizing **3**) or PMBCl (1.2 eq, for synthesizing **4**) was added. The reaction was stirred at 50 °C for 2 h. The solution was then diluted with water and extracted with EtOAc for 3 times. The combined organic layer was washed with brine, dried over Na_2SO_4,

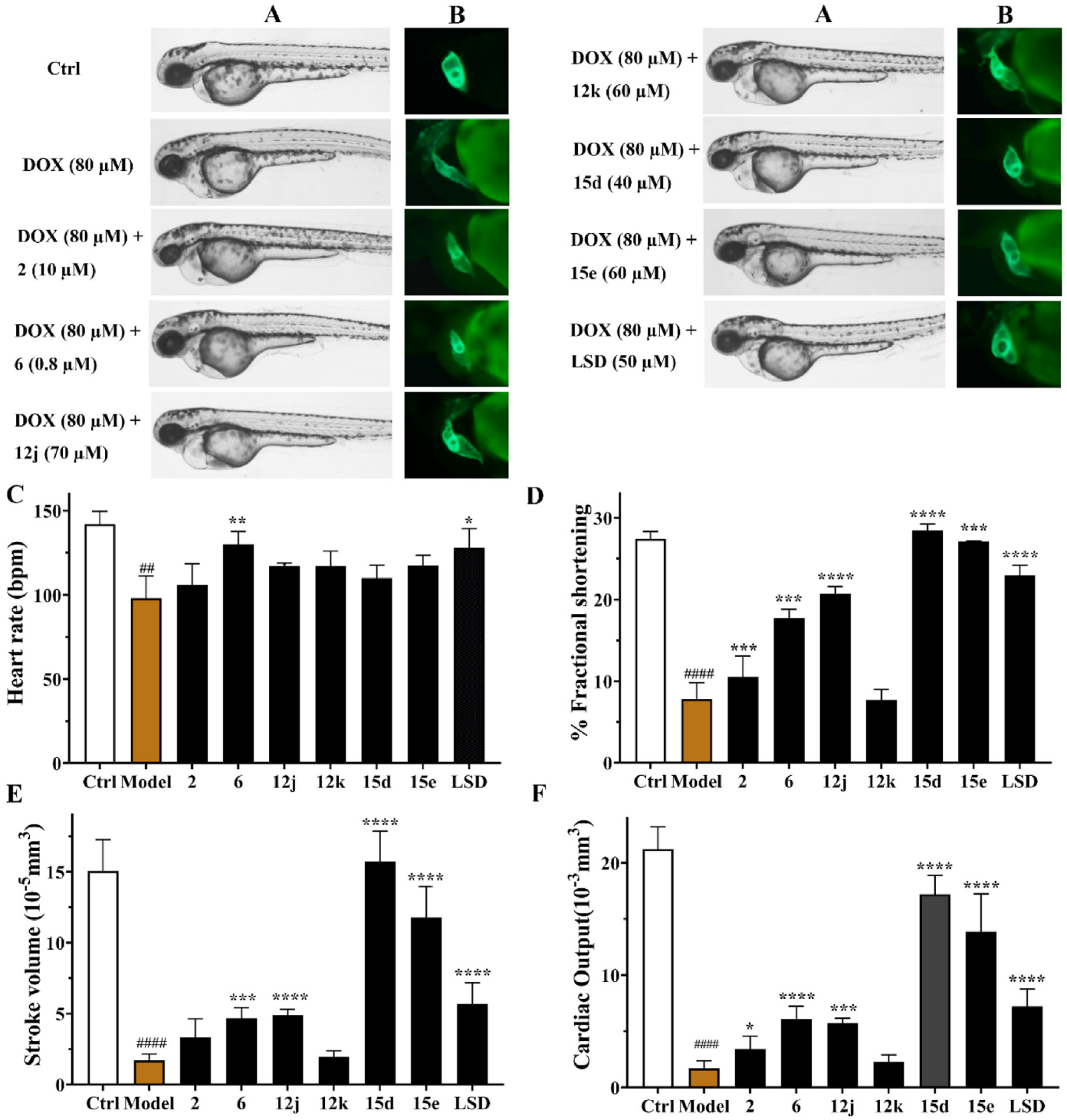


Fig. 5. The cardioprotective effects of **2**, **6**, **12j**, **12k**, **15d**, **15e**, and LSD on DOX-induced cardiomyopathy in zebrafish Tg(cmlc2:GFP). Tg(cmlc2:GFP) zebrafish embryos were treated with DOX (80 μM) in the absence or presence of each of test compound at their optimum concentration at 24 hpf. Four cardiovascular parameters including heartbeat, stroke volume, cardiac output and fractional shortening, were measured at 72 hpf to evaluate the cardiac function. (A) Light microscopy image of the Tg(cmlc2:GFP) zebrafish. (B) Representative fluorescent microscopy image of atrium and ventricle. (C) Graph of heart rate (D) Graph of fraction shortening (E) graph of stroke volume (F) Graph of cardiac output. Data are presented as mean ± S.D. (n = 4). $^{\#\#}P < 0.01$ and $^{\#\#\#\#}P < 0.0001$ vs. the control group, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 vs. the DOX treated only group.

and concentrated in vacuo to give the crude product, which was chromatographed using a silica gel column to yield the pure product (**3** and **4**).

*5.1.1.1. Benzyl ent-16-oxobeyeran-19-oate (**3**).* Yield: 117 mg (91%), starting from 100 mg of **2**; white solid; mp: 100.4–100.9 °C; $[\alpha]^{25}_D$ −59 (c 1.6, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42–7.27 (5H, overlap, 2,3,4,5,6-Ph), 5.10 (2H, m, CH_2-Ph), 2.55 (1H, dd, J = 3.8,18.7 Hz, H-15), 2.21 (1H, d, J = 13.3 Hz, H-3), 1.21 (3H, s, H-18), 0.97 (3H, s, H-17), 0.61 (3H, s, H-20), 1.90–0.80 (18H, m, CH,

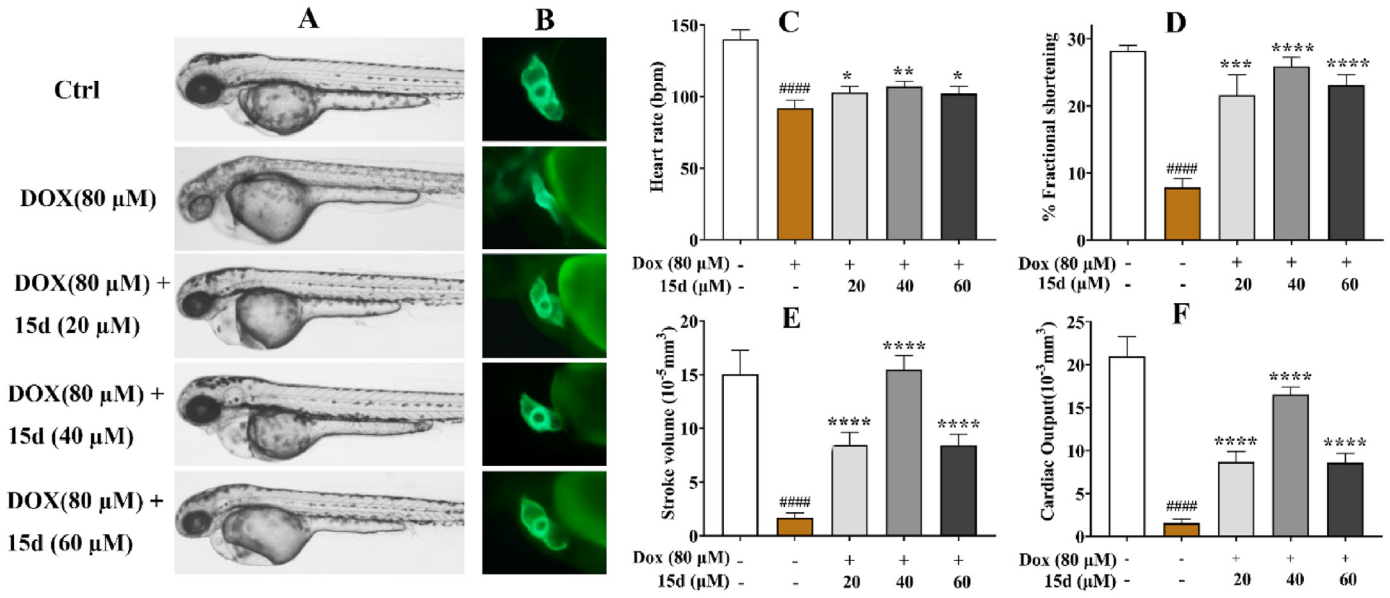


Fig. 6. The cardioprotective effects of **15d** at 20, 40 and 60 μM, on DOX-induced cardiomyopathy in zebrafish Tg(cmlc2:GFP). (A) Light microscopy image of the Tg(cmlc2:GFP) zebrafish. (B) Representative fluorescent microscopy image of atrium and ventricle. (C) Graph of heart rate (D) Graph of fraction shortening. (E) Graph of stroke volume. (F) Graph of cardiac output. Data are presented as mean ± S.D. (n = 4). ####P < 0.0001 vs. the control group, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 vs. the DOX treated only group.

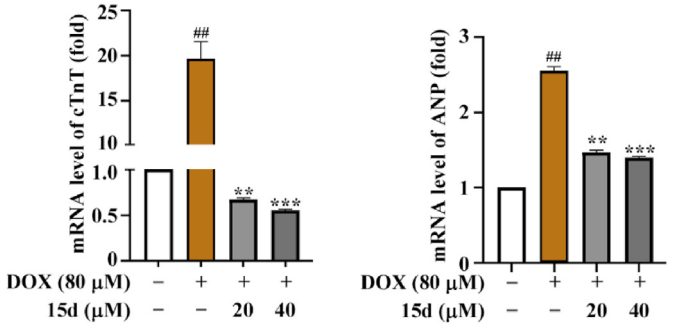


Fig. 7. Relative mRNA level of the cardiomyopathy biomarkers ANP and cTnT in control zebrafish embryos and DOX-treated embryos with or without **15d**. Data are presented as mean ± S.D. (n = 4). ##P < 0.01 vs. the control group, **P < 0.01 and ***P < 0.001 vs. the DOX treated only group.

CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.46, 176.99, 135.99, 128.51, 128.51, 128.40, 128.40, 128.17, 66.12, 57.18, 54.66, 54.28, 48.69, 48.37, 43.90, 41.50, 39.80, 39.43, 38.01, 37.96, 37.31, 28.96, 21.73, 20.31, 19.89, 18.97, 13.32; HRMS (ESI, m/z) calcd for $C_{27}H_{36}O_3Na$, 431.2562 [M + Na^+]; found, 431.2560.

*5.1.1.2. 4-Methoxybenzyl ent-16-oxobeyeran-19-oate (**4**).* Yield: 109 mg (79%), starting from 100 mg of **2**; white solid; mp 79.2–80.7 °C; $[\alpha]^{25}_D$ −57 (c 1.6, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28 (2H, m, 2,6-Ph), 6.88 (2H, m, 3,5-Ph), 5.01 (2H, m, CH_2-Ph), 3.82 (3H, s,OCH_3), 2.56 (1H, dd, J = 3.8, 18.7, Hz, H-15), 2.19 (1H, d, J = 13.3 Hz, H-3), 1.19 (3H, s, H-18), 0.97 (3H, s, H-17), 0.60 (3H, s, H-20), 1.90–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.63, 177.10, 159.53, 130.17, 130.17, 128.13, 113.86, 113.86, 65.90, 57.20, 55.29, 54.67, 54.29, 48.72, 48.40, 43.88, 41.51, 39.82, 39.45, 38.03, 37.96, 37.33, 28.95, 21.72, 20.32, 19.87, 18.97, 13.35; HRMS (ESI, m/z) calcd for $C_{28}H_{38}O_4Na$, 461.2668 [M + Na^+]; found, 461.2661.

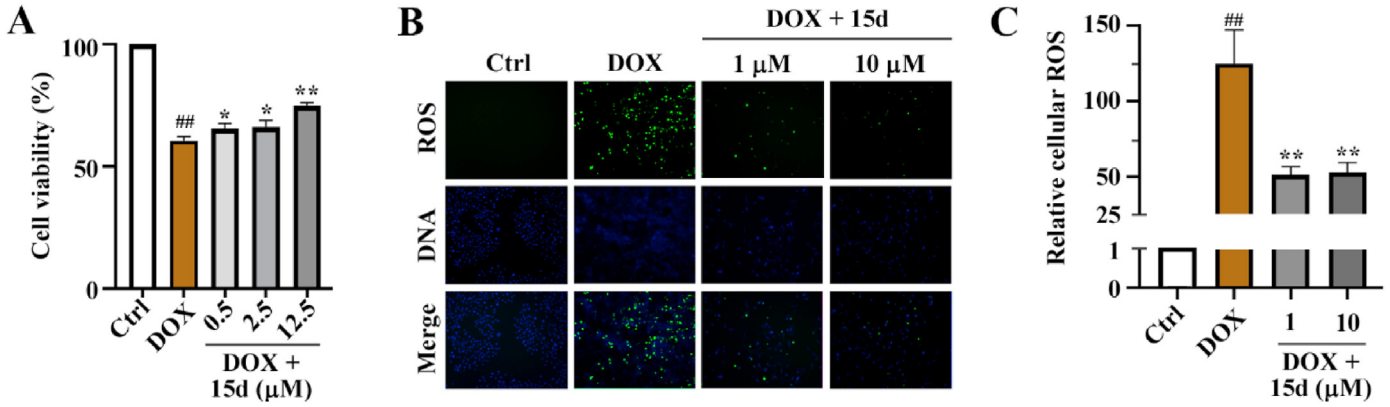


Fig. 8. Derivative **15d** inhibited the DOX-induced cytotoxicity and ROS overaccumulation in H9c2 Cells. (A) The protective effect 15d on cell viability. (B) Confocal images of ROS (Scale bar: 100 μm). (C) Graph of ROS level. Data are presented as mean ± S.D. (n = 3). ##P < 0.01 vs. the control group, *P < 0.05, and **P < 0.01 vs. the DOX treated only group.

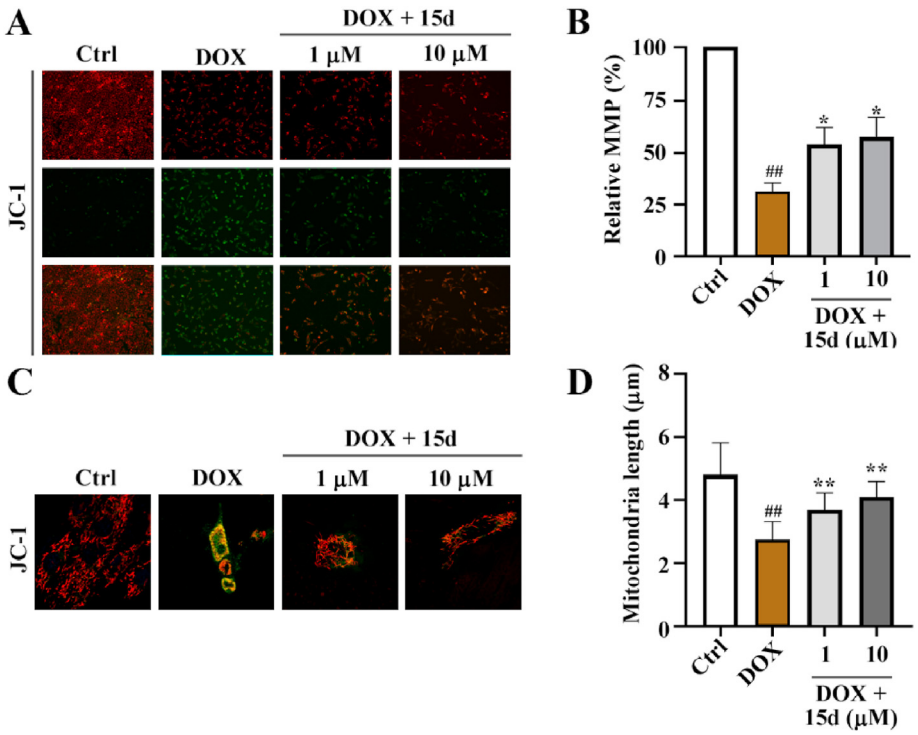


Fig. 9. The effect of **15d** on mitochondrial membrane potential and mitochondrial morphology in H9c2 Cells treated with DOX. (A) Confocal images of JC-1stained mitochondrial potential (Scale bar: 50 μm). (B) Graph of the red to green (R/G) fluorescence intensities. (C) Confocal images of JC-1stained mitochondrial morphology (Scale bar: 50 μm). (D) Graph of the length of the mitochondrial. Data are presented as mean ± S.D. (n = 3). $^{\#\#}P < 0.01$ vs. the control group, $^{*}P < 0.05$, and $^{**}P < 0.01$ vs. the DOX treated only group.

*5.1.2. Synthesis of methyl ent-16-oxobeyeran-19-oate (**5**)*

Isosteviol (**2**) (100 mg, 0.3 mmol), CH_3I (0.04 mL, 0.6 mmol) and TBAF (0.9 mL, 1 M in THF, 0.9 mmol) were dissolved in anhydrous THF (2 mL). The mixture was stirred at room temperature until all starting material was consumed. The solution mixture was then diluted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to give 78 mg of **5** (75%), white solid; mp 215.9–216.8 °C; $[\alpha]^{25}_D$ −46 (c 0.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.64 (3H, s, $COCH_3$), 2.63 (1H, dd, J = 3.8, 18.6 Hz, H-15), 2.18 (1H, d, J = 13.5 Hz, H-3), 1.19 (3H, s, H-18), 0.98 (3H, s, H-17), 0.69 (3H, s, H-20), 1.95–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.54, 177.86, 57.08, 54.75, 54.33, 51.27, 48.72, 48.48, 43.79, 41.50, 39.83, 39.46, 37.96, 37.96, 37.33, 28.86, 21.72, 20.34, 19.87, 18.96, 13.18; HRMS (ESI, m/z) calcd for $C_{21}H_{33}O_3$, 333.2429 [M + H^+]; found, 333.2428.

*5.1.3. Synthesis of ent-4-amino-19-norbeyeran-16-one (**6**)*

Isosteviol (**2**) (250 mg, 0.8 mmol), DPPA (0.2 mL, 0.9 mmol) and Et_3N (0.25 mL, 1.8 mmol) were dissolved in anhydrous tert-butanol (6 mL). The mixture was heated to reflux and stirred for 7 h. After concentration under vacuum, the residue was chromatographed using a silica gel column to give 205 mg of **6** (90%), white solid; mp 122.5–123.9 °C; $[\alpha]^{25}_D$ −70 (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.73 (1H, dd, J = 3.8, 18.7 Hz, H-15), 1.38 (3H, s, H-18), 1.01 (3H, s, H-17), 0.98 (3H, s, H-20), 1.90–0.80 (19H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.20, 59.08, 55.11, 54.76, 54.49, 48.86, 48.70, 41.54, 40.34, 39.18, 38.52, 37.49, 37.11, 31.88, 19.87, 19.87, 19.83, 17.96, 14.06; HRMS (ESI, m/z) calcd for $C_{19}H_{32}NO$, 290.2484 [M + H^+]; found, 290.2472.

*5.1.4. Synthesis of ent-16α-hydroxy-15β-hydroxymethylbeyeran-19-oic acid (**7**)*

Isosteviol (**2**) (50 mg, 0.2 mmol), NaOH (31.4 mg, 0.8 mmol) and paraformaldehyde (37.8 mg, 1.3 mmol) were dissolved in anhydrous EtOH (2 mL). The mixture was stirred at 80 °C until all starting material was consumed. The mixture was diluted with EtOAc, neutralized with HCl (1N). The solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed using a silica gel column to give 32mg of **7** (58%), white solid; mp 199.6–200.8 °C; $[\alpha]^{25}_D$ −31 (c 0.8, CH_3OH); 1H NMR (CD_3OD, 400 MHz) δ 3.83 (1H, dd, J = 5.2, 10.6, Hz, H-1′a), 3.56 (1H, d, J = 4.7 Hz, H-16), 3.48 (1H, dd, J = 8.5, 10.6 Hz, H-1′b), 2.10 (1H, m, H-3), 2.02 (1H, m, H-15), 1.17 (3H, s, H-18), 0.90 (3H, s, H-17), 0.88 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.40, 84.62, 63.02, 57.97, 57.06, 54.19, 49.84, 42.56, 40.47, 39.62, 38.15, 37.82, 34.69, 33.25, 29.36, 28.22, 24.15, 22.08, 19.19, 18.72, 12.47; HRMS (ESI, m/z) calcd for $C_{21}H_{34}O_4Na$, 373.2355 [M + Na^+]; found, 373.2357.

*5.1.5. Synthesis of ent-16α-hydroxy-15β-(tosyloxy)methylbeyeran-19-oic acid (**8**)*

Compound **7** (100 mg, 0.3 mmol) and TsCl (114.4 mg, 0.6 mmol) were dissolved in anhydrous pyridine (2 mL). The mixture was heated to reflux and stirred for 5 h. The reaction mixture was then diluted with EtOAc, neutralized with HCl (1N). The solution was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed using a silica gel column to give 46 mg of **8** (32%), white solid; mp 106.6–107.2 °C; $[\alpha]^{25}_D$ −55 (c 1.1, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (2H, d, J = 8.3 Hz, 2,6-Ph), 7.36 (2H, d, J = 8.3 Hz, 3,5-Ph), 4.32 (1H, dd, J = 4.7, 9.5 Hz, H-1′a), 3.96 (1H, t, J = 9.2 Hz, H-1′b), 3.39 (1H, d, J = 4.5 Hz, H-16), 2.45 (3H, s, CH_3-Ph), 2.15–2.06 (2H, overlap, H-15,

H-3), 1.20 (3H, s, H-18), 0.85 (3H, s, H-17), 0.72 (3H, s, H-20); 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.41, 144.99, 132.79, 129.97, 128.01, 84.17, 77.23, 72.40, 57.41, 56.84, 53.86, 47.93, 43.46, 43.02, 40.85, 39.32, 38.35, 37.66, 34.53, 32.94, 28.73, 24.87, 21.84, 21.65, 19.55, 18.70, 13.85, 12.71; HRMS (ESI, m/z) calcd for $C_{28}H_{40}O_6SNa$, 527.2444 [M + Na^+]; found, 527.2433.

*5.1.6. Synthesis of ent-16-oxo-5β-(tosyloxy)methylbeyeran-19-oic acid (**9**)*

To a solution of **8** (100 mg, 0.2 mmol) in anhydrous DMF (2 mL) was added PDC (225.7 mg, 0.6 mmol). The reaction was stirred at room temperature for 24 h. The mixture was then filtered and concentrated. The residue was chromatographed using a silica gel column to yield 65 mg of **9** (65%), white solid; mp 163.5–164.8 °C; $[\alpha]^{25}_D$ −31 (c 0.7, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, d, J = 8.3 Hz, 2,6-Ph), 7.35 (2H, d, J = 8.3 Hz, 3,5-Ph), 4.26 (2H, m, H-1′), 2.51 (1H, m, H-15), 2.45 (3H, s, CH_3-Ph), 2.17 (1H, d, J = 13.4 Hz, H-3), 1.25 (3H, s, H-18), 0.91 (3H, s, H-17), 0.70 (3H, s, H-20); 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 220.59, 182.68, 144.99, 132.26, 129.87, 129.87, 128.09, 128.09, 67.63, 57.02, 56.88, 52.86, 51.24, 48.07, 43.59, 40.52, 39.57, 38.40, 37.68, 37.12, 35.00, 28.91, 21.70, 21.44, 19.60, 19.53, 18.75, 13.21; HRMS (ESI, m/z) calcd for $C_{28}H_{38}O_6SNa$, 525.2287 [M + Na^+]; found, 525.2280.

*5.1.7. Synthesis of ent-15-methylene-16-oxobeyeran-19-oic acid (**10**)*

To a solution of **9** (100 mg, 0.2 mmol) in anhydrous pyridine (2 mL) was added DMAP (36.7 mg, 0.3mmol). The reaction was heated to reflux and stirred for 3 h. The mixture was then diluted with EtOAc, neutralized with HCl (1N), and washed with saturated brine. The solution was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product, which was chromatographed using a silica gel column to produce 30 mg of **10** (46%), white solid; mp 177.7–178.3 °C; $[\alpha]^{25}_D$ −101 (c 1.4, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.07 (1H, s, CH_2 =), 5.49 (1H, s, CH_2 =), 2.18 (1H, d, J = 12.9 Hz, H-3), 1.28 (3H, s, H-18), 1.02 (3H, s, H-17), 0.69 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.83, 183.18, 154.40, 116.17, 56.96, 56.63, 53.47, 46.80, 43.78, 40.41, 38.81, 38.13, 37.87, 37.85, 29.71, 29.10, 21.76, 21.04, 20.12, 18.91, 12.41; HRMS (ESI, m/z) calcd for $C_{21}H_{30}O_3Na$, 353.2093 [M + Na^+]; found, 353.2087.

*5.1.8. Synthesis of ent-15β -acetoxymethyl-16α-hydroxybeyeran-19-oic acid (**11a**)*

To the solution of **7** (200 mg, 0.6 mmol) in anhydrous pyridine (5 mL) was added Ac_2O (0.07 mL, 0.7 mmol) and DMAP (73.3 mg, 0.6 mmol). The reaction was stirred at room temperature until all starting material was consumed. The solution was then diluted with EtOAc, neutralized with HCl (1N), and washed with brine. The solution was dried over Na_2SO_4 and concentrated. The residue was chromatographed using a silica gel column to give 78 mg of **11a** (35%), white solid; mp 157.0–158.4 °C; $[\alpha]^{25}_D$ −87 (c 0.6, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.22 (1H, dd, J = 6.2, 10.8 Hz, H-1′a), 4.05 (1H, dd, J = 8.9, 10.8 Hz, H-1′b), 3.45 (1H, d, J = 4.6 Hz, H-16), 2.20 (1H, m, H-15), 2.16–2.06 (4H, overlap, H-3, $COCH_3$), 1.21 (3H, s, H-18), 0.91 (3H, s, H-17), 0.84 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.67, 171.56, 85.27, 66.32, 57.48, 56.97, 54.01, 47.36, 43.56, 42.87, 40.81, 39.46, 38.36, 37.72, 34.72, 33.22, 29.70, 28.80, 24.98, 21.91, 19.49, 18.77, 12.93; HRMS (ESI, m/z) calcd for $C_{23}H_{36}O_5Na$, 415.2461 [M + Na^+]; found, 415.2455.

*5.1.9. General procedures for the synthesis of **11b**–**11n***

Compound (**7**, 1 eq), EDCI (5 eq), DMAP (2 eq) and appropriate acid (1.5 eq) were dissolved in anhydrous DCM. The mixture was stirred at room temperature until all starting material was consumed. The solution was then diluted with DCM, washed brine, dried over anhydrous Na_2SO_4, and concentrated in vacuum. The residue was chromatographed using a silica gel column to give the pure products (**11b**–**11n**).

*5.1.9.1. Ent-15β-((cyclohexanecarbonyl)oxy)methyl-16α-hydroxybeyeran-19-oic acid (**11b**).* Yield: 92 mg (35%), starting from 200 mg of **7**; white solid; mp 91.1–91.5 °C; $[\alpha]^{25}_D$ −57 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.21 (1H, dd, J = 4.8, 10.8 Hz, H-1′a), 4.00 (1H, m, H-1′b), 3.52 (1H, d, J = 4.6 Hz, H-16), 2.33 (1H, m, COCH), 2.24–2.11 (2H, overlap, H-15, H-3), 1.25 (3H, s, H-18), 0.93 (3H, s, H-17), 0.87 (3H, s, H-20), 1.90–0.80 (27H, m, CH, CH_2 in ent-beyerane skeleton or carbon chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.23, 173.13, 85.69, 66.21, 57.46, 57.18, 54.13, 47.50, 45.25, 43.28, 42.76, 40.93, 39.40, 38.32, 38.01, 34.85, 33.14, 30.21, 29.22, 29.09, 28.13, 25.74, 25.48, 25.42, 25.07, 19.61, 18.76, 13.98; HRMS (ESI, m/z) calcd for $C_{28}H_{44}O_5Na$, 483.3087 [M + Na^+]; found, 483.3091.

*5.1.9.2. Ent-16α-hydroxy-15β-(nicotinoyloxy)methylbeyeran-19-oic acid (**11c**).* Yield: 87 mg (33%), starting from 200 mg of **7**; white solid; mp 113.5–114.0 °C; $[\alpha]^{25}_D$ −55 (c 1.4, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.21 (1H, s, 2-pyrimidine), 8.75 (1H, d, J = 4.9 Hz, 4-pyrimidine), 8.30 (1H, d, J = 8.0 Hz, 6-pyrimidine), 7.38 (1H, dd, J = 4.9, 8.0 Hz, 5-pyrimidine), 4.52 (1H, dd, J = 4.9, 10.9 Hz, H-1′a), 4.27 (1H, m, H-1′b), 3.64 (1H, d, J = 4.8 Hz, H-16), 2.34 (1H, m, H-15), 1.27 (3H, s, H-18), 0.94 (3H, s, H-17), 0.92 (3H, s, H-20); 2.25–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); 13C NMR ($CDCl_3$, 100 MHz) δ 173.22, 165.52, 153.38, 150.88, 137.28, 126.13, 123.51, 85.40, 67.34, 57.46, 57.16, 54.19, 47.41, 45.30, 42.81, 41.06, 39.35, 38.35, 37.99, 34.93, 33.13, 28.11, 25.06, 22.00, 19.59, 18.80, 14.04; HRMS (ESI, m/z) calcd for $C_{27}H_{38}NO_5$, 456.2750 [M + H^+]; found, 456.2740.

*5.1.9.3. Ent-16α-hydroxy-15β-(isonicotinoyloxy)methylbeyeran-19-oic acid (**11d**).* Yield: 84 mg (32%), starting from 200 mg of **7**; white solid; mp 139.2–140.4 °C; $[\alpha]^{25}_D$ −44 (c 1.6, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.74 (2H, d, J = 5.8 Hz, 3,5-pyrimidine), 7.85 (2H, d, J = 5.8 Hz, 2,6-pyrimidine), 4.50 (1H, dd, J = 5.4, 11.0 Hz, H-1′a), 4.25 (1H, dd, J = 8.7, 11.0 Hz, H-1′b), 3.58 (1H, d, J = 4.7 Hz, H-16), 2.35 (1H, m, H-15), 2.17 (1H, d, J = 13.2 Hz, H-3), 1.27 (3H, s, H-18), 0.97–0.91 (6H, overlap, H-17, H-20), 2.00–0.85 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.11, 165.41, 150.61, 137.35, 122.99, 85.56, 67.73, 57.43, 57.19, 54.14, 53.44, 47.34, 45.29, 42.77, 41.02, 39.34, 38.33, 37.98, 34.89, 33.07, 29.71, 28.20, 25.02, 21.98, 19.54, 18.84, 14.09; HRMS (ESI, m/z) calcd for $C_{27}H_{38}NO_5$, 456.2750 [M + H^+]; found, 456.2738.

*5.1.9.4. Ent-15β-(benzoyloxy)methyl-16α-hydroxybeyeran-19-oic acid (**11e**).* Yield: 108 mg (42%), starting from 200 mg of **7**; white solid; mp 96.7–96.9 °C; $[\alpha]^{25}_D$ −54 (c 1.1, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.03 (2H, d, J = 7.4 Hz, 2,6-Ph), 7.56 (1H, t, J = 7.4 Hz, 4-Ph), 7.43 (2H, t, J = 7.4 Hz, 3,5-Ph), 4.45 (1H, dd, J = 4.9, 10.9 Hz, H-1′a), 4.22 (1H, m, H-1′b), 3.63 (1H, d, J = 4.7 Hz, H-16), 2.32 (1H, m, H-15), 2.17 (1H, d, J = 13.0 Hz, H-3), 1.27 (3H, s, H-18), 0.96–0.90 (6H, overlap, H-17, H-20); 2.0–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.21, 166.64, 148.84, 125.57, 124.28, 122.98, 116.55, 86.48, 67.95, 61.66, 57.60, 57.21, 56.22, 54.01, 47.82, 45.30, 42.72, 41.02, 39.48, 38.33, 34.99, 33.12, 28.15, 25.12, 22.71, 22.18, 18.78, 13.93; HRMS (ESI, m/z) calcd for $C_{28}H_{37}O_5$, 453.2641 [M-H^+]; found, 453.2650.

*5.1.9.5. Ent-15β-((4-fluorobenzoyl)oxy)methyl-16α-hydroxybeyeran-19-oic acid (**11f**).* Yield: 95 mg (35%), starting from 200 mg of **7**; white solid; mp 108.1–109.1 °C; $[\alpha]^{25}_D$ −59 (c 1.4, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.05 (2H, m, 2,6-Ph), 7.10 (2H, m, 3,5-Ph), 4.44 (1H, m, H-1′a), 4.20 (1H, m, H-1′b), 3.59 (1H, d, J = 4.7 Hz, H-16), 2.32 (1H, m, H-15), 2.17 (1H, d, J = 13.4 Hz, H-3), 1.27 (3H, s, H-18), 0.96–0.90 (6H, overlap, H-17, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.14, 165.90, 132.23, 132.14, 126.38, 126.35, 115.78, 115.56, 85.71, 67.03, 57.47, 57.21, 54.18, 47.53, 45.29, 42.77, 40.98, 39.37, 38.34, 38.01, 34.90, 33.10, 28.17, 25.05, 22.03, 19.60, 18.81, 14.06; HRMS (ESI, m/z) calcd for $C_{28}H_{37}FO_5Na$, 495.2523 [M + Na^+]; found, 495.2539.

*5.1.9.6. Ent-16α-hydroxy-15β-((4-methoxybenzoyl)oxy)methylbeyeran-19-oic acid (**11g**).* Yield: 75 mg (27%), starting from 200 mg of **7**; white solid; mp 201.7–202.5 °C; $[\alpha]^{25}_D$ −53 (c 0.3, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98 (2H, d, J = 8.9 Hz, 3,5-Ph), 6.91 (2H, d, J = 8.9 Hz, 2,6-Ph), 4.40 (1H, dd, J = 5.0, 10.9 Hz, H-1′a), 4.18 (1H, dd, J = 8.9, 10.9 Hz, H-1′b), 3.86 (3H, s, OCH_3), 3.61 (1H, d, J = 4.7 Hz, H-16), 2.34 (1H, m, H-15), 2.17 (1H, d, J = 13.5 Hz, H-3), 1.26 (3H, s, H-18), 0.96–0.91 (6H, overlap, H-17, H-20), 1.80–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton);^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.13, 166.59, 163.42, 131.64, 131.64, 122.54, 113.77, 113.77, 85.63, 66.57, 57.51, 57.19, 55.44, 54.21, 47.63, 45.30, 42.80, 40.95, 39.40, 38.34, 38.03, 34.91, 33.16, 28.11, 25.09, 22.07, 19.63, 18.80, 14.07; HRMS (ESI, m/z) calcd for $C_{29}H_{40}O_6Na$, 507.2723 [M + Na^+]; found, 507.2711.

*5.1.9.7. Ent-15β-((2,3-dimethoxybenzoyl)oxy)methyl-16α-hydroxybeyeran-19-oic acid (**11h**).* Yield: 102 mg (35%), starting from 200 mg of **7**; white solid; mp 112.0–113.5 °C; $[\alpha]^{25}_D$ −52 (c 1.1, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41 (1H, dd, J = 1.8, 7.6 Hz, 6-Ph), 7.18–7.00 (2H, overlap, 3,4-Ph), 4.49 (1H, dd, J = 4.5, 10.3 Hz, H-1′a), 4.20 (1H, dd, J = 10.3, 12.1 Hz, H-1′b), 3.86 (6H, s, 2 × OCH_3), 3.57 (1H, d, J = 4.4 Hz, H-16), 2.30 (1H, m, H-15), 2.21 (1H, d, J = 3.5 Hz, H-3), 1.28 (3H, s, H-18), 0.95 (3H, s, H-17), 0.90 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.16, 166.84, 133.03, 130.14, 129.93, 129.59, 129.59, 128.51, 128.51, 85.64, 66.90, 57.48, 57.18, 54.19, 47.55, 45.28, 42.80, 41.00, 39.39, 38.34, 38.01, 34.91, 33.14, 28.11, 25.07, 24.88, 22.07, 19.62, 18.78, 14.04; HRMS (ESI, m/z) calcd for $C_{30}H_{42}O_7Na$, 537.2829 [M + Na^+]; found, 537.2843.

*5.1.9.8. Ent-15β-((3,4-dimethoxybenzoyl)oxy)methyl-16α-hydroxybeyeran-19-oic acid (**11i**).* Yield: 116 mg (40%), starting from 200 mg of **7**; white solid; mp 169.3–170.8 °C; $[\alpha]^{25}_D$ −65 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.68 (1H, d, J = 8.4 Hz, 6-Ph), 7.55 (1H, s, 2-Ph), 6.89 (1H, d, J = 8.4 Hz, 5-Ph), 4.41 (1H, dd, J = 4.7, 10.9 Hz, H-1′a), 4.22 (1H, dd, J = 8.6, 10.9 Hz, H-1′b), 3.93 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.64 (1H, d, J = 4.6 Hz, H-16), 2.32 (1H, m, H-15), 2.17 (1H, d, J = 13.5 Hz, H-3), 1.27 (3H, s, H-18), 0.96–0.90 (6H, overlap, H-17, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.15, 166.63, 153.06, 148.69, 123.64, 122.65, 112.01, 110.44, 85.47, 66.56, 57.51, 57.19, 56.04, 56.04, 54.26, 47.59, 45.29, 42.79, 40.97, 39.39, 38.34, 38.01, 34.92, 33.17, 28.12, 25.11, 22.08, 19.64, 18.79, 14.06; HRMS (ESI, m/z) calcd for $C_{30}H_{42}O_7K$, 553.2568 [M + K^+]; found, 553.2562.

*5.1.9.9. Ent-16α-hydroxy-15β-((2,3,4-trimethoxybenzoyl)oxy)methylbeyeran-19-oic acid (**11j**).* Yield: 70 mg (23%), starting from 200 mg of **7**; white solid; mp 96.6–97.2 °C; $[\alpha]^{25}_D$ −54 (c 2.1, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.62 (1H, d, J = 9.0 Hz, 6-Ph), 6.68 (1H, d, J = 9.0 Hz, 5-Ph), 4.40 (1H, dd, J = 4.6, 10.2 Hz, H-1′a), 4.09 (1H, m, H-1′b), 3.84 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.49 (1H, d, J = 4.5 Hz, H-16), 3.21 (1H, s, 16-OH), 2.23 (1H, m, H-15), 2.12 (1H, d, J = 13.7 Hz, H-3), 1.21 (3H, s, H-18), 0.89 (3H, s, H-17), 0.83 (3H, s, H-20), 1.85–0.75 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.13, 165.07, 156.57, 153.08, 141.89, 126.68, 116.36, 106.47, 85.73, 66.72, 60.99, 60.04, 56.58, 56.18, 55.11, 53.03, 46.88, 44.28, 41.61, 39.94, 38.44, 37.31, 33.95, 32.11, 28.68, 27.15, 24.11, 21.16, 18.67, 17.77, 12.92; HRMS (ESI, m/z) calcd for $C_{31}H_{44}O_8Na$, 567.2934 [M + Na^+]; found, 567.2909.

*5.1.9.10. Ent-16α-hydroxy-15β-((3,4,5-trimethoxybenzoyl)oxy)methylbeyeran-19-oic acid (**11k**).* Yield: 97 mg (31%), starting from 200 mg of **7**; white solid; mp 114.4–115.8 °C; $[\alpha]^{25}_D$ −35 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (2H, s, 2,6-Ph), 4.43 (1H, dd, J = 4.3, 10.9 Hz, H-1′a), 4.27 (1H, dd, J = 8.8, 10.9 Hz, H-1′b), 3.90 (3H, s, OCH_3), 3.90 (6H, s, 2 × OCH_3), 3.69 (1H, d, J = 4.7 Hz, H-16), 2.35 (1H, m, H-15), 2.17 (1H, d, J = 13.4 Hz, H-3), 1.28 (3H, s, H-18), 0.94 (3H, s, H-17), 0.92 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.18, 166.43, 153.02, 153.02, 142.28, 125.21, 106.83, 106.83, 85.36, 66.66, 60.94, 57.48, 57.18, 56.28, 56.28, 54.31, 47.47, 45.25, 42.80, 41.04, 39.36, 34.93, 33.16, 31.45, 30.20, 28.13, 25.12, 22.11, 19.65, 18.73, 14.04; HRMS (ESI, m/z) calcd for $C_{31}H_{44}O_8K$, 583.2673 [M + K^+]; found, 583.2665.

*5.1.9.11. Ent-15β-(((E)-3-(3,5-dimethoxyphenyl)acryloyl)oxy)methyl-16α-hydroxybeyeran-19-oic acid (**11l**).* Yield: 120 mg (39%), starting from 200 mg of **7**; white solid; mp 127.3–128.5 °C; $[\alpha]^{25}_D$ −56 (c 1.9, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.61 (1H, d, J = 16.0 Hz, CH_2 =), 6.68 (2H, d, J = 2.3 Hz, 2,6-Ph), 6.49 (1H, t, J = 2.3 Hz, 4-Ph), 6.44 (1H, d, J = 16.0 Hz, CH_2 =), 4.33 (1H, dd, J = 5.0, 10.8 Hz, H-1′a), 4.14 (1H, m, H-1′b), 3.81 (6H, s, 2 × OCH_3), 3.56 (1H, d, J = 4.6 Hz, H-16), 2.26 (1H, m, H-15), 2.16 (1H, d, J = 13.1 Hz, H-3), 1.26 (3H, s, H-18), 0.93 (3H, s, H-17), 0.89 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.14, 167.16, 161.02, 161.02, 145.18, 136.27, 118.40, 106.07, 106.07, 102.82, 85.76, 66.72, 57.45, 57.17, 55.47, 55.47, 54.08, 47.44, 45.28, 42.81, 40.99, 39.38, 38.32, 34.88, 33.16, 29.72, 28.08, 25.04, 22.05, 19.62, 18.77, 13.94; HRMS (ESI, m/z) calcd for $C_{32}H_{44}O_7Na$, 563.2985 [M + Na^+]; found, 563.2996.

*5.1.9.12. Ent-16α-hydroxy-15β-(2-(4-methoxyphenyl)acetoxy)methylbeyeran-19-oic acid (**11m**).* Yield: 84 mg (30%), starting from 200 mg of **7**; white solid; mp 75.0–76.4 °C; $[\alpha]^{25}_D$ −60 (c 0.9, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.18 (2H, d, J = 8.6 Hz, 2,6-Ph), 6.85 (2H, d, J = 8.6 Hz, 3,5-Ph), 4.21 (1H, dd, J = 5.1, 10.7 Hz, H-1′a), 3.96 (1H, t, J = 10.2 Hz, H-1′b), 3.78 (3H, s, OCH_3), 3.59 (2H, s, $COCH_2$), 3.33 (1H, d, J = 4.6 Hz, H-16), 2.20–2.08 (2H, overlap, H-15, H-3), 1.23 (3H, s, H-18), 0.87 (3H, s, H-17), 0.86 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.22, 172.09, 158.76, 130.30, 130.30, 126.20, 114.15, 114.15, 85.49, 66.88, 57.41, 57.15, 55.31, 53.95, 47.39, 45.24, 42.70, 40.85, 40.71, 39.34, 38.29, 37.98, 34.88, 34.78, 33.10, 28.09, 24.94, 22.02, 19.58, 13.92; HRMS (ESI, m/z) calcd for $C_{30}H_{43}O_6$, 499.3059 [M + H^+]; found, 499.3080.

*5.1.9.13. Ent-16α-hydroxy-15β-(2-(3,4,5-trimethoxyphenyl)acetoxy)methylbeyeran-19-oic acid (**11n**).* Yield: 109 mg (34%), starting from 200 mg of **7**; white solid; mp 174.0–175.4 °C; $[\alpha]^{25}_D$ −51 (c 2.6, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.51 (2H, s, 2,6-Ph), 4.25 (1H, dd, J = 5.1, 10.8 Hz, H-1′a), 3.99 (1H, t, J = 10.0 Hz, H-1′b), 3.85 (6H, s, 2 × OCH_3), 3.82 (3H, s, OCH_3), 3.59 (2H, s, $COCH_2$), 3.37 (1H, d, J = 4.7 Hz, H-16), 2.23–2.07 (2H, overlap, H-15, H-3), 1.24 (3H, s, H-18), 0.90–0.81 (6H, overlap, H-17, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.20,

171.69, 153.34, 137.19, 129.63, 106.33, 106.33, 85.39, 66.87, 60.87, 57.37, 57.14, 56.16, 56.16, 53.94, 47.45, 45.24, 42.70, 41.85, 40.82, 39.30, 38.29, 37.96, 34.78, 33.02, 31.45, 28.15, 24.93, 22.01, 19.55, 18.74, 13.94; HRMS (ESI, m/z) calcd for $C_{32}H_{45}O_8$, 557.3115 [M-H$^+$]; found, 557.3111.

*5.1.10. General procedures for the synthesis of **12a**–**12n***

Products **12a**–**12n** were synthesized from **11a**–**11n** following the same procedure described for preparation of **9**.

*5.1.10.1. Ent-15β -acetoxymethyl-16-oxobeyeran-19-oic acid **12a**.* Yield: 47 mg (47%), starting from 100 mg of **11a**; white solid; mp 159.6–160.3 °C; $[\alpha]^{25}_D$ −30 (c 1.4, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.30 (2H, m, H-1′), 2.66 (1H, m, H-15), 2.16 (1H, d, J = 13.3 Hz, H-3), 2.03 (3H, s, $COCH_3$), 1.25 (3H, s, H-18), 0.97 (3H, s, H-17), 0.78 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.74, 183.53, 170.67, 62.28, 56.93, 53.00, 51.21, 48.11, 43.59, 40.65, 39.51, 38.45, 35.47, 31.52, 30.15, 29.71, 28.92, 21.46, 20.85, 19.79, 19.60, 18.75, 13.17; HRMS (ESI, m/z) calcd for $C_{23}H_{34}O_5Na$, 413.2304 [M + Na$^+$]; found, 413.2301.

*5.1.10.2. Ent-15β-((cyclohexanecarbonyl)oxy)methyl-16-oxobeyeran-19-oic acid (**12b**).* Yield: 46 mg (45%), starting from 103 mg of **11b**; white solid; mp 145.3–146.6 °C; $[\alpha]^{25}_D$ −68 (c 2.4, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.34 (1H, dd, J = 4.5, 11.4 Hz, H-1′a), 4.23 (1H, dd, J = 3.2, 11.4 Hz, H-1′b), 2.58 (1H, m, H-15), 2.32–2.13 (2H, overlap, COCH, H-3), 1.26 (3H, s, H-18), 0.98 (3H, s, H-17), 0.82 (3H, s, H-20), 1.90–0.80 (27H, m, CH, CH_2 in ent-beyerane skeleton or carbon chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.47, 175.54, 172.85, 61.81, 57.19, 56.98, 53.09, 50.70, 48.14, 45.26, 43.14, 40.55, 39.42, 38.40, 37.93, 37.13, 35.33, 29.71, 28.99, 28.91, 28.16, 25.73, 25.43, 25.37, 21.57, 19.79, 18.74, 14.19; HRMS (ESI, m/z) calcd for $C_{28}H_{43}O_5$, 459.3110 [M + H$^+$]; found, 459.3090.

*5.1.10.3. Ent-15β-(nicotinoyloxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12c**).* Yield: 68 mg (66%), starting from 106 mg of **11c**; white solid; mp 108.9–109.6 °C; $[\alpha]^{25}_D$ −40 (c 1.1, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.15 (1H, s, 2-pyrimidine), 8.77 (1H, d, J = 3.7 Hz, 4-pyrimidine), 8.27 (1H, m, 6-pyrimidine), 7.39 (1H, dd, J = 4.8, 8.0 Hz, 5-pyrimidine), 4.66 (1H, dd, J = 4.9, 11.5 Hz, H-1′a), 4.51 (1H, dd, J = 3.6, 11.5 Hz, H-1′b), 2.75 (1H, m, H-15), 2.21 (1H, d, J = 12.8 Hz, H-3), 1.26 (3H, s, H-18), 1.03 (3H, s, H-17), 0.90 (3H, s, H-20), 2.00–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.01, 172.91, 164.93, 153.53, 150.75, 137.27, 125.76, 123.51, 62.95, 57.13, 56.86, 53.23, 50.72, 48.28, 45.31, 40.60, 39.34, 38.44, 37.08, 35.45, 31.45, 28.19, 21.57, 19.96, 19.70, 18.80, 14.28; HRMS (ESI, m/z) calcd for $C_{27}H_{35}NO_5Na$, 476.2413 [M + Na$^+$]; found, 476.2382.

*5.1.10.4. Ent-15β-(isonicotinoyloxy)methyl-16-oxobeyeranbeyeran-19-oic acid **(12d)**.* Yield: 16 mg (35%), starting from 46 mg of **11d**; white solid; mp 72.3–72.9 °C; $[\alpha]^{25}_D$ −56 (c 2.0, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.76 (2H, d, J = 5.5 Hz, 3,5-pyrimidine), 7.81 (2H, d, J = 5.5 Hz, 2,6-pyrimidine), 4.61 (1H, dd, J = 5.6, 11.5 Hz, H-1′a), 4.50 (1H, dd, J = 3.8, 11.5 Hz, H-1′b), 2.79 (1H, m, H-15), 2.20 (1H, d, J = 13.6 Hz, H-3), 1.26 (3H, s, H-18), 1.03 (3H, s, H-17), 0.92 (3H, s, H-20), 2.00–0.85 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 220.82, 172.93, 164.88, 150.69, 150.69, 136.97, 124.00, 122.91, 63.34, 57.15, 56.81, 53.18, 50.84, 48.26, 45.32, 40.64, 39.31, 38.45, 37.05, 35.52, 31.45, 30.21, 28.23, 21.55, 19.99, 18.84, 14.33; HRMS (ESI, m/z) calcd for $C_{27}H_{35}NO_5K$, 492.2152 [M + K$^+$]; found, 492.2167.

*5.1.10.5. Ent-15β-(benzoyloxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12e**).* Yield: 54 mg (58%), starting from 93 mg of **11e**; white solid; mp 98.0–98.9 °C; $[\alpha]^{25}_D$ −79 (c 1.7, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (2H, d, J = 7.7 Hz, 2,6-Ph), 7.54 (1H, t, J = 7.7 Hz, 4-Ph), 7.42 (2H, t, J = 7.7 Hz, 3,5-Ph), 4.60 (1H, dd, J = 4.8, 11.4 Hz, H-1′a), 4.49 (1H, dd, J = 3.3, 11.4 Hz, H-1′b), 2.73 (1H, m, H-15), 2.21 (1H, d, J = 13.1 Hz, H-3), 1.30 (3H, s, H-18), 1.04 (3H, s, H-17), 0.90 (3H, s, H-20), 2.0–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.32, 172.87, 166.27, 133.08, 129.61, 128.47, 129.61, 128.47, 62.57, 57.18, 56.96, 53.22, 50.93, 48.24, 45.31, 40.63, 39.40, 38.45, 37.94, 37.17, 35.44, 28.18, 22.70, 21.60, 19.93, 19.73, 18.81, 14.29; HRMS (ESI, m/z) calcd for $C_{28}H_{36}O_5Na$, 475.2461 [M + Na$^+$]; found, 475.2465.

*5.1.10.6. Ent-15β-((4-fluorobenzoyl)oxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12f**).* Yield: 102 mg (63%), starting from 162 mg of **11f**; white solid; mp 205.4–206.2 °C; $[\alpha]^{25}_D$ −90 (c 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (2H, m, 2,6-Ph), 7.09 (2H, m, 3,5-Ph), 4.56 (1H, dd, J = 5.5, 11.4 Hz, H-1′a), 4.46 (1H, dd, J = 3.6, 11.4 Hz, H-1′b), 2.76 (1H, m, H-15), 2.20 (1H, d, J = 13.4 Hz, H-3), 1.30 (3H, s, H-18), 1.02 (3H, s, H-17), 0.91 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.13, 172.88, 165.34, 132.26, 132.26, 132.17, 115.78, 115.78, 115.56, 62.71, 57.21, 56.89, 53.21, 51.04, 48.21, 45.32, 40.65, 39.36, 38.44, 37.92, 37.11, 35.49, 28.27, 21.58, 19.96, 19.69, 18.84, 14.32; HRMS (ESI, m/z) calcd for $C_{28}H_{35}FO_5Na$, 493.2367 [M + Na$^+$]; found, 493.2342.

*5.1.10.7. Ent-15β-((4-methoxybenzoyl)oxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12g**).* Yield: 41 mg (41%), starting from 100 mg of **11g**; white solid; mp 202.2–202.9 °C; $[\alpha]^{25}_D$ −80 (c 1.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (2H, d, J = 8.9 Hz, 3,5-Ph), 6.89 (2H, d, J = 8.9 Hz, 2,6-Ph), 4.54 (1H, dd, J = 5.1, 11.5 Hz, H-1′a), 4.46 (1H, dd, J = 3.3, 11.5 Hz, H-1′b), 3.84 (3H, s, OCH_3), 2.73 (1H, m, H-15), 2.20 (1H, d, J = 13.4 Hz, H-3), 1.26 (3H, s, H-18), 1.03 (3H, s, H-17), 0.90 (3H, s, H-20), 1.80–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.42, 172.81, 166.01, 163.46, 131.68, 131.68, 122.18, 113.74, 113.74, 62.32, 57.22, 56.96, 55.42, 53.23, 51.12, 48.20, 45.31, 40.65, 39.41, 38.44, 35.45, 31.45, 30.21, 28.24, 21.61, 19.95, 19.72, 18.83, 14.33; HRMS (ESI, m/z) calcd for $C_{29}H_{38}O_6Na$, 505.2566 [M + Na$^+$]; found, 505.2575.

*5.1.10.8. Ent-15β-((2,3-dimethoxybenzoyl)oxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12h**).* Yield: 29 mg (30%), starting from 29 mg of **11h**; white solid; mp 117.3–118.6 °C; $[\alpha]^{25}_D$ −67 (c 2.2, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (1H, d, J = 2.0 Hz, 6-Ph), 7.15–6.98 (2H, overlap, 3,4-Ph), 4.58 (1H, dd, J = 5.6, 11.5 Hz, H-1′a), 4.50 (1H, dd, J = 3.4, 11.5 Hz, H-1′b), 3.87 (6H, s, 2 × OCH_3), 2.74 (1H, m, H-15), 2.17 (1H, d, J = 13.2 Hz, H-3), 1.24 (3H, s, H-18), 0.98 (3H, s, H-17), 0.82 (3H, s, H-20), 2.00–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.42, 172.94, 165.44, 153.48, 149.40, 125.70, 123.76, 122.23, 115.95, 62.47, 61.67, 57.20, 57.02, 56.10, 52.88, 50.66, 48.21, 45.28, 40.59, 39.46, 38.43, 37.98, 37.22, 35.34, 28.15, 21.64, 19.80, 19.73, 18.73, 14.23; HRMS (ESI, m/z) calcd for $C_{30}H_{39}O_7$, 511.2696 [M-H$^+$]; found, 511.2647.

*5.1.10.9. Ent-15β-((3,4-dimethoxybenzoyl)oxy)methyl-16-oxobeyeranbeyeran-19-oic acid (**12i**).* Yield: 60 mg (58%), starting from 104 mg of **11i**; white solid; mp 105.3–106.0 °C; $[\alpha]^{25}_D$ −72 (c 0.7, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (1H, dd, J = 2.0, 8.5 Hz, 6-Ph), 7.47 (1H, d, J = 2.0 Hz, 2-Ph), 6.88 (1H, d, J = 8.5 Hz, 5-Ph), 4.57 (1H, dd, J = 4.8, 11.4 Hz, H-1′a), 4.50 (1H, dd, J = 3.3, 11.4 Hz, H-1′b), 3.92 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 2.72 (1H, m, H-15), 2.20 (1H, d, J = 13.4 Hz, H-3), 1.26 (3H, s, H-18), 1.04 (3H, s, H-17), 0.89 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane

skeleton); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 221.36, 172.86, 166.01, 153.10, 148.66, 122.28, 111.92, 110.37, 62.40, 57.17, 56.99, 56.02, 56.02, 53.24, 50.97, 48.20, 45.31, 39.43, 38.45, 37.95, 37.16, 35.43, 31.45, 30.20, 28.15, 21.61, 19.96, 19.73, 18.78, 14.28; HRMS (ESI, m/z) calcd for $C_{30}H_{40}O_7K$, 551.2411 [M + K^+]; found, 551.2438.

*5.1.10.10. Ent-16-oxo-15β-((2,3,4-trimethoxybenzoyl)oxy)methyl-beyeran-19-oic acid (**12j**).* Yield: 49 mg (70%), starting from 70 mg of **11j**; white solid; mp 96.7–97.6 °C; $[\alpha]^{25}_D$ −54 (c 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (1H, d, J = 9.0 Hz, 6-Ph), 6.68 (1H, d, J = 9.0 Hz, 5-Ph), 4.52 (1H, dd, J = 4.7, 11.4 Hz, H-1′a), 4.46 (1H, dd, J = 3.6, 11.4 Hz, H-1′b), 3.90 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 2.69 (1H, m, H-15), 2.20 (1H, d, J = 13.4 Hz, H-3), 1.25 (3H, s, H-18), 1.01 (3H, s, H-17), 0.88 (3H, s, H-20), 2.00–0.75 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.44, 172.88, 164.65, 157.22, 155.03, 142.90, 126.91, 117.40, 106.83, 62.15, 61.87, 61.05, 57.19, 57.01, 56.05, 52.90, 50.83, 48.21, 45.30, 40.61, 39.46, 38.44, 37.99, 37.22, 35.39, 28.16, 21.64, 19.82, 19.73, 18.75, 14.26; HRMS (ESI, m/z) calcd for $C_{31}H_{42}O_8K$, 581.2517 [M + K^+]; found, 581.2511.

*5.1.10.11. Ent-16-oxo-15β-((3,4,5-trimethoxybenzoyl)oxy)methyl-beyeran-19-oic acid (**12k**).* Yield: 75 mg (58%), starting from 130 mg of **11k**; white solid; mp 109.9–110.8 °C; $[\alpha]^{25}_D$ −50 (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 (2H, s, 2,6-Ph), 4.60 (1H, dd, J = 4.4, 11.4 Hz, H-1′a), 4.53 (1H, dd, J = 3.4, 11.4 Hz, H-1′b), 3.78–3.95 (9H, overlap, 3 × OCH_3), 2.71 (1H, d, J = 4.1 Hz, H-15), 2.20 (1H, d, J = 13.2 Hz, H-3), 1.30 (3H, s, H-18), 1.05 (3H, s, H-17), 0.87 (3H, s, H-20), 1.90–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.26, 172.89, 165.84, 152.97, 152.97, 124.78, 107.64, 106.79, 106.79, 60.94, 57.18, 57.01, 56.63, 56.25, 53.25, 50.76, 48.21, 48.18, 45.28, 43.48, 40.65, 38.45, 37.15, 35.42, 28.12, 21.59, 19.98, 19.75, 18.76, 14.26, 13.33; HRMS (ESI, m/z) calcd for $C_{31}H_{42}O_8Na$, 565.2778 [M + Na^+]; found, 565.2752.

*5.1.10.12. Ent-15β-(((E)-3-(3,5-dimethoxyphenyl)acryloyl)oxy) methyl-16-oxobeyeran-19-oic acid (**12l**).* Yield: 53 mg (53%), starting from 100 mg of **11l**; white solid; mp 149.3–150.0 °C; $[\alpha]^{25}_D$ −99 (c 1.1, CH_2Cl_2); 1H NMR ($CDCl_3$, 400 MHz) δ 7.57 (1H, d, J = 16.0 Hz, CH2 =), 6.66 (2H, d, J = 2.3 Hz, 2,6-Ph), 6.49 (1H, t, J = 2.3 Hz, 4-Ph), 6.34 (1H, d, J = 16.0 Hz, CH2 =), 4.45 (2H, m, H-1′), 3.80 (6H, s, 2 × OCH3), 2.71 (1H, m, H-15), 2.15 (1H, d, J = 13.4 Hz, H-3), 1.24 (3H, s, H-18), 1.01 (3H, s, H-17), 0.81 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); 13C NMR ($CDCl_3$, 150 MHz) δ 221.78, 182.02, 166.46, 161.01, 161.01, 145.16, 136.21, 118.22, 106.10, 106.10, 102.53, 62.43, 56.98, 56.98, 55.43, 55.43, 53.05, 51.24, 48.18, 43.52, 40.76, 39.55, 38.46, 37.69, 37.22, 35.51, 28.84, 21.55, 19.90, 19.64, 18.76, 13.26; HRMS (ESI, m/z) calcd for $C_{32}H_{43}O_7$, 539.3009 [M + H^+]; found, 539.3019.

*5.1.10.13. Ent-15β-(2-(4-methoxyphenyl)acetoxy)methyl-16-oxobeyeran-19-oic acid (**12m**).* Yield: 54 mg (56%), starting from 96 mg of **11m**; white solid; mp 87.1–87.9 °C; $[\alpha]^{25}_D$ −84 (c 1.3, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 (2H, d, J = 8.6 Hz, 2,6-Ph), 6.84 (2H, d, J = 8.6 Hz, 3,5-Ph), 4.34 (1H, dd, J = 4.9, 11.4 Hz, H-1′a), 4.24 (1H, dd, J = 3.2, 11.4 Hz, H-1′b), 3.78 (3H, s, OCH_3), 3.52 (2H, d, J = 3.5 Hz, $COCH_2$), 2.55 (1H, m, H-15), 2.16 (1H, d, J = 13.5 Hz, H-3), 1.27 (3H, s, H-18), 0.88 (3H, s, H-17), 0.80 (3H, s, H-20); 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.45, 172.90, 171.40, 158.74, 130.45, 130.45, 125.81, 114.07, 114.07, 62.38, 57.14, 56.91, 55.32, 52.65, 50.74, 48.01, 45.26, 40.56, 40.44, 39.38, 38.37, 37.94, 37.07, 35.23, 28.14, 21.53, 19.66, 19.63, 18.74, 14.17; HRMS (ESI, m/z) calcd for $C_{30}H_{40}O_6Na$, 519.2723 [M + Na^+]; found, 519.2747.

*5.1.10.14. Ent-16-oxo-15β-(2-(3,4,5-trimethoxyphenyl)acetoxy)methylbeyeran-19-oic acid (**12n**).* Yield: 51 mg (50%), starting from 102 mg of **11n**; white solid; mp 85.1–86.0 °C; $[\alpha]^{25}_D$ −56 (c 1.9, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.44 (2H, s, 2,6-Ph), 4.38 (1H, dd, J = 4.8, 11.4 Hz, H-1′a), 4.25 (1H, dd, J = 3.2, 11.4 Hz, H-1′b), 3.84 (6H, s, 2 × OCH_3), 3.82 (3H, s, OCH_3), 3.60–3.43 (2H, m, $COCH_2$), 2.56 (1H, m, H-15), 2.16 (1H, d, J = 13.6 Hz, H-3), 1.27 (3H, s, H-18), 0.86 (3H, s, H-17), 0.80 (3H, s, H-20), 1.85–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.35, 172.89, 170.99, 153.29, 153.29, 137.07, 129.31, 106.35, 106.35, 62.50, 60.84, 57.10, 56.85, 56.11, 56.11, 52.62, 50.66, 48.00, 45.27, 41.79, 40.43, 39.33, 38.38, 37.91, 37.00, 35.26, 28.15, 21.50, 19.63, 19.59, 18.75, 14.20; HRMS (ESI, m/z) calcd for $C_{32}H_{44}O_8K$,595.2673 [M + K^+]; found, 595.2688.

*5.1.11. Synthesis of ent-4-amino-15β-hydroxymethyl-19-norbeyeran-16α-ol (**13**)*

Compound **13** was synthesized from **7** following the same procedure described for preparation of **6**. Yield: 34 mg (37%), starting from 100 mg of **7**; white solid; mp 180.2–181.6 °C; $[\alpha]^{25}_D$ −49 (c 1.6, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.97 (1H, dd, J = 5.1, 9.8 Hz, H-1′a), 3.62 (1H, d, J = 4.9 Hz, H-16), 3.52 (1H, t, J = 10.2 Hz, H-1′b), 2.12 (1H, m, H-15), 1.34 (3H, s, H-18), 1.07 (3H, s, H-17), 0.94 (3H, s, H-20); 2.0–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 84.07, 64.88, 59.14, 57.55, 55.45, 55.15, 50.35, 47.80, 42.03, 41.58, 40.85, 40.14, 38.34, 33.85, 32.97, 31.87, 24.95, 19.16, 17.88, 13.76; HRMS (ESI, m/z) calcd for $C_{20}H_{36}NO_2$, 322.2746 [M + H^+]; found, 322.2725.

*5.1.12. General procedures for the synthesis of **14a–14e***

Products 14a-14e were synthesized from 13 following the same procedure described for preparation of 11a-11n.

*5.1.12.1. Ent-4-amino-16α-hydroxy-19-norbeyeran-15β-methyl nicotinate (**14a**).* Yield: 113 mg (43%), starting from 200 mg of **13**; white solid; mp 76.9–77.3 °C; $[\alpha]^{25}_D$ −45 (c 1.6, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.25 (1H, s, 2-pyrimidine), 8.77 (1H, dd, J = 1.8, 4.9 Hz, 4-pyrimidine), 8.32 (1H, m, 6-pyrimidine), 7.41 (1H, dd, J = 4.9, 7.9 Hz, 5-pyrimidine), 4.59 (1H, dd, J = 6.1, 11.0 Hz, H-1′a), 4.29 (1H, dd, J = 8.2, 11.0 Hz, H-1′b), 3.59 (1H, d, J = 5.0 Hz, H-16), 2.47 (1H, m, H-15), 1.33 (3H, s, H-18), 1.11 (3H, s, H-17), 0.94 (3H, s, H-20), 1.95–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.56, 153.48, 150.94, 137.08, 126.13, 123.48, 85.32, 67.20, 59.08, 57.45, 54.98, 54.44, 47.44, 42.53, 41.68, 40.93, 38.34, 37.68, 33.73, 32.96, 31.84, 24.95, 20.38, 19.12, 17.87, 13.76; HRMS (ESI, m/z) calcd for $C_{26}H_{39}N_2O_3$, 427.2960 [M + H^+]; found, 427.2951.

*5.1.12.2. Ent-4-amino-16α-hydroxy-19-norbeyeran-15β-methyl 4-methoxybenzoate (**14b**).* Yield: 93 mg (33%), starting from 200 mg of **13**; white solid; mp 173.4–175.2 °C; $[\alpha]^{25}_D$ −54 (c 0.4, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (2H, d, J = 8.2 Hz, 3,5-Ph), 6.87 (2H, d, J = 8.2 Hz, 2,6-Ph), 4.43 (1H, dd, J = 6.5, 11.0 Hz, H-1′a), 4.16 (1H, dd, J = 7.7, 11.0 Hz, H-1′b), 3.79 (3H, s, OCH_3), 3.48 (1H, d, J = 5.0 Hz, H-16), 2.37 (1H, m, H-15), 1.25 (3H, s, H-18), 1.05 (3H, s, H-17), 0.86 (3H, s, H-20), 1.95–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.60, 162.45, 130.58, 130.58, 121.51, 112.78, 112.78, 84.41, 65.41, 58.07, 56.48, 54.43, 53.94, 53.46, 46.69, 41.49, 40.78, 39.77, 37.36, 36.67, 32.70, 31.96, 30.84, 23.95, 19.38, 18.13, 16.88, 12.75; HRMS (ESI, m/z) calcd for $C_{28}H_{42}NO_4$, 456.3114 [M + H^+]; found, 456.3079.

*5.1.12.3. Ent-4-amino-16α-hydroxy-19-norbeyeran-15β-methyl 2,3-dimethoxybenzoate (**14c**).* Yield: 110 mg (36%), starting from 200 mg of **13**; white solid; mp 164.4–165.2 °C; $[\alpha]^{25}_D$ −67 (c 0.7,

CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40 (1H, dd, J = 1.8, 7.7 Hz, 6-Ph), 7.12 (2H, m, 3,4-Ph), 4.63 (1H, dd, J = 5.1, 10.4 Hz, H-1′a), 4.13 (1H, t, J = 10.9 Hz, H-1′b), 3.91 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.60 (1H, m, H-16), 3.08 (1H, s, 16-OH), 2.35 (1H, m, H-15), 1.86 (1H, dd, J = 5.2, 13.8 Hz, H-3), 1.37 (3H, s, H-18), 1.03 (3H, s, H-17), 0.96 (3H, s, H-20), 1.95–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.85, 154.52, 153.52, 125.70, 124.37, 122.70, 116.49, 86.34, 67.83, 61.68, 57.92, 56.22, 56.08, 55.86, 54.12, 47.77, 42.53, 41.00, 38.69, 37.68, 35.70, 34.22, 32.90, 26.95, 25.04, 19.70, 19.14, 17.43, 14.29; HRMS (ESI, m/z) calcd for $C_{29}H_{43}NO_5Na$, 508.3039 [M + Na^+]; found, 508.3063.

*5.1.12.4. Ent-4-amino-16α-hydroxy-19-norbeyeran-15β-methyl 2,3,4-trimethoxybenzoate (**14d**).* Yield: 79 mg (55%), starting from 90 mg of **13**; white solid; mp 51.7–52.9 °C; $[\alpha]^{25}_D$ −70 (c 0.4, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.71 (1H, d, J = 8.9 Hz, 2-Ph), 6.76 (1H, d, J = 8.9 Hz, 3-Ph), 4.56 (1H, dd, J = 5.0, 10.3 Hz, H-1′a), 4.18 (1H, dd, J = 10.3, 11.9 Hz, H-1′b), 3.94 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.58 (1H, d, J = 4.7 Hz, H-16), 2.40 (1H, m, H-15), 1.87 (1H, m, H-3), 1.35 (3H, s, H-18), 1.09 (3H, s, H-17), 0.97 (3H, s, H-20), 1.95–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.24, 157.59, 154.08, 142.90, 127.64, 117.48, 107.49, 86.61, 67.69, 62.00, 61.07, 59.07, 57.53, 56.13, 55.05, 54.35, 47.91, 42.40, 41.72, 40.92, 38.36, 37.68, 33.78, 32.98, 31.88, 25.08, 20.48, 19.22, 17.87, 13.64; HRMS (ESI, m/z) calcd for $C_{30}H_{46}NO_6$, 516.3325 [M + H^+]; found, 516.3315.

*5.1.12.5. Ent-4-amino-16α-hydroxy-19-norbeyeran-15β-methyl 3,4,5-trimethoxybenzoate (**14e**).* Yield: 61 mg (38%), starting from 100 mg of **13**; white solid; mp 75.4–76.6 °C; $[\alpha]^{25}_D$ −24 (c 1.7, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33 (2H, s, 2,6-Ph), 4.55 (1H, dd, J = 5.5, 10.9 Hz, H-1′a), 4.26 (1H, dd, J = 8.3, 10.9 Hz, H-1′b), 3.91 (6H, s, 2 × OCH_3), 3.90 (3H, s, OCH_3), 3.64 (1H, d, J = 5.0 Hz, H-16), 2.44 (1H,m, H-15), 1.34 (3H, s, H-18), 1.10 (3H, s, H-17), 0.94 (3H, s, H-20), 1.95–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.45, 153.03, 153.03, 142.31, 125.20, 106.81, 106.81, 85.38, 66.69, 60.93, 59.08, 57.55, 56.25, 56.25, 55.04, 54.55, 47.59, 42.57, 41.73, 40.92, 38.38, 37.69, 33.75, 32.98, 31.86, 25.01, 20.38, 19.18, 17.88, 13.78; HRMS (ESI, m/z) calcd for $C_{30}H_{46}NO_6$, 516.3325 [M + H^+]; found, 516.3319.

*5.1.13. General procedures for the synthesis of **15a**–**15e***

Products **15a**–**15e** were synthesized from **14a**–**14e** following the same procedure described for preparation of **12a**–**12n**.

*5.1.13.1. Ent-4-amino-16-oxo-19-norbeyeran-15β-methyl nicotinate (**15a**).* Yield: 45 mg (57%), starting from 80 mg of **14a**; white solid; mp 124.8–125.6 °C; $[\alpha]^{25}_D$ −48 (c 0.9, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.18 (1H, s, 2-pyrimidine), 8.78 (1H, d, J = 4.9 Hz, 4-pyrimidine), 8.26 (1H, d, J = 8.0 Hz, 6-pyrimidine), 7.42 (1H, dd, J = 4.9, 8.0 Hz, 5-pyrimidine), 4.67 (1H, dd, J = 6.6, 11.6 Hz, H-1′a), 4.50 (1H, dd, J = 3.3, 11.6 Hz, H-1′b), 2.91 (1H, m, H-15), 1.96 (1H, d, J = 13.8 Hz, H-3), 1.35 (3H, s, H-18), 1.07 (3H, s, H-17), 1.02 (3H, s, H-20), 1.95–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 220.92, 165.03, 153.65, 150.83, 137.00, 125.69, 123.54, 63.28, 58.97, 56.74, 55.12, 53.39, 51.39, 48.19, 41.43, 40.48, 38.31, 37.79, 36.88, 34.40, 31.81, 19.93, 19.93, 19.23, 17.86, 14.14; HRMS (ESI, m/z) calcd for $C_{26}H_{37}N_2O_3$, 425.2804 [M + H^+]; found, 425.2797.

*5.1.13.2. Ent-4-amino-16-oxo-19-norbeyeran-15β-methyl 4-methoxybenzoate (**15b**).* Yield: 35 mg (52%), starting from 68 mg of **14b**; white solid; mp 118.1–119.5 °C; $[\alpha]^{25}_D$ −48 (c 2.7, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.95 (2H, d, J = 8.7 Hz, 3,5-Ph), 6.93 (2H, d, J = 8.7 Hz, 2,6-Ph), 4.56 (1H, dd, J = 7.1, 11.5 Hz, H-1′a), 4.42 (1H, dd, J = 2.9, 11.5 Hz, H-1′b), 3.85 (3H, s, OCH_3), 2.92 (1H, m, H-15), 1.96 (1H, m, H-3), 1.34 (3H, s, H-18), 1.09 (3H, s, H-17), 1.01 (3H, s, H-20), 1.95–0.80 (17H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.28, 166.10, 163.55, 131.59, 131.59, 122.12, 113.84, 113.84, 62.70, 58.98, 56.78, 55.45, 55.11, 53.36, 51.85, 48.11, 41.53, 40.55, 38.34, 37.80, 36.93, 34.44, 31.83, 19.97, 19.93, 19.24, 17.90, 14.16; HRMS (ESI, m/z) calcd for $C_{28}H_{40}NO_4$, 454.2957 [M + H^+]; found, 454.2945.

*5.1.13.3. Ent-4-amino-16-oxo-19-norbeyeran-15β-methyl 2,3-dimethoxybenzoate (**15c**).* Yield: 53 mg (53%), starting from 100 mg of **14c**; white solid; mp 152.3–153.2 °C; $[\alpha]^{25}_D$ −89 (c 0.8, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28 (1H, m, 6-Ph), 7.13–7.05 (2H, overlap, 3,4-Ph), 4.55 (1H, dd, J = 6.9, 11.5 Hz, H-1′a), 4.44 (1H, dd, J = 3.4, 11.5 Hz, H-1′b), 3.89 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 2.82 (1H, m, H-15), 1.34 (3H, s, H-18), 0.99 (3H, s, H-17), 0.99 (3H, s, H-20), 2.00–0.75 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.04, 165.68, 154.55, 153.65, 125.53, 123.91, 122.04, 116.02, 62.74, 61.74, 57.10, 56.09, 56.00, 53.44, 52.90, 51.47, 48.14, 40.52, 38.69, 37.79, 36.91, 35.66, 34.84, 26.95, 19.81, 19.19, 19.05, 17.44, 14.66; HRMS (ESI, m/z) calcd for $C_{29}H_{42}NO_5$, 484.3063[M + H^+]; found,484.3082.

*5.1.13.4. Ent-4-amino-16-oxo-19-norbeyeran-15β-methyl 2,3,4-trimethoxybenzoate (**15d**).* Yield: 57 mg (57%), starting from 100 mg of **14d**; white solid; mp 109.2–109.9 °C; $[\alpha]^{25}_D$ −70 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.55 (1H, d, J = 8.9 Hz, 6-Ph), 6.71 (1H, d, J = 8.9 Hz, 5-Ph), 4.52 (1H, dd, J = 6.7, 11.5 Hz, H-1′a), 4.43 (1H, dd, J = 3.3, 11.5 Hz, H-1′b), 3.92 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 2.88 (1H, m, H-15), 1.26 (3H, s, H-18), 1.06 (3H, s, H-17), 1.00 (3H, s, H-20), 2.00–0.75 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.35, 164.71, 157.31, 155.10, 142.96, 126.72, 117.28, 106.92, 62.46, 61.88, 61.04, 58.97, 56.82, 56.06, 55.11, 53.15, 51.63, 48.11, 41.60, 40.51, 38.35, 37.79, 36.97, 34.41, 31.85, 29.71, 19.84, 19.24, 17.89, 14.12; HRMS (ESI, m/z) calcd for $C_{30}H_{44}NO_6$, 514.3168 [M + H^+]; found, 514.3148.

*5.1.13.5. Ent-4-amino-16-oxo-19-norbeyeran-15β-methyl 3,4,5-trimethoxybenzoate (**15e**).* Yield: 65 mg (54%), starting from 120 mg of **14e**; white solid; mp 64.0–64.8 °C; $[\alpha]^{25}_D$ −66 (c 1.1, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.24 (2H, s, 2,6-Ph), 4.62 (1H, m, H-1′a), 4.50 (1H, m, H-1′b), 3.89 (9H, overlap, 3 × OCH_3), 2.86 (1H, m, H-15), 1.35 (3H, s, H-18), 1.05 (3H, s, H-17), 1.03 (3H, s, H-20), 2.0–0.80 (18H, m, CH, CH_2 in ent-beyerane skeleton); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 221.20, 165.92, 153.00, 153.00, 142.37, 124.73, 106.75, 106.75, 62.91, 60.93, 59.00, 56.94, 56.19, 56.19, 55.09, 53.44, 51.39, 48.14, 41.73, 40.52, 38.38, 37.80, 36.94, 34.36, 31.81, 19.95, 19.95, 19.27, 17.85, 14.09; HRMS (ESI, m/z) calcd for $C_{30}H_{44}NO_6$, 514.3168 [M + H^+]; found, 514.3158.

5.2. Zebrafish embryo collection and drug treatment

The maintenance of wild-type zebrafish and transgenic zebrafish Tg(cmlc2:GFP) were performed following standard procedures. And the fertilized zebrafish embryos were obtained as previously described [32,33,41]. Zebrafish experiments were complied with the local and national animal use protocols. In brief, zebrafish (3–12 months old), kept at aquaculture system with light cycle, were fed with brine shrimp twice a day. The fertilized zebrafish embryos were produced from pairwise breeding and selected by microscopic examination.

5.2.1. Survival of the DOX-treated zebrafish embryos in the absence or presence of test compound

A previously described assay was used [33]. Briefly, the wild-

type zebrafish of 24 hpf were distributed into a 24-well plate (20 embryos with 1 mL egg water per well), and were treated with DOX (100 μM) with or without each of the test compound (5, 15, 40 μM) for another 48 h. The survival of zebrafish was assessed at 96 hpf using inverted fluorescence microscope (ZEISS, Germany). The zebrafish, which lack of motion on touch, or lost contractility in both atrium and ventricle, were regarded as dead ones.

5.2.3. Cardioprotective effect evaluation

The transgenic zebrafish Tg(cmlc2:GFP) embryos of 24 hpf were transferred into 24-well plates (20 embryos per well) and were treated with DOX (80 μM) with or without the test compound for another 48 h. The parameters including heartbeat, fractional shortening, stroke volume, and cardiac output, were examined and calculated to evaluate the cardiac functions following the methods previously reported [41].

5.2.4. Quantitative polymerase chain reaction

Twenty embryos from each treatment were subjected to RNA extraction using a standard TRIzol protocol (Generay Biotech). RNA concentration and quality were manus measured using Nanodrop 2000 S spectrophotometer (Thermo Scientific). Total RNA was amplified by qRT-PCR using a two-step RT-PCR kit (Vazyme, Nanjing, China). The qRT-PCR analyses were repeated at least three times. Primers for qPCR are listed as following: β-actin L, CCT ACT AAT ACA CAG CCA TGG ATG A; β-actin R, GTC CCA TGC CAA CCA TCA C; cTnT-L, GTC TGC ACT TCG GCG GTT ACA; cTnT-R, AGG TAA AAT CTA TAT TGT TCA GTG AAA TCT AAC CG; ANP-L, ATG GCC GGG GGA CTA ATT CT; ANP-R, AGA GTT GCA ACC GAG GGT GC.

5.3. Cell viability assay

The H9c2 cell were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin, in a humidified incubator with 5% CO_2 at 37 °C. The cells were used until they reached 70% confluence. The Cell Counting Kit-8 (CCK8) assays (Beyotime) were carried out to evaluate the Cell viability. Briefly, H9c2 cells were incubated in a 96-well plate with medium containing CCK-8 (0.5 mg/mL) for 4 h, after the treatment of DOX with or without the test compound for 48 h. The optical density was measured at 450 nm by Spectra Max M5 plate reader.

5.4. Assessment of intracellular reactive oxygen species (ROS)

The intracellular reactive oxygen species were assayed using the using DCFH-DA (Sigma-Aldrich). Briefly, H9c2 cells, seeded in 24-well plate were treated with or without the test compound for 48 h. DCFH-DA diluted with DMEM to final concentration of 5 μmol/L was then added to H9c2cells. The mixture was incubated for 40 min at 37 °C. The dye was then removed, and the wells were washed three times with phosphate buffered saline (PBS). The fluorescence intensity was then measured with confocal microscopy (Carl Zeiss) to determine the reactive oxygen species level.

5.5. Measurement of mitochondrial membrane potential ($\Delta\psi m$)

Mitochondrial membrane potential was measured using the cationic JC-1 (Sigma-Aldrich) dye. Briefly, H9c2 cells, seeded in confocal petri dishes were treated with or without the test compound for 48 h. The JC-1 working solution, prepared to a final concentration of 1 μg/mL, was added to H9c2 cells with 500 μL per well. The mixture was incubated at 37 °C for 20 min. The dye was then removed, and the wells were washed three times with PBS. The H9c2cells were observed using a confocal microscope (Carl Zeiss), and the ratio of red-to-green fluorescence intensities were analyzed to measure the $\Delta\psi$.

Author contributions

Y.Z. designed the overall study. H.Y.Z. designed and synthesis the compounds. H.Y.Z. performed biological experiments in zebrafish. G.X., C.X., E.O. and J.S.L participated in synthesis and biological evaluation in zebrafish. X.O.S. supervised the biological experiments in H9c2 cell. X.O.S and B.L performed the biological experiments in H9c2 cells. Y.Z. and H.Y.Z. wrote the manuscript. Y. Z. supervised the overall study.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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