Diterpenoids with thioredoxin reductase inhibitory activities from *Jatropha multifida*

Jian-Yong Zhu [a,#], Chun-Yang Zhang [a,#], Jing-Jing Dai [b], Khalid Rahman[c] and Hong Zhang [a,*]

[a]*Central Laboratory, Seventh People's Hospital of Shanghai University of TCM, Shanghai 200137, P. R. China*

[b]*School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu Sichuan 611137, P. R. China*

[c]*Faculty of Science, School of Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, U.K.*

* Corresponding author
E-mail address: hqzhang51@126.com
[#] Jian-Yong Zhu and Chun-Yang Zhang are co-first authors

ABSTRACT

Chemical investigation of the *Jatropha multifida* has led to the isolation of nine diterpenoids (**1**–**9**), including a new jatromulone A, four podocarpane diterpenoids (**2**–**5**), two lathyrane-type diterpenoids (**6** and **7**), and two dinorditerpenoids (**8** and **9**). Their structures were elucidated by spectroscopic analysis, and the absolute configurations of **1** were determined by CD analysis. All of the diterpenoids were screened for inhibitory activity against thioredoxin reductase (TrxR), which is a potential target for cancer chemotherapy with redox balance and antioxidant functions. Compounds **6** and **7** exhibited stronger activity (IC_{50}: 23.4 and 10.6 μM, respectively) than the positive control, curcumin (IC_{50} = 25.0 μM). Compounds **2**–**9** were isolated from *J. multifida* for the first time.

Keywords: *Jatropha multifida*; Podocarpane; Lathyrane; Dinorditerpenoid; TrxR inhibitory activity

1. Introduction

The genus *Jatropha* belongs to the Euphorbiaceae and comprises ca. 200 species which are distributed mainly in the tropical and subtropical regions of Americas and Africa (Zhang et al. 2009). Previous chemical investigations on the genus *Jatropha* have resulted in the isolation of alkaloids, cyclic peptides, terpenes (monoterpene, sesquiterpenes, diterpenoids, and triterpenes), flavonoids, lignans, coumarins, and some others (Zhang et al. 2009; Sabandar et al. 2013;). Extracts and pure compounds of plants from this *Jatropha* species are reported for extensive biological activities (Sabandar et al. 2013; Perea-Domínguez et al. 2017). The chloroform fraction of *J. multifida* caused cell cycle arrest at G1 checkpointand apoptosis in MCF-7 and revealed the probable application of JMR-Ch in cancer therapy (Erharuyi et al. 2014). However, the chemistry of *J. multifida* have not been extensively researched previously. Up to now, two novel jatrophane diterpenoids (Das et al. 2009a, 2009c), twelve lathyrane-type diterpenoid (Das et al. 2008, 2009b, 2010; Kanth et al. 2011), a flavone (Das et al. 2008), and a coumarino-lignan (Das et al. 2008) were isolated from *J. multifida* collected from Botanical Garden, Osmania University, Hyderabad, in August, 2006. In our continuing program aimed at the discovery of novel TrxR inhibitors from natural resources, the petroleum ether (PE) fraction of the ethanolic extract of *J. multifida* showed inhibitory activity against TrxR. Subsequent chemical investigation led to the isolation of nine diterpenoids, including one new podocarpane diterpenoid jatromulone A, together with eight known compounds (**2**-**9**) (Figure 1). All of the diterpenoids were screened for inhibitory activity against thioredoxin reductase (TrxR), compounds **6** and **7** exhibited stronger activity than the positive control, curcumin (IC_{50} = 25.0 μM) with IC_{50} values of 23.4 and 10.6 μM, respectively. Herein, details of the isolation, structural elucidation, TrxR inhibitory activities of these compounds are described.

2. Results and discussion

Compound **1**, a yellow powder, had the molecular formula $C_{18}H_{24}O_3$, as determined by HRESIMS at *m*/*z* 311.1611 [M + Na]$^+$ (calcd 311.1618), corresponding to seven degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyl (3400 cm^{-1}) and ketone carbonyl (1711 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table S1) of **1** showed four methyl singlets [δ_H 2.19 (3H, s, CH_3-15), 1.26 (3H, s, CH_3-18), 1.16 (3H, s, CH_3-20), and 0.79 (3H, s, CH_3-19)], an oxygenated methine [δ_H 3.98 (1H, d, J = 2.3, H-3)], two aromatic protons [δ_H 6.53 (1H, s, H-11) and 6.84 (1H, s, H-12)], and a series of aliphatic methylene multiplets. The ^{13}C NMR spectrum (Table S1) in combination with DEPT experiments resolved 18 carbon resonances attributable to a ketone group (δ_C 210.6), one benzene ring (δ_C 152.2, 145.8, 131.5, 126.7, 122.2, and 110.3), two sp^3 quaternary carbons, two sp^3 methines (one oxygenated), three sp^3 methylenes, and four methyls. As five of the seven degrees of unsaturation were accounted for by one ketone and one benzene ring, the remaining degrees of unsaturation required that **1** was tricyclic system. The above-mentioned data implied that **1** possessed most of the structural features of podocarpane diterpenoids, which showed high similarity to those of co-isolated 3β,12-dihydroxy-13-methylpodocarpane-8,10,13-triene (**2**) (Ravindranath et al. 2004). In comparison with **2**, the major differences being the presence of a ketone canbonyl (δ_C 210.6) instead of a methylene (δ_C 27.9) in **2**, indicating that **1** was the oxygenated derivative of **2**. The location of the additional ketone canbonyl was assigned at C-2 by HMBC correlations from H_2-1 to C-2 and from H-3 (δ_H 3.98) to C-2. This was supported by the downfield-shifted C-1 signal in **1** with respect to that in **2** (δ_C 51.7 in **1**; δ_C 37.0 in **2**). The planar structure of **1** was further secured by detailed analyses of its 2D NMR data (Figure S1). The relative configuration of **1** was established by NOESY experiment and by comparison of its 1D NMR data with those of **2**. The NOE interactions (Figure S2) of H-3/Hα-1, H-3/H_3-18, H-3/H-5 and H_3-18/H-5 suggested that H-3, H_3-18, and H-5 were cofacial and were assigned to be α-oriented randomly. H_3-19/H_3-20 and Hβ-1/ H_3-20 assigned CH_3-20 as β. The absolute configuration of compound **1** was determined from its CD curve cotton effects at 294 nm ($\Delta\varepsilon$ −5.37) and 275 ($\Delta\varepsilon$ +3.03) (Figure S3). According to the octant rule (Kirk, 1986), the hydroxyl group has to be in the rear top-right octant as depicted in Figure

S4. Ring C (Figure S4) with its substituents is in the second sphere and expected to have a strong negative contribution to the Cotton effect. Hence, compound **1** was determined as depicted and given the trivial name jatromulone A .

By comparison of their observed and reported NMR data, the known compounds **2**–**9** were identified as 3*β*,12-dihydroxy-13-methylpodocarpane-8,10,13-triene (**2**) (Ravindranath et al. 2004), (2*S*,3*R*,5*S*,10*R*)-2,3-dihydroxy-15,16-dinor-ent-pimar-8,11,13-triene (**3**) (Li et al. 2015), (2*S*,3*R*,5*S*,10*R*)-2-acetoxy-3-hydroxy-15,16-dinor-ent-pimar-8,11,13-triene (**4**) (Li et al. 2015), gossweilone (**5**) (Ngouela et al. 2003), jatrointelone A(**6**) (Zhu et al. 2015), jatrophodione A (**7**) (Xu et al. 2011), 1,2-dihydroheudelotinol (**8**) (Kimbu et al. 1991), and heudelotinone (**9**) (Kimbu et al. 1991; Xu et al. 2011).

Compounds **1**−**9** were tested for their inhibitory activity against TrxR, and curcumin, a well-known TrxR inhibitor (Zhu et al. 2015), was used as a positive control. Compound exhibiting inhibition less than 50% at 50 *μ*M is considered as inactive. As summarized in Table 1, jatrointelone A (**6**) and jatrophodione A (**7**) showed stronger inhibitory activities with IC_{50} values of 23.4 and 10.6 *μ*M, respectively, and gossweilone (**5**) and heudelotinone (**9**) exhibited moderate activities, while other compounds were inactive .

3. Experimental

3.1. General methods

Optical rotations were measured on a Rudolph Autopol I automatic polarimeter. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. NMR spectra were measured on a Bruker AM-400 spectrometer at 25°C. HRESIMS was performed on a Waters Micromass Q-TOF spectrometer. A Shimadzu LC-20 AT equipped with an SPD-M20A PDA detector was used for HPLC. A YMC-pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm) were used for semi-preparative HPLC separation. Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), C18 reversed-phase silica gel (12 nm, S-50 μm, YMC Co., Ltd.), and Sephadex LH-20 gel

(Amersham Biosciences) were used for column chromatography (CC). All solvents were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). TrxR was purchased from Sigma-Aldrich (St. Louis, USA).

3.2. Materials

Leaves and stems of *J. multifida* were collected from the campus of Guangzhou University of Traditional Chinese Medicine, Guangzhou city, P. R. China, in September 2015. The plant material was identified by Professor Lu-Ping Qin and A voucher specimen (Accession number SJMZ-201509) has been deposited at Central Laboratory, Shanghai Seventh People's Hospital, Shanghai University of TCM.

3.3. Extraction and isolation

The air-dried powder of the leaves and stems of *J. multifida* (3 kg) was extracted with 95% EtOH (3 × 3 L) at rt to give 160 g of crude extract. The extract was suspended in H_2O (1 L) and successively partitioned with petroleum ether (PE, 3 × 1 L), EtOAc (3 × 1 L), and *n*-BuOH (3 × 1 L) to yield three corresponding portions. The PE extract (33 g) was subjected to MCI gel CC eluted with a MeOH/H_2O gradient (3:7 → 10:0) to afford Fr. I−Fr. VI. Fr. II (5.5 g) was loaded onto Sephadex LH-20 column and eluted with EtOH led to Fr. IIa–Fr. IIc. Fr. IIb (1.0 g) was chromatographed over a C_{18} reversed-phase (RP-C_{18}) column eluted with MeOH/H_2O (6:4 → 10:0) to afford Fr. IIa1–Fr. IIa3. Fr. IIa2 was loaded onto a Sephadex LH-20 column and eluted with CH_2Cl_2-MeOH (1:1) to give **9** (16.5 mg) and **8** (17.8 mg). Fr. IIa3 was purified using RP-HPLC (MeOH/H_2O, 7:3, 3 mL/min), to give **6** (7 mg, t_R 15 min) and **7** (9 mg, t_R 17 min). Separation of the Fr. III (3.6 g) by Sephadex LH-20 eluted with MeOH led to Fr. IIIa–Fr. IIId. Fr. IIIb (1.1 g) was subjected to silica gel chromatography using PE/EtOAc mixtures (v/v 1:0 → 0:1) to afford Fr. IIIb1–Fr. IIIb3. Fr. IIIb2 was purified using RP-HPLC (MeOH/H_2O, 8:2, 3 mL/min), to give **3** (8 mg, t_R 14 min) and **4** (11 mg, t_R 15 min). Fr. IIIc (0.9 g) was chromatographed over a C_{18} reversed-phase (RP-C_{18}) column eluted with MeOH/H_2O (7:3 → 10:0) to afford Fr. IIIc1–Fr. IIIc2. Fr. IIIc1 was purified using RP-HPLC (MeOH/H_2O, 7:3, 3 mL/min), to give **1** (6 mg, t_R 16 min) and **2** (20 mg, t_R 17 min). Fr. IIIc2 was loaded onto a Sephadex LH-20 column and eluted with MeOH to give **5** (10 mg).

Jatromulone A, a colorless powder; $[\alpha]^{25}_{D}$ −26.7 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 293 (4.34) nm; CD (*c* 1.0 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 275 ($\Delta\varepsilon$ +3.03), 294 ($\Delta\varepsilon$ −5.37) nm; IR (KBr) ν_{max} 2971, 1711, 1393, 1272, 1197, 1110, 865 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S1; HRESIMS *m/z* 311.1611 $[M + Na]^{+}$ (calcd for $C_{18}H_{24}O_{3}$, 311.1618).

3.4. Evaluation of the TrxR inhibitory activities

For determining the TrxR inhibitory activity of the compounds, the DTNB reduction assay was employed (Huang et al. 2014). All assays were conducted at 25°C in a total volume of 40μL. In each measurement, 0.3 μL of TrxR (0.04 μM) was added to an assay buffer containing 1 M potassium phosphate (pH 7.0), 500 mM EDTA (pH 7.4), NADPH (0.48 mM) and 1μL of inhibitor at various concentrations. After 5 min pre-incubation, the reaction was initiated with the addition of 3.2μL of DTNB (final concentration of 5.0 mM). The control was incubated with the same amount of DMSO (2.5 %, v/v). The increase in absorbance at 412 nm ($\Delta\varepsilon$ 412 =13.6 mM^{-1} cm^{-1}) was monitored in the initial 120s. The IC_{50} values were calculated to represent the TrxR inhibitory effect of compounds.

4. Conclusions

Chemical investigation of the *Jatropha multifida* has led to the isolation of nine diterpenoids (**1**–**9**), including a new jatromulone A, compounds **2**–**9** were isolated from *J. multifida* for the first time. All the diterpenoids were screened for their inhibitory activity against thioredoxin reductase (TrxR). The results showed that two compounds (**6** and **7**) exhibited stronger inhibitory activities, and compounds (**5** and **9**) exhibited moderate activities, while other compounds were inactive. The bioactive data of compounds (**6**) and (**7**) featured with the presence of α,β-unsaturated ketone motif were consistent with the reported lathyrane-type diterpenoids, which were believed to be the potential TrxR inhibitors (Zhu et al. 2015).

Supplementary material

Supplementary data: Tables S1 (1D data of **1**), figures for 2D NMR correlations of **1**, CD, IR, HRESIMS, 1D and 2D NMR spectra of **1**, ^{1}H and ^{13}C NMR spectra of known compounds **2**–**9**.

Disclosure statement

No potential conflict interest was reported by the authors.

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