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Design, synthesis and *in vitro* NO-releasing activities of ocotillol-type furoxans

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A series of novel ocotillol-type furoxan derivatives was synthesized by coupling various furoxans to 3-OH of 6-deoxy ocotillol, and their *in vitro* nitric oxide (NO) releasing capability was studied. The discharge of NO was examined after 30 min at two different concentrations, the results showed that all of the compounds tested could release NO in a dose-dependent manner. All of the synthesized compounds released similar amounts of NO at 100 μM, whereas at 500 μM these compounds showed more difference, in which compound **II$_1$**, **II$_3$**, **II$_4$**, **III$_2$** displayed higher potency in releasing NO at this concentration. Analysis of the *in vitro* data showed that the derivatives bearing the same furoxan group on different ocotillol cores possessed various NO releasing capacity, suggesting that the structure of carrier of NO releasing groups may affect the NO release. Indeed, except compound **II$_2$,** 24(*S*)-6-deoxy ocotillol derivatives from compound **6** with different furoxan substitutions at 3-OH and **III$_2$** displayed enhanced NO releasing capacity, compared to other compounds derived from compounds **5** and **9**. The results illustrated that the functional group and the stereochemistry on the ocotillol structure may affect the NO release of furoxans.

1. Introduction

Nitric oxide (NO) is a simple and significant bioactive molecule in mammals, which is involved in various physiological and pathological processes (Moncada 1999). Known as the endothelium-derived relaxing factor (EDRF), NO is produced by nitric oxide synthase (NOS) enzymes and results in powerful vasodilatation (Smits et al. 1995). Besides the effect on blood vessels, NO is also engaged in central and peripheral nervous systems as a neurotransmitter to modulate cGMP production (Vincent 2010). Furthermore, NO is a bactericidal agent and can be generated through phagocytes by the human immune system responding to bacterial infection (Coleman 2001). Briefly, the biological effects of nitric oxide and its autoxidation products are highly complex and are still under investigation.

Currently NO is used to treat neonatal pulmonary diseases (Finer and Barrington 2006) and adult hypoxemia (Griffiths and Evans 2005). However, the gaseous form of NO renders the dose inaccurate. The pulmonary toxicities associated with inspired NO_2 and the hypertension caused by acute withdrawal of NO also limit the application of gaseous nitric oxide (Hayward et al. 1999).

The research on NO donor drugs has drawn attention since the last century. Three NO donor drugs are presently commercially available: organic nitrates for treatment of angina, sodium nitroprusside for hypertensive crisis and molsidomine as an oral-active, long acting vasodilator (Megson and Webb 2002; Rosenkranz et al. 2014), and various other NO donor compounds have also been discovered (Miller and Megson 2007). Furoxans (Fig. 1) are one of the NO releasing groups that have been used in NO donor drug discovery (Gasco and Schoenafinger 2005), and several reports showed that hybrid compounds by combination of vasodilators, antihistamines and protein pump inhibitors to furoxan groups improved the pharmacological effect of the core drugs (Boschi et al. 2006; Coruzzi et al.2000; Sorba et al. 2003). The triterpenoid saponin, ocotillol was isolated from *Fouquieria splendens Engelm* (Warnhoff and Halls 1965) (Fig. 1). It can also be synthesized from 20(*S*)-protopanaxadiol (**PPD**), one of the main components of *Panax ginseng* (Fujita et al. 1995), which contains mainly triterpenoid saponins and demonstrated various beneficial effects (Kiefer and Pantuso 2003). In our previous study, ocotillol-type triterpenoids derived from **PPD** displayed significant protective effect against myocardial ischemia (Bi et al. 2011; Han et al. 2011; Wang et al. 2010) and excellent antibacterial activity without cytotoxicity in human cell lines (Zhou et al. 2013; Bi et al. 2014).

Mechanistically, plant-derived triterpenoid saponins are employed in plant defense systems by perturbing the membrane of bacteria and enveloped viruses (Hill and Connolly 2012; Augustin et al. 2011), as well as improving the membrane permeability of mammalian cells (Melzig et al. 2001). Consequently, triterpenoid saponins possessing affinity to membranes could be developed as appropriate carriers for NO releasing functional groups. Furthermore, the hybrid compounds may also improve the biological effect of ocotillol-type triterpenoids. Inspiring this combinatorial strategy, we synthesized a series of ocotillol-type furoxans and initially tested their *in vitro* NO releasing capacity.

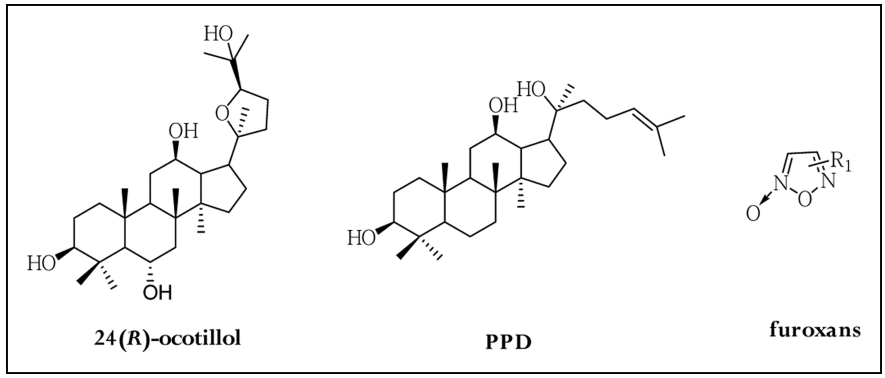
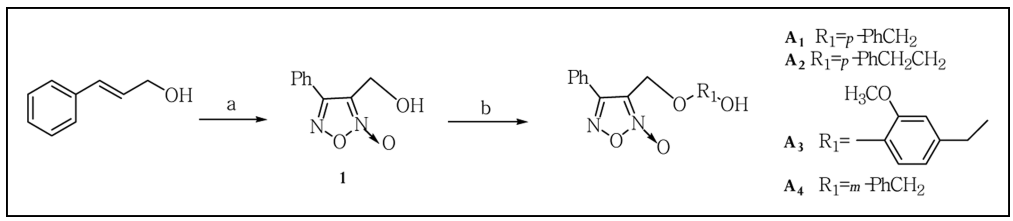


Fig. 1: 24(*R*)-Ocotillol, **PPD** and furoxans.



Scheme 1: Synthesis of furoxans $\mathbf{A_{1\text{-}4}}$ *Reagents and conditions*: a) $NaNO_2$, HOAc, r.t., 1 h; b) (i) $SOCl_2$, pyridine, CH_2Cl_2, r.t., 8 h; (ii) HOR_1OH, K_2CO_3, KI, CH_3CN, r.t., 3 h.

2. Investigations and results

2.1. Synthesis of compounds

Cinnamyl alcohol reacted with $NaNO_2$ provided the furoxan **1**. Addition of thionyl chloride formed the corresponding chlorinated compound, which was subjected to nucleophilic substitution with para-(hydroxyalkyl)phenols to give phenol ethers $\mathbf{A_{1\text{-}4}}$ (Chen et al. 2008; Fang et al. 2008).(Scheme 1).

As previously described, PPD was transformed to epimeric triols **3** and **4** by a straightforward three-step procedure: protection as diacetate **PA** followed by epoxidation and base treatment furnished the desired products. The reaction of compound **3** or compound **4** with succinic anhydride in the presence of DMAP provided carboxylic acids **5** and **6** (Scheme 2). **PA** was oxidized to compound **7** by addition of chromium trioxide. After deprotection, lactone **8** was obtained in good yields. The reaction of compound **8** with succinic anhydride in the presence of DMAP provided carboxylic acid **9** (Scheme 2).

The coupling reaction between furoxans $\mathbf{A_{1\text{-}4}}$ and compounds **5**, **6** or **9** by esterification in the presence of DMAP and EDCI yielded compounds $\mathbf{I_{1\text{-}4}}$, $\mathbf{II_{1\text{-}4}}$, $\mathbf{III_{1\text{-}4}}$, respectively (Scheme 3). The structures of the aimed compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS or ESI-MS.

2.2. Screening

The *in vitro* NO-releasing capacity was measured by the Griess method (Coneski and Schoenfisch 2012), the azo dye formed from NO_2^- and NO_3^- by reaction of released NO with oxygen under aqueous conditions can be detected by UV absorbance. All of the twelve compounds could significantly release nitric oxide in 30 min in the presence of L-cysteine. As shown in Fig. 2, most of the compounds showed similar NO releasing capability at 100 μM, however at 500 μM, compounds $\mathbf{II_1}$, $\mathbf{II_3}$, $\mathbf{II_4}$, $\mathbf{III_2}$ discharged NO quantities superior to other analogues. Furthermore, all of the compounds showed higher NO release capability at 500 μM than 100 μM.

NO release of the compounds with the four furoxan groups we prepared, displayed similar results at 100 μM, while at 500 μM the differences in NO release was more appropriate for analysis. The derivatives with different ocotillol cores bearing the same furoxan group discharged various amounts of nitric oxide, which suggested that the NO releasing capacity of furoxan may be affected by carrier's structure. When different furoxan groups were present, the comparison of NO release at 500 μM is more convincing: $\mathbf{II_1}$, $\mathbf{II_3}$, $\mathbf{II_4}$ derived from compound **6** with 24(*S*) configuration and $\mathbf{III_2}$ from **9** possess superior NO release capacity than other NO donor compounds synthesized from **5** and **9**, which suggested the stereochemistry and the functional group at C-24 of ocotillol derivatives may affect the release of NO even though the furoxan group substitutions situated at the other extremity of the ocotillol core. As the reference, sodium nitroprusside (SNP) was similar to our compounds for NO release at 100 μM after 30 min, but multiplied its potency with a correlation to the concentration at 500 μM. Since SNP is an inorganic compound releasing NO in minutes for treatment of acute hypertensive crises, the releasing effect of our compounds seem to require longer acting time.

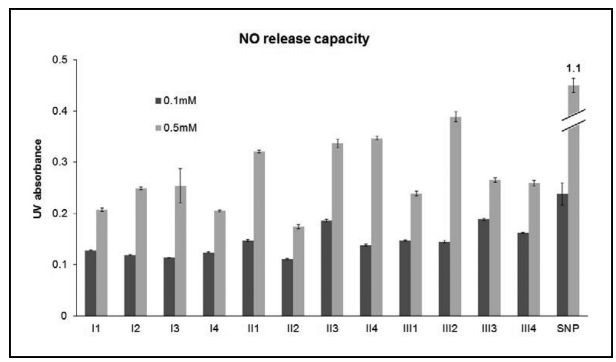
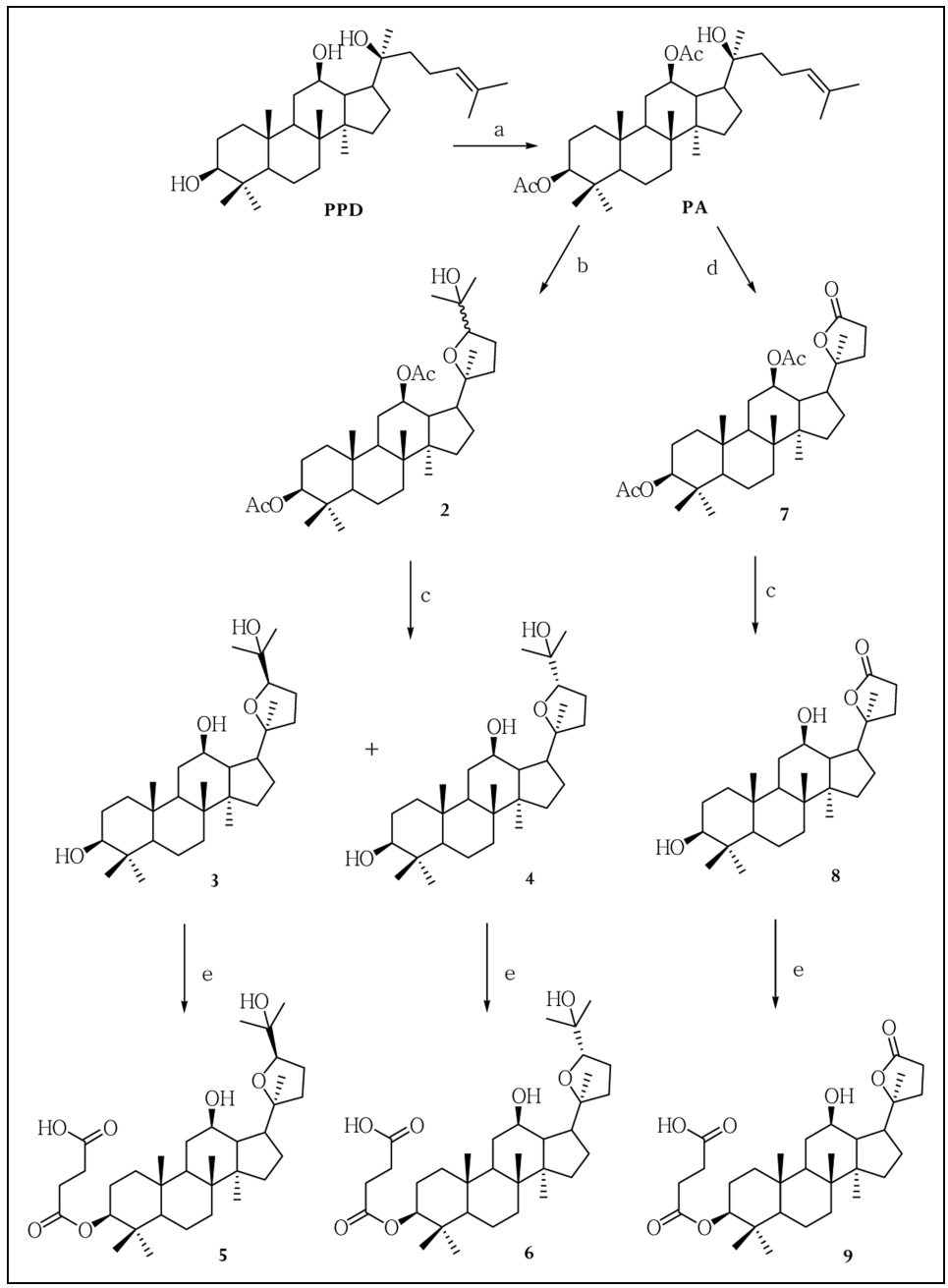


Fig. 2: NO-release of final products at 30 min at 100 and 500 μM.

Many studies on the discovery of NO donor drugs focused on the relationship of drug structures and their pharmacological effects, the quantitative structure-activity relationship (QSAR) of hybrid NO donor drugs demonstrated that the structural diversification of NO donor compounds, including different NO releasing groups and substitutions on the core drug could dramatically affect the bioactivity (Gasco et al. 2008). However, our results showed that minor changes in the structure of ocotillol core could already cause differences in *in vitro* NO release in the presence

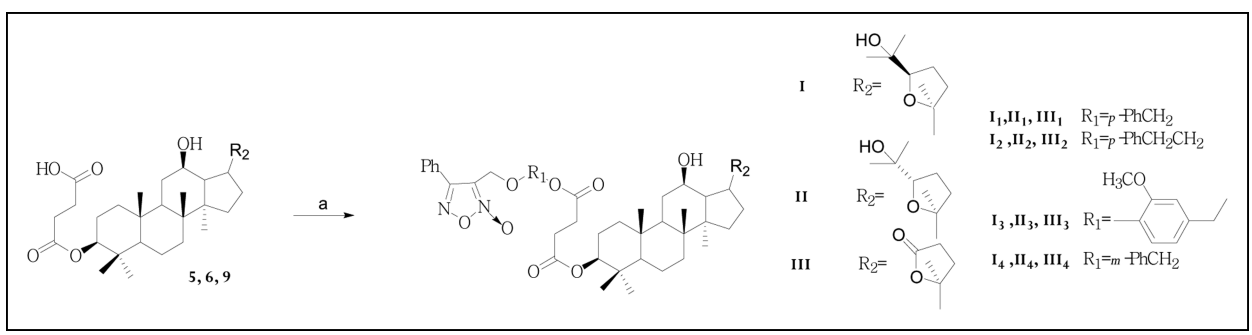


Scheme 2: Synthesis of the derivatives from **PPD** (compounds **5**, **6**, **9**) *Reagents and conditions*: a) $(CH_3CO)_2O$, DMAP, $CHCl_3$, r.t. 12 h; b) *m*-CPBA, CH_2Cl_2, -3 °C, 5 h; c) NaOH, H_2O, CH_3OH, reflux, 6 h; d) CrO_3, CH_3COOH, H_2O, r.t, 3 h; e) Succinic anhydride, DMAP, $CHCl_3$, 42 °C, 6 h.

of the same furoxan group. As shown in Fig. 2, the NO release at 500 μM of compound $\mathbf{II_2}$ improved when the hydroxyl tetrafuran ring was replaced by a lactone in $\mathbf{III_2}$, which suggested that the intramolecular influence amongst the functional groups in drug compounds may affect the capability of NO release.

2.3. Conclusion

In summary, we designed and synthesized a series of ocotillol-type furoxans. All of the compounds demonstrated NO releasing capability in a dose-dependent manner. The NO release from



Scheme 3: Synthesis of final products (compounds $\mathbf{I_{1\text{-}4}}$, $\mathbf{II_{1\text{-}4}}$, $\mathbf{III_{1\text{-}4}}$) *Reagents and conditions*: a) $\mathbf{A_{1\text{-}4}}$, DMAP, EDCI, 25 °C, CH_2Cl_2, 6 h.

these compounds was similar at 100 μM clearly different at 500 μM. Compounds **III$_2$** showed superior NO-releasing quantity to other compounds, and most of the derivatives from compounds **6** with a 24(*S*) configuration displayed relatively greater NO-releasing capacity than those derived from compounds **5** and **9**, which suggested that the stereochemistry and the functional group of ocotillol core may affect the NO release of furoxans. The preliminary results illustrated that the carrier structure of NO-releasing groups was important for the NO release capability to some extent, which should be considered in further NO donor drug design and pharmacological evaluation. As the *in vitro* testing of NO release was promising, the pharmacological study and the further expansion of hybrid compound library formed by NO releasing moieties bound to appropriate carrier will be carried out in our laboratories.

3. Experimental

3.1. General

All chemicals and solvents were analytical grade and, when necessary, purified and dried using standard methods. TLC analyses were carried out on silica gel G (Yantai Chemical group, Yantai, China). All chemicals were purchased from the Tianjin Damao Chemical Factory (Tianjin, China), or the Tianjin Basifu Chemical Factory (Tianjin, China). All solvents were purchased from the Tianjin Bodi Chemical Factory (Tianjin, China). Silica gels were produced by the Yantai Dexin Biological Science and Technology Factory (Yantai, China).

Melting points were measured on an XT3A micro-melting point apparatus and are uncorrected (Beijing Keyi Company, Beijing, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER-AV400 instrument or a Bruker AV-300 (Bruker, Ettlingen, Germany) in the indicated solvents (TMS as internal standard): the values of the chemical shifts expressed in δ values (ppm) and the coupling constants (*J*) in Hz. Mass spectra were recorded using an HP 1100 LC/MSD spectrometer (HP, Palo Alto, USA). High resolution mass spectra were determined on an Agilent QTOF 6520 Accurate-Mass Q-TOF LC/MS (Agilent, Palo Alto, USA). Absorbance was measured by a SpectraMax® M3 multi-functional microplate reader (Molecular Devices, Silicon Valley, USA).

Compounds **1** and **A$_{1\text{-}4}$** were prepared following published procedures (Chen et al. 2008; Fang et al. 2008), 20(S)-protopanaxadiol (**PPD**) was isolated from ginseng total saponin by the authors (Li et al. 2005), Compound **3, 4, 8** were prepared following published procedures (Zhou et al. 2013).

3.2. Preparation of compounds 5,6,9

Butanedioic anhydride (60 mg, 0.60 mmol) was added to a solution of **3**, **4**, **8** (0.30 mmol) and DMAP (0.16 mmol) in dry CH_2Cl_2 (8 mL). The reaction mixture was stirred at 35 °C for 5 h at room temperature, then the organic solution was washed with 10% HCl (3 × 10 mL), water (3 × 20 mL) and NaCl (3 × 10 mL) successively, dried over anhydrous Na_2SO_4.The mixture was concentrated and purified by silica gel column chromatography (20:1 dichloromethane: methanol) to afford compounds **5**, **6**, **9**.

*3.2.1. (20S, 24R)-Epoxy-3β-O-(3-carboxy propionyl)-dammarane-12β, 25-diol (**5**)*

White solid, yield 87%,1H NMR ($CDCl_3$, 400 MHz) δ 4.50 (t, J = 8.9 Hz, 6.9 Hz, 1H), 3.85 (t, J = 8.0 Hz, 7.3 Hz, 1H), 3.52 (dd, J = 10.1 Hz, 6.3 Hz, 1H), 2.63-2.67 (m, 4H), 2.15-2.22 (m, 1H), 1.29 (s, 3H), 1.26 (s, 6H), 1.00 (s, 3H), 0.98(s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); 13C NMR (CDCl3, 75 MHz) δ 177.16, 172.26, 86.93, 85.73, 81.70, 71.38, 70.72, 56.47, 52.43, 50.80, 49.68, 48.33, 40.16, 39.00, 38.30, 37.46, 35.14, 32.99, 31.63, 31.57, 29.81, 29.40, 28.98, 28.30, 28.23, 27.94, 26.45, 25.40, 24.01, 18.54 (overlapping signal), 16.82, 16.74, 15.76.HR-MS (ESI) m/z: 577.4106 $[M+H]^+$.

*3.2.2. (20S, 24S)-Epoxy-3β-O-(3-carboxy propionyl)-dammarane-12β, 25-diol (**6**)*

White solid, yield 83%,^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.47 (t, J = 17.7 Hz, 4.0 Hz, 1H), 3.86 (t, J = 17.9 Hz, 4.2 Hz, 1H), 3.52 (t, J = 8.2 Hz, 7.6 Hz, 1H), 2.13-2.28 (m, 1H), 1.29 (s, 3H), 1.24 (s, 6H), 1.05 (s, 3H), 0.98(s, 3H), 0.93 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H); 13C NMR (CDCl3, 75 MHz) δ 176.61, 172.12, 87.73, 87.43, 81.51, 70.84, 70.50, 56.30, 52.41, 50.37, 49.09, 48.89, 40.01, 38.82, 38.17, 37.33, 34.94, 32.48, 31.91, 31.69, 29.72, 29.50, 29.31, 29.06, 28.18, 28.02, 25.44, 24.26, 23.87, 18.42, 18.01, 16.70, 16.58, 15.71.HR-MS (ESI) m/z: 577.4104 $[M+H]^+$.

*3.2.3. 20S-Epoxy-3β-O-(3-carboxy propionyl)-12β-hydroxy-dammarane-24-one (**9**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.41 (td, J = 17.3 Hz, 4.1 Hz, 1H), 3.79 (td, J = 17.8 Hz, 4.1 Hz, 1H), 3.49 (t, J = 8.2 Hz, 7.6 Hz, 1H), 2.33-2.31 (m, 4H), 2.13-2.28 (m, 1H), 1.27 (s, 3H), 1.02 (s, 3H), 0.98(s, 3H), 0.93 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H).ESI-MS m/z 533.5 [M + H]+

3.3. Preparation of compunds **I$_{1\text{-}4}$, II$_{1\text{-}4}$, III$_{1\text{-}4}$**

Compounds **A$_1$**, **A$_2$**, **A$_3$** or **A$_4$** (0.05 mmol) were added to a solution of compounds **5**, **6** or **9** (0.03 mmol) in CH_2Cl_2, then EDCI (0.05 mmol) and DMAP (7 mg, 0.05 mmol) were added. The resulting mixture was stirred at 25 °C for 6 h. The reaction was quenched with 5% hydrochloric acid, washed with saturated sodium bicarbonate, water and brine successively, dried over anhydrous Na_2SO_4, concentrated in vacuum and the residue purified over silica gel to provide compounds **I$_{1\text{-}4}$**, **II$_{1\text{-}4}$**, **III$_{1\text{-}4}$**.

*3.3.1. 4-[(20S,24R)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} benzyl alcohol ester (**I$_1$**)*

Colorless, oleosus liquid, yield 71%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.67-2.61 (m, 4H), 3.54-3.48 (td, 1H, *J* = 10.4 Hz, 4.4 Hz), 3.86-3.83 (m, 1H), 4.51-4.46 (dd, 1H, *J* = 10.8 Hz, 5.6 Hz), 6.97 (d, 2H), 5.10 (s, 2H), 5.07 (s, 2H), 7.31 (d, 2H), 7.58-7.49 (m, 3H), 7.85-7.83 (dd, 2H, *J* = 8.2 Hz, 6.6 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.31, 16.32, 16.37, 18.08, 18.11, 23.56, 24.95, 26.08, 27.56, 27.86, 28.53, 29.22, 29.45, 29.64, 31.14, 31.27, 32.56, 34.67, 36.99, 37.82, 38.53, 39.68, 47.88, 49.32, 50.33, 51.94, 55.99, 58.31, 65.95, 70.03, 76.68, 70.87, 77.20, 81.16, 86.45, 111.92, 114.87, 126.05, 127.63, 129.33, 129.76, 131.25, 131.38, 156.88, 171.80, 172.12. HR-MS (ESI) *m/z*: 857.497 $[M+H]^+$.

*3.3.2. 4-[(20S,24R)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{ 4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} phenethyl alcohol ester (**I$_2$**)*

Colorless liquid, yield 71%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.61 (s, 4H), 2.90-2.87 (t, 2H), 3.54-3.48 (td, 1H, *J* = 10.4 Hz, 4.4 Hz), 3.86-3.83(dd, 1H, *J* = 8.6 Hz, 6.9 Hz), 4.28-4.24 (t, 2H), 4.51-4.47 (dd, 1H, *J* = 10.4 Hz, 6.0 Hz), 5.08 (s, 2H), 6.94-6.92 (d, 2H), 7.17-7.13 (d, 2H), 7.58-7.50 (m, 3H), 7.86-7.84 (dd, 2H, *J* = 8.1 Hz, 6.6 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.35, 16.35, 16.40, 18.14, 23.61, 24.98, 26.11, 27.59, 27.89, 28.56, 29.47, 29.20, 29.67, 31.17, 31.30, 32.59, 34.15, 34.71, 37.03, 37.87, 38.56, 39.72, 47.92, 49.36, 50.37, 51.98, 56.02, 58.43, 65.17, 70.07, 70.91, 77.20, 81.17, 85.39, 86.49, 112.07, 113.87, 115.00, 126.05, 127.70, 129.34, 130.23, 131.37, 131.72, 155.71, 171.91, 172.23. HR-MS (ESI) *m/z*: 871.5121 $[M+H]^+$.

*3.3.3. 4-[(20S,24R)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}-3-methoxy benzyl alcohol ester (**I$_3$**)*

White, amorphous powder, yield 66%, m.p. 61-62 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.70-2.62 (m, 4H), 3.54-3.48 (td, 1H, *J* = 10.4 Hz, 4.4 Hz), 3.84(s, 4H), 4.50-4.46 (dd, 1H, *J* = 10.4 Hz, 5.9 Hz), 5.07 (s, 2H), 6.90-6.88 (d, 2H), 5.13 (s, 2H), 7.00 -7.98 (d, 1H), 7.59-7.51(m, 3H), 7.94-7.92 (dd, 2H, *J* = 8.0 Hz, 6.6 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.34, 16.33, 16.39, 18.11, 23.60, 24.97, 26.10, 27.58, 27.88, 28.55, 29.24, 29.46, 29.66, 31.16, 31.29, 32.58, 34.69, 37.01, 37.85, 38.55, 39.71, 47.90, 49.34, 50.36, 51.96, 55.82, 56.02, 60.05, 66.22, 70.06, 70.90, 77.20, 81.21, 85.38, 86.48, 112.08, 112.43, 116.54, 121.06, 126.19, 127.93, 129.22, 131.26, 131.48, 150.46, 157.27, 171.85, 172.15. HR-MS (ESI) *m/z*: 887.5078 $[M+H]^+$.

*3.3.4. 4-[(20S,24R)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{3-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}benzyl alcohol ester (**I$_4$**)*

White, amorphous powder, yield 69%, m.p. 65-66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.70-2.61 (m, 4H), 3.55-3.48 (td, 1H, *J* = 10.4 Hz, 4.4 Hz), 3.86-3.83 (dd, 1H, *J* = 8.4 Hz, 6.8 Hz), 4.49-4.45 (dd, 1H, *J* = 16.3 Hz, 9.1 Hz), 5.12 (s, 4H), 6.96-6.94 (d, 1H),7.02-7.00 (d, 2H), 7.32-7.28 (t, 1H), 7.59-7.51 (m, 3H), 7.86-7.84 (d, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.33, 16.32, 16.38, 18.10, 18.13, 23.57, 24.97, 26.10, 27.58, 27.88, 28.55, 29.21, 29.45, 31.17, 31.28, 32.58, 34.68, 37.00, 37.83, 38.51, 39.71, 47.91, 49.35, 50.32, 51.97, 55.96, 58.33, 65.89, 70.06, 70.90, 81.21, 85.38, 86.48, 111.94, 114.19, 114.71, 121.81, 126.10, 127.68, 129.36, 129.97, 131.39, 137.89, 157.06, 157.15, 171.86, 172.10. HR-MS (ESI) *m/z*: 857.4965 $[M+H]^+$.

*3.3.5. 4-[(20S,24S)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} benzyl alcohol ester (***II$_1$***)*

White, amorphous powder, yield 73%, m.p. 60-61 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.68-2.62 (m, 4H), 3.49 (s, 2H), 3.56-3.49 (td, 1H, *J* = 10.1 Hz, 4.2 Hz), 3.90-3.86 (dd, 1H, *J* = 10.8 Hz, 5.2 Hz), 4.52-4.48 (dd, 1H, *J* = 11.1 Hz, 5.4 Hz), 5.07 (s, 2H), 5.10 (s, 2H), 6.99-6.97 (d, 2H), 7.33-7.3 (d, 2H), 7.57-7.50 (m, 3H), 7.86-7.84 (dd, 2H, *J* = 8.1 Hz, 1.5 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.43, 16.31, 16.42, 17.74, 18.13, 23.61, 24.22, 25.00, 27.90, 28.04, 28.51, 28.85, 29.25, 29.47, 29.67, 31.58, 31.66, 32.16, 34.63, 37.03, 37.85, 38.51, 39.71, 48.76, 48.87, 50.08, 52.12, 55.97, 58.34, 65.98, 69.97, 70.44, 77.20, 81.18, 87.12, 87.35, 111.94, 114.90, 126.07, 127.66, 129.35, 129.79, 130.28, 131.40, 156.90, 171.81, 172.16. HR-MS (ESI) *m/z*: 857.4966 $[M+H]^+$.

*3.3.6. 4-[(20S,24S)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}phenethyl alcohol ester (***II$_2$***)*

White, amorphous powder, yield 71%, m.p. 64-65 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.61 (s, 4H), 2.90-2.87 (t, 2H), 3.56-3.50 (td, 1H, *J* = 10.0 Hz, 4.3 Hz), 3.89-3.86 (dd, 1H, *J* = 10.2 Hz, 5.2 Hz), 4.52-4.48 (dd, *J* 1H, = 10.5 Hz, 5.8 Hz), 5.08 (s, 2H), 6.94-6.92 (d, 2H), 7.58-7.51 (m, 3H), 7.18-7.15 (d, 2H), 7.86-7.85 (d, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.42, 16.30, 16.42, 17.73, 18.14, 23.63, 24.21, 25.00, 27.91, 28.02, 28.50, 28.85, 29.19, 29.46, 29.66, 31.57, 31.65, 32.16, 34.64, 37.03, 37.86, 38.51, 39.71, 48.77, 48.87, 50.08, 52.11, 55.98, 58.43, 65.16, 69.98, 70.43, 74.11, 77.19, 81.16, 87.11, 87.33, 112.05, 115.00, 126.13, 127.69, 129.33, 130.22, 131.36, 131.71, 155.70, 157.07, 171.87, 172.21. HR-MS (ESI) *m/z*: 871.512 $[M+H]^+$.

*3.3.7. 4-[(20S,24S)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}-3-methoxy benzyl alcohol ester (***II$_3$***)*

Colorless, oleic-like liquid, yield 70%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.69-2.58 (m, 4H), 3.54-3.49 (m, 1H), 3.84 (s,3H), 3.90-3.86 (m, 1H), 4.51-4.47 (dd, 1H, *J* = 10.6 Hz, 5.4 Hz), 5.07 (s, 2H), 5.13 (s, 2H), 6.90-6.88 (d, 2H), 7.03-7.00 (d, 2H), 7.58-7.49 (m, 3H), 7.94-7.92 (dd, 2H, *J* = 8.0 Hz, 6.6 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 15.41, 16.29, 16.42, 17.73, 18.13, 22.66, 23.62, 24.19, 25.00, 27.90, 28.00, 28.50, 28.84, 29.25, 29.46, 29.66, 31.57, 31.65, 32.16, 34.63, 37.03, 37.85, 38.51, 39.71, 48.76, 48.86, 50.08, 52.11, 55.82, 55.98, 60.06, 66.22, 69.99, 70.43, 74.11, 81.22, 87.11, 87.33, 112.08, 112.44, 116.56, 121.06, 126.19, 127.94, 129.22, 131.26, 131.49, 146.17, 150.48, 157.26, 171.83, 172.15. HR-MS (ESI) *m/z*: 887.5064 $[M+H]^+$.

*3.3.8. 4-[(20S,24S)-Epoxy-12β,25-diol-dammarane-3β-O]-4-oxo-butyric acid{3-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} benzyl alcohol ester (***II$_4$***)*

White, amorphous powder, yield 71%, mp 74-75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.72-2.63 (m, 4H), 3.57-3.50 (td, 1H, *J* = 10.2 Hz, 4.5 Hz), 3.90-3.86 (dd, 1H, *J* = 10.7 Hz, 5.3 Hz), 4.50-4.46 (dd, 1H, *J* = 16.4 Hz, 9.5 Hz), 5.12 (s, 4H), 6.96-6.94 (dd, 1H, *J* = 8.0 Hz, 2.2 Hz), 7.01-7.00 (d, 2H), 7.32-7.28 (t, 1H), 7.58-7.50 (m, 3H), 7.86-7.84 (dd, 2H, *J* = 7.6 Hz, 1.2 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.10, 15.42, 16.28, 16.41, 17.73, 18.12, 23.59, 24.17, 25.01, 27.88, 28.00, 28.51, 28.84, 29.21, 29.44, 29.66, 31.57, 31.62, 32.17, 34.61, 37.00, 37.83, 38.46, 39.70, 48.75, 48.85, 50.04, 52.12, 55.91, 58.31, 65.90, 70.43, 77.20, 81.21, 87.12, 87.33, 111.94, 114.19, 114.70, 121.81, 126.09, 127.68, 129.36, 129.97, 131.39, 131.88, 157.06, 157.14, 171.85, 172.12; HR-MS (ESI) *m/z*: 857.4974 $[M+H]^+$.

*3.3.9. 4-[20(S)-Dammarane-12β-hydroxy-20,24-γ-lactone-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} benzyl alcohol ester (***III$_1$***)*

White, amorphous powder, yield 65%, mp 75-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.57 (m, 6H), 3.62 (td, 1H, *J* = 4.9 Hz), 4.47 (dd, 1H, *J* = 10.7 Hz, 5.5 Hz), 5.07 (s, 2H), 5.10 (s, 2H), 6.96, 7.30 (m, 4H), 7.50, 7.83 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 14.08, 15.51, 16.11, 16.45, 17.57, 18.10, 22.66, 23.55, 26.21, 26.44, 27.92, 28.58, 29.28, 29.33, 29.50, 29.67, 30.00, 31.41, 31.70, 31.90, 34.54, 36.96, 37.87, 38.53, 39.65, 48.87, 49.53, 49.88, 52.08, 55.90, 58.42, 65.97, 70.82, 81.11, 89.13, 114.95, 127.68, 129.35, 129.86, 130.27, 131.40, 156.94, 171.80, 172.11, 176.04; ESI-MS *m/z*: 835.2 $[M+K]^+$.

*3.3.10. 4-[20(S)-Dammarane-12β-hydroxy-20,24-γ-lactone-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}phenethyl alcohol ester (***III$_2$***)*

White, amorphous powder, yield 68%, m.p. 85-88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.52 (m, 6H), 2.86 (t, 2H, *J* = 7.0 Hz), 3.57 (td, 1H), 4.24 (t, 2H, *J* = 7.0 Hz), 4.47 (dd, 1H, *J* = 5.8 Hz, 10.4 Hz), 5.08 (2H, s), 6.92, 7.15 (4H, m), 7.70, 7.84 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 13.69, 13.86, 14.08, 15.51, 16.12, 16.45, 17.57, 18.11, 19.15, 22.65, 23.57, 26.23, 26.44, 27.93, 28.57, 29.21, 29.32, 29.43, 29.48, 29.66, 30.55, 31.41, 31.70, 31.89, 34.17, 34.54, 36.96, 37.88, 38.53, 39.65, 48.88, 49.53, 49.89, 52.08, 55.90, 58.51, 61.86, 65.16, 65.53, 70.16, 70.49, 70.82, 72.47, 77.20, 81.10, 89.11, 112.06, 115.06, 126.18, 127.71, 128.81, 129.33, 130.22, 130.86, 131.36, 131.75, 155.74, 171.87, 172.19, 176.04. ESI-MS *m/z*: 849.2 $[M+K]^+$.

*3.3.11. 4-[20(S)-Dammarane-12β-hydroxy-20,24-γ-lactone-3β-O]-4-oxo-butyric acid{4-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy}-3-methoxy benzyl alcohol ester (***III$_3$***)*

White, amorphous powder, yield 72%, m.p. 88-91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.56 (m, 6H), 3.62 (td, 1H, *J* = 10.6 Hz, 5.0 Hz), 3.8 (s, 3H), 4.47 (dd, 1H, *J* = 10.5 Hz, 5.8 Hz), 5.06 (2H, s), 5.13 (2H, s), 5.30 (s, 1H), 6.87, 6.98 (m, 3H), 7.50, 7.92 (m, 5H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ: 15.48, 16.11, 16.44, 17.56, 18.07, 23.54, 26.27, 26.43, 27.90, 28.55, 29.24, 29.32, 29.46, 29.66, 31.42, 31.63, 34.49, 36.92, 37.84, 38.48, 39.61, 48.79, 49.47, 49.84, 52.07, 55.82, 55.86, 60.06, 66.22, 70.80, 81.12, 89.12, 112.10, 112.45, 116.56, 121.06, 126.18, 127.93, 129.22, 131.27, 131.48 (Ph), 146.17, 150.47, 171.84, 172.14, 176.07; ESI-MS *m/z*: 865.2 $[M+K]^+$.

*3.3.12. 4-[20(S)-Dammarane-12β-hydroxy-20,24-γ-lactone-3β-O]-4-oxo-butyric acid{3-(4-phenyl-1,2,5-oxdiazole-2-oxides)-3-methoxy} benzyl alcohol ester (***III$_4$***)*

White, amorphous powder, yield 64%, mp 81-83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.53 (m, 6H), 3.60 (td, 1H), 4.45 (dd, 1H, *J* = 9.8 Hz, 6.6 Hz), 5.10 (d, 4H), 5.30 (s, 1H), 6.94(s, 1H), 7.00(s, 1H), 7.28(s, 1H), 7.51, 7.84 (m, 5H); ^{13}C NMR 400 MHz, $CDCl_3$) δ: 15.47, 16.09, 16.43, 17.55, 18.06, 23.52, 26.28, 26.44, 27.17, 27.89, 28.55, 29.20, 29.32, 29.44, 29.66, 31.42, 31.63, 34.49, 36.90, 37.83, 38.43, 39.61, 48.79, 49.48, 49.80, 52.08, 55.80, 58.32, 61.84, 65.89, 70.80, 81.12, 89.13, 111.95, 114.17, 114.71, 121.79, 126.09, 127.67, 129.37, 129.96, 131.40, 137.90, 157.16, 171.87, 172.11, 176.08; ESI-MS *m/z*: 835.2 $[M+K]^+$.

3.4. In vitro nitric oxide-release assay

In vitro nitric oxide release was measured by determination of nitrite (NO_2^-) and nitrate (NO_3^-) as described Griess reaction (Coneski and Schoenfisch 2012). L-Cysteine in phosphate (PBS) buffer was prepared by dissolving KH_2PO_4 (0.2694 g), K_2HPO_4 (1.8310 g), L-cysteine (218 mg) in distilled water (100 mL) to a final concentration of 18 mM. The Griess reagent was then prepared by dissolving sulfanilamide (4.0 g) and *N*-napthylenediamine·2HCl (0.2 g) in a mixture of 85% H_3PO_4 (10 mL) and distilled water (90 mL).

The test compounds (6 μmol) were first dissolved in 0.6 mL dimethyl sulfoxide, then diluted into L-cysteine in PBS buffer to 500 μM and 100 μM, respectively. A control blank was prepared by diluting equal amount of dimethyl sulfoxide with PBS solution. Griess reagent (250 μL) was added to the compound solution (200 μL) after 30 min, the mixture was then inoculated at 37 °C for 30 min, the absorbance at 540 nm was measured and repeated twice.

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