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Natural Thioredoxin Reductase Inhibitors from *Jatropha integerrima*†

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Nine new diterpenoids, jatrointelones A−I (**1**−**9**), including seven lathyranes (**1**−**7**) and two jatropholanes (**8** and **9**), along with 12 known analogues (**10**−**21**) were isolated from the trunks of *Jatropha integerrima*. The structures were elucidated by spectroscopic analysis, and the absolute configurations of **1**−**7** were determined by combination of single crystal X-ray diffraction, CD analysis (exciton chirality and $Rh_2(OCOCF_3)_4$-induced methods), and chemical correlations. All of the isolates were screened for inhibitory activity against thioredoxin reductase (TrxR), which is a potential target for cancer chemotherapy with redox balance and antioxidant functions. Compounds **1**, **3**, **6**, **7**, and **15**−**21** exhibited stronger activity than the positive control, curcumin (IC_{50} = 25.0 μM), in which **17** and **19** represented the most active compounds with IC_{50} values of 9.4 and 6.8 μM, respectively. The active diterpenoids represent the rare examples of non-aromatic TrxR inhibitors from nature, and a preliminary structure−activity relationship is also proposed.

Introduction

Thioredoxin reductase (TrxR), along with NAPDH and thioredoxin (Trx), is the major regulator of the intracellular redox homeostasis, which plays important roles in diverse biological processes such as cell-growth regulation, apoptosis escapes, DNA synthesis, and angiogenesis.[1-3] Overexpressed TrxR in cancer cells is potentially related to the imbalanced deoxynucleotide pools and may accelerate the development of the malignant phenotype by gene amplification, genetic rearrangements, and even therapy resistance.[4,5] On the contrary, deactivation of TrxR does not only change the redox state and activity of Trx, but may also convert TrxR into a reactive oxygen species generator,[6] and further lead to the inhibition of proliferation, and even induction of necrosis or apoptosis of cells.[7] In recent years, accumulating evidence suggests that TrxR is a potential target for cancer chemotherapy.[8,9]

Jatropha integerrima Jacq (Euphorbiaceae), is a common ornamental shrub widely distributed in subtropical and tropical areas of the world.[10] Previous chemical investigation of this plant led to the isolation of several macrocyclic diterpenes,[11] sesquiterpenes,[12] and cyclic peptides,[13] some of which showed antituberculosis[12] and antimicrobial activities.[14] In our early work on the polar fraction of the ethanolic extract of this plant, seven pairs of lignan enantiomers with NO inhibitory activities were isolated.[15] 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. integerrima* showed inhibitory activity against TrxR. Subsequent chemical investigation led to the isolation of 21

diterpenoids, including nine new compounds. Bioassay verified that 14 compounds were responsible for the TrxR inhibitory activities of the PE fraction, with IC_{50} values ranging from 6.8 to 39.1 μM. Herein, details of the isolation, structural elucidation, TrxR inhibitory activities of these compounds, and a preliminary structure-activity relationship (SAR) is described.

Results and discussion

The air-dried powder of the trunks of *J. integerrima* was extracted with 95% EtOH at room temperature (rt) to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether (PE), EtOAc, and *n*-BuOH. Various column chromatographic separations of the PE extract afforded compounds **1**–**21** (Figure 1).

Compound **1**, colorless crystals, had the molecular formula $C_{20}H_{28}O_4$, as established by a HRESIMS ion at *m*/*z* 355.1889 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1879), corresponding to seven degrees of unsaturation. The IR spectrum exhibited absorption bands for α,β-unsaturated ketone groups (1682 and 1637 cm^{-1}). The ^1H NMR spectrum exhibited signals for five methyl groups [δ_H 1.83 (3H, d, J = 1.2 Hz), 1.82 (3H, d, J = 0.8 Hz), 1.19 (3H × 2, s), and 1.18 (3H, s)], two olefinic protons [δ_H 7.44 (1H, d, J = 1.2 Hz) and 6.76 (1H, dd, J = 11.5 and 0.8 Hz)], and a series of aliphatic methylene or methine multiplets. The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 20 carbon resonances attributable to two ketone groups (δ_C 213.2 and 199.3), two trisubstituted double bonds (δ_C 160.2,

140.3, 149.5, and 132.5), five methyls, three sp^3 methines, three sp^3 methylenes, and three sp^3 quaternary carbons (including two oxygenated ones). As four of the seven degrees of unsaturation were accounted for by two ketones and two double bonds, the remaining degrees of unsaturation required that **1** was tricyclic system. The above-mentioned information was quite similar to that of jatrowediol[16] reported from the same genus. In comparison with jatrowediol, the major differences of **1** were due to the replacement of a sp^3 methylene (C-12) and a sp^3 methine (C-13) in jatrowediol by a trisubstituted double bond [δ_H 6.76; δ_C 149.5 and 132.5], indicating that **1** was a 12,13-didehydrogenated derivative of jatrowediol. This was supported by the downfield-shifted H-11 signal in **1** with respect to that in jatrowediol (δ_H 1.55 in **1**; δ_H 0.79 in jatrowediol). The location of the Δ^{12} was further confirmed by HMBC correlations from H-12 (δ_H 6.77) to C-14, C-13, C-11, and C-9 and from H_3-20 (δ_H 1.82) to C-14, C-13 and C-12. The planar structure of **1** was further established by detailed interpretation of its 2D NMR data (Figure 2). The relative configuration of **1** was established on the basis of NOESY experiment. The NOE interactions of H-9/H-11 indicated that the dimethyl cyclopropane was *cis*-oriented and was arbitrarily assigned as β. Thus, the NOE correlations of H-8β /CH_3-19 and H_3-17 assigned H_3-17 as β, while correlations of H-9/CH_3-18 and H-7α and H-7α/H-4 assigned H-4 as α. The structure including absolute configuration (AC) of **1** was confirmed by a single crystal X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation (Figure 3). Therefore, compound **1** was determined to be (–)-(1*Z*,12*E*,4*R*,6*S*,9*S*,11*S*,15*R*)-lathyr-1,12-dien-6,15-diol-3,14-dione and was

given a trivial name jatrointelone A.

Compound **2** had the molecular formula $C_{20}H_{26}O_4$, two mass units less than that of **1**. The NMR spectra of **2** revealed similar structural features to those found in **1**, except for the presence of an additional double bond (δ_C 149.9 and 139.9) instead of a methine and a methylene (δ_C 47.9 and 33.4) in **1**, indicating that **2** was the dehydrogenated derivative of **1**. The location of the additional double bond was assigned at Δ^4 by HMBC correlations from H-1 (δ_H 6.91) to C-4 and from H-5 (δ_H 5.62) to C-3, C-4, C-6, C-7, and C-15. The planar structure of **2** was further established by detailed interpretation of its 2D NMR data. The relative configuration of **2** was assigned to be the same as that of **1** by comparison of their NOE and NMR data. The absolute configuration of **2** was established by CD exciton chirality method.[17,18] The UV spectrum of **2** exhibited a strong absorption at 267 nm (log ε 3.78) attributable to two α,β-unsaturated ketone groups. Consistent with this UV maximum, the CD spectrum of **2** showed a negative Cotton effect at 320 nm ($\Delta\varepsilon$ –2.56, $n \rightarrow \pi^*$ transition) and a positive Cotton effect at 260 nm ($\Delta\varepsilon$ +1.32, $\pi \rightarrow \pi^*$ transition) due to the transition interaction between two identical α,β-unsaturated ketone chromophores, indicating a negative chirality for **2**. The negative chirality of **2** revealed that the transition dipole moments of two chromophores were oriented in a counterclockwise manner (ESI† S96), and the absolute stereochemistry of **2** was assigned. Therefore, compound **2** was determined to be (–)-(1*Z*,4*E*,12*E*,4*R*,6*S*,9*S*,11*S*,15*R*)-lathyr-1,4,12-trien-6,15-diol-3,14-dione and was given a trivial name jatrointelone B.

Compound **3**, an amorphous solid, exhibited a molecular formula of $C_{20}H_{28}O_3$ as determined by HRESIMS and ^{13}C NMR data. The NMR data of **3** were very similar to those of the known compound (–)-(12*E*,2*S*,3*S*,4*R*,5*R*,6*R*,9*S*,11*S*,15*R*)-15-acetoxy-5,6-epoxylathyr-12-en-3-ol-14-one isolated from the *Euphorbia micractina*,[19] except for the presence of a tetrasubstituted double bond (δ_C 149.1 and 141.9) instead of an acetyl group and two sp^3 carbons (one methine and one oxygenated quaternary carbon), indicating that a molecule of acetic acid was eliminated in the above known compound to form a double bond in **3**. The location of $\Delta^{4(15)}$ was assigned by HMBC correlations from both H-2 (δ_H 2.13) and H-5 (δ_H 3.79) to C-4 and C-15. The planar structure of **3** was further secured by detailed analyses of its 2D NMR data (Figure 2). The relative configuration of **3** was established on the basis of NOESY experiment. The NOE interaction between H-11 and CH_3-20 assigned Δ^{12} as *E*. The NOE interactions of H-9/H-11 indicated that the dimethyl cyclopropane was *cis*-oriented and was arbitrarily assigned as β. Thus, the NOE correlations of CH_3-19/H-8β and H-12 and H-8β/H-5 and H-12 assigned H-5 as β, while correlations of H-9/CH_3-18 and H-7α and H-7α/CH_3-17 assigned CH_3-17 as α. Finally, the α-orientation of H-2 and H-3 were assigned by NOE correlations of H-5/CH_3-16 and H-3/CH_3-17, respectively (Figure 4). The AC of **3** was assigned by the $Rh_2(OCOCF_3)_4$-induced CD analysis. On the basis of the bulkiness rule for secondary alcohols, a positive Cotton effect at around 350 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum indicated a *S*-configuration, while negative Cotten effect implied a *R*-configuration.[20] Thus, a positive Cotton effect at 353 nm

(the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **3** assigned the 3*S* configuration (ESI† S97). Therefore, compound **3** was determined to be (–)-(4*Z*,12*E*,2*S*,3*S*,5*R*,6*R*,9*S*,11*S*)-5,6-epoxylathyr-4,12-dien-3-ol-14-one and was given a trivial name jatrointelone C.

Compound **4**, an amorphous solid, gave a $[M + Na]^+$ ion at *m*/*z* 355.1877 in the HRESIMS corresponding to a molecular formula of $C_{20}H_{28}O_4$, an oxygen atom more than that of **3**. The NMR spectra of **4** revealed similar structural features to those found in **3**, except for the absence of one methyl group and the presence of a hydroxymethyl (δ_H 4.58 and 4.42; δ_C 56.8) in **4**, indicating that **4** was the hydroxylated derivative of **3**. HMBC correlations from the hydroxymethyl protons (δ_H 4.58 and 4.42) to C-12 (δ_C 142.8), C-13 (δ_C 140.8), and C-14 (δ_C 194.4) suggested that the hydroxyl group was located at C-20. The planar structure of **4** was further established by detailed interpretation of its 2D NMR data. The relative configuration of **4** was assigned to be the same as that of **3** by comparison of their NOE and ^{13}C NMR data. The AC of **4** was assigned to be the same as that of **3** by comparison of their CD spectra, which showed similar cotton effects at 360 nm ($\Delta\varepsilon$ –2.23), 315 nm ($\Delta\varepsilon$ +1.45), 270 nm ($\Delta\varepsilon$ +1.81), and 244 nm ($\Delta\varepsilon$ –1.64), due to the $n \rightarrow \pi^*$ (above 300 nm) and $\pi \rightarrow \pi^*$ (230-300 nm) transition of the α,β-unsaturated ketone moiety (ESI†, S98). Therefore, compound **4** was determined to be (–)-(4*Z*,12*E*,2*S*,3*S*,5*R*,6*R*,9*S*,11*S*)-5,6-epoxylathyr-4,12-dien-3,20-diol-14-one and was given a trivial name jatrointelone D.

Compound **5** had a molecular formula of $C_{20}H_{28}O_4$ as determined by HRESIMS, an

oxygen atom more than that of **3**. The NMR data of **5** were similar to those of **3** except for the absence of one methyl group in **3** and the presence of a hydroxymethyl [(δ_H 3.45, 2H, s); δ_C 71.9] in **5**, indicating that **5** was a hydroxylated derivative of **3**. HMBC correlations from the hydroxymethyl protons (δ_H 3.45, 2H, H_2-18) to C-9 (δ_C 30.8), C-10 (δ_C 30.1), and C-11 (δ_C 23.9), together with NOE correlations between H_2-18, H-11, and H-9 suggested that the hydroxyl group was located at C-18. The planar structure of **5** was further established by detailed interpretation of its 2D NMR data. The relative configuration of **5** was assigned to be the same as that of **3** by comparison of their NOE and ^{13}C NMR data. The AC of **5** was assigned to be same as that of **3** and **4** by comparison of their CD spectra (ESI†, S98). Therefore, compound **5** was determined to be (–)-(4*Z*,12*E*,2*S*,3*S*,5*R*,6*R*,9*S*,10*S*,11*S*)-5,6-epoxylathyr-4,12-dien-3,18-diol-14-one and was given a trivial name jatrointelone E.

Compound **6**, an amorphous solid, had the molecular formula $C_{20}H_{26}O_4$, as established by a HRESIMS ion at *m*/*z* 353.1715 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1723), corresponding to eight degrees of unsaturation. The 1D NMR spectra of **6** showed high similarity to those of curculathyrane B, isolated from *Jatropha crucus* and assigned by single-crystal X-ray diffraction method.[21] In comparison with curculathyrane B, the major differences of **6** were due to the replacement of a sp^3 methylene (C-12) and a sp^3 methine (C-13) in curculathyrane B by a trisubstituted double bond [δ_H 6.02; δ_C 145.8 and 137.8] in **6**, indicating that **6** was a 12,13-didehydrogenated derivative of curculathyrane B. This was supported by the

downfield-shifted H-11 signal in **6** with respect to that in curculathyrane B (δ_H 1.64 in **6**; δ_H 0.54 in curculathyrane B). The location of the Δ^{12} was further confirmed by HMBC correlations from H-12 (δ_H 6.02) to C-14, C-13, C-11, and C-9 and from CH_3-20 (δ_H 2.01) to C-14, C-13 and C-12. The relative configuration of **6** was established by comparison of its NOE and ^{13}C NMR data with those of known analogues. The NOE interactions of H-9/H-11 indicated that the dimethyl cyclopropane was *cis*-oriented and was arbitrarily assigned as *β*. Thus, the NOE correlations of CH_3-19/H-8*β* and H-12 and H-8*β*/H-5 and H-12 assigned H-5 as *β*. As no NOE correlation of CH_3-17 was observed, the configuration of the epoxy ring in **6** was determined by comparison of its ^{13}C NMR data with those of **3a**, a model compounds derived from **3** by pyridinium chlorochromate oxidation. The maximum $\Delta\delta_C$ between **6** and **3a** regarding the epoxy ring area (C-5, C-6, C-7, and C-17) was less than 0.8 ppm, indicating that the epoxy ring in **6** has the same configuration as that of **3a**. The CH_3-16 (δ_H 1.34 and δ_C 26.1) of **6** was assigned as *β* by comparison of its NMR data with those of curculathyrane B (δ_H 1.34 and δ_C 26.2).[21,22] The AC of **6** was assigned to be the same as that of **3a** by comparison of their CD spectra, which showed similar cotton effects at 377 nm ($\Delta\varepsilon$ –0.62), 330 nm ($\Delta\varepsilon$ +1.02), and 260 nm ($\Delta\varepsilon$ –2.96), (ESI†, S99). Thus compound **6** was determined to be (–)-(4*E*,12*E*,2*R*,5*R*,6*R*,9*S*,11*S*)-5,6-epoxylathyr-4,12-dien-2-ol-3,14-dione and was given a trivial name jatrointelone F.

Compound **7** had a molecular formula same as that of **6**. Its NMR data were very similar to those of **6**, with the slight differences being due to the tertiary methyl

group at C-2 (δ_H 1.37 and δ_C 25.0 in **7**; δ_H 1.34 and δ_C 26.1 in **6**), indicating that **7** was an C-2 epimer of **6**.[22] This was supported by NOESY experiments that CH_3-16 only correlated with H_a-1 (δ_H 3.34) in **7** while only correlated with H_b-1 (δ_H 2.74) in **6**. Thus the CH_3-16 group was α-oriented in **7**. The AC of **7** was assigned to be same as that of **6** by comparison of their CD spectra (ESI†, S99). Thus, compound **7** was determined to be (–)-(4*E*,12*E*,2*S*,5*R*,6*R*,9*S*,11*S*)-5,6-epoxylathyr-4,12-dien-2-ol-3,14-dione and was given a trivial name jatrointelone G.

Compound **8**, a white powder, exhibited a molecular formula of $C_{22}H_{26}O_4$ as determined by HRESIMS and ^{13}C NMR data. The 1D NMR spectra of **8** bore a resemblance to those of *epi*-jatrophol,[23] previously isolated from *J. crucus*, except for the presence of an additional acetyl group [δ_H 2.11 (3H, s); δ_C 20.9, 171.3] in **8**, indicating that **8** was the acetylated derivative of *epi*-jatrophol. The location of the acetyl group was assigned at C-18 by an HMBC correlation from H_2-18 to the carbonyl group at δ_C 171.3. This was supported by the downfield-shifted H_2-18 signals in **8** with respect to those in *epi*-jatrophol (δ_H 4.10 and 3.96 in **8**; δ_H 3.55 and 3.48 in *epi*-jatrophol). The relative configuration of **8** was established by NOESY experiment and by comparison of its 1D NMR data with those of *epi*-jatrophol. The NOE interactions of H-9/H-11 indicated that the dimethyl cyclopropane was *cis*-oriented and was arbitrarily assigned as β. Thus, the NOE interactions of CH_2-18/H-9 and H-11 assigned CH_2-18 as α. The structure of **8** was further confirmed by chemical transformation from **8** to *epi*-jatrophol via alkaline hydrolysis (ESI†,

S100 and S101). Thus, the structure of **8** was determined as depicted and given a trivial name jatrointelone H.

Compound **9** had the same molecular formula as that of **8**. The NMR data of **9** were very similar to that of **8**, with the slight differences being due to the methyl group at C-2 (δ_C 17.2 in **9**; δ_C 15.8 in **8**), indicating that **9** was an C-2 epimer of **8**. Thus, the CH_3-16 group was α-oriented in **9**. This was consistent with the ^{13}C chemical shift of the CH_3-16 in other known analogues with the α-CH_3-16, as CH_3-16 adopted β orientation usually resonate around 15.8 ppm.[24] Compound **9** has the same planar structure as compound acetoxyjatropholone,[25] differing only in the orientation of the acetoxy group at C-10. The structure of **9** was finally confirmed by alkaline hydrolysis of **9**, which yielded the co-isolated known compound **12** (ESI†, S102 and S103). Hence, the structure of **9** was deduced as shown and named jatrointelone I.

The known compounds jatropholone A (**10**),[26] jatropholone B (**11**),[26] jatrophol (**12**),[27] *epi*-jatrophaldehyde (**13**),[23] 4-*epi*-curcusone E (**14**),[28] curcusone A (**15**),[29] curcusone B (**16**),[29] curcusone C (**17**),[29] curcusone D (**18**),[29] 2-*epi*-jatrogrossidione (**19**),[30] jatrogrossidione (**20**),[30] and jatrophadiketone (**21**),[31] were identified by comparison of their spectroscopic data with those in the literature.

Compounds **1**−**21** were tested for their inhibitory activity against TrxR, and curcumin, a well-known TrxR inhibitor,[32] was used as a positive control. Compound exhibiting inhibition less than 50% at 50 μM is considered as inactive. As summarized in Table 3, jatropholane diterpenes (**8**–**13**) did not inhibit TrxR, while lathyrane (**1**–**7**) and rhamnofolane (**15**–**21**) diterpenes showed moderate inhibitory

activities with IC_{50} values ranging from 6.8 to 39.1 μM. Compounds **17** and **19** represented the most active compounds with IC_{50} values at 9.4 and 6.8 μM, respectively. Generally, compounds with a -CO-C=C-CO- motif (**6**–**7**, and **15**–**21**) exhibit more potency than compounds without these structural features (**1**, **2**, **4**, **5**, and **8–14**) except for compound **3**. Compared with **3**, the hydroxylation of the methyl groups at either C-20 (in **4**) or C-18 (in **5**) decreased the activity by some extent.

Among the rhamnofolane diterpenes (**15**–**21**), hydroxylation of C-2 or C-3 obviously increase the activity (**15** vs **17** and **19**; **16** vs **18** and **20**). Moreover, compounds **15**, **17**, and **19** with a β-CH_3-16 at C-2 showed better activities than their α-counterparts, **16**, **18**, and **20**, respectively, indicating that the β-orientation of CH_3-16 contributed more activities than the α-orientation of CH_3-16 in rhamnofolane diterpenes. Thus, 2-*epi*-jatrogrossidione (**19**) with all of the above-mentioned favorable features was presented as the most active compound.

Conclusions

In summary, nine new diterpenoids, jatrointelones A−I (**1**−**9**), along with 12 known analogues (**10**−**21**) were isolated from the trunks of *J. integerrima*. The structures were determined by a combination of spectroscopic analysis, single crystal X-ray diffraction, and chemical correlations. Compounds **1**, **3**, **6**, **7**, and **15**−**21** exhibited inhibitory activity against TrxR, and **17** and **19** represented the most active compounds with IC_{50} values at 9.4 and 6.8 μM, respectively. The preliminary structure−activity relationship of this group of compounds was also discussed.

In the last decades, numerous TrxR-targeting compounds, such as platinum, arsenic, and gold-related chemoprevention agents, have been developed as potential

TrxR inhibitors for anticancer treatment.[33] Naturally occurring TrxR inhibitors, such as resveratrol, curcumin, pleurotin and some flavonoids, have been reported recently,[33-35] and most of them were structurally characterized with the conjugated aromatic systems. The active diterpenoids reported in the current study represented the rare example of non-aromatic TrxR inhibitors in nature.[34,35] They may serve as a novel structural motif for the design of TrxR inhibitors in the future. However, the mechanism of inhibition on TrxR and the potential usage of these compounds in anticancer therapy require further investigation.

Experimental section

General experimental procedures

X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Melting points were measured on an X-4 melting instrument and are uncorrected. 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.), C_{18} 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 (Guangzhou Chemical Reagents Company, Ltd.). TrxR was purchased from Sigma-Aldrich (St. Louis, USA).

Plant material

Trunks of *J. integerrima* were collected in the East campus of Sun Yat-sen University in the Guangzhou city, P. R. China, in November 2013, and were authenticated by Associate Professor Lin Jiang of Sun Yat-sen University. A voucher specimen (accession number: YJIZ-201311) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and isolation

The air-dried powder of the trunks of *J. integerrima* (6 kg) was extracted with 95% EtOH (3 × 10 L) at rt to give 462 g of crude extract. The extract was suspended in H_2O (2 L) and successively partitioned with petroleum ether (PE, 3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L) to yield three corresponding portions. The PE extract (76.6 g) was subjected to MCI gel CC eluted with a MeOH/H_2O gradient (2:8 → 10:0) to afford Fr. I– Fr. X. Separation of the Fr. III (5.5 g) by Sephadex LH-20 eluted with MeOH led to Fr. IIIa– Fr. IIId. Fr. IIIa (1.5 g) was subjected to silica gel chromatography using PE/acetone mixtures (v/v 1:0 → 0:1) to afford Fr. IIIa1– Fr. IIIa3. Fr. IIIa2 was loaded onto a Sephadex LH-20 column and eluted with CH_2Cl_2-MeOH (1:1) to give **14** (6.2 mg). Fr. IIIa3 was purified on a semi-preparative reversed-phase (RP) HPLC system equipped with a YMC column (MeOH/H_2O, 8:2, 3 mL/min), to give **21** (5.8 mg, t_R 11 min). Fr. IIIb (3.5 g) was subjected to silica gel chromatography using PE/EtOAc

mixtures (v/v 1:0 → 0:1) to afford Fr. IIIb1–Fr. IIIb4. Yellow crystals of **20** (60.3 mg) and **19** (12.0 mg) from Fr. IIIb2 and Fr. IIIb3 were recrystallized in petroleum ether-EtOAc (10:1), respectively. Fr. IIIb3 was purified using RP-HPLC (CH_3OH/H_2O, 7:3, 3 mL/min) to give **17** (8.2 mg, t_R 10 min) and **18** (12.1 mg, t_R 12 min). Fr. IIIb4 was purified using RP-HPLC (CH_3OH/H_2O, 8:2, 3 mL/min), to give **15** (9.2 mg, t_R 12 min) and **16** (12.3 mg, t_R 14 min). Fractionation of Fr. IV (6.5 g) by Sephadex LH-20 eluted with EtOH led to Fr. IVa–Fr. IVe. Fr. IVa (3.5 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. IVa1– Fr. IVa4. Fr. IVa2 was loaded onto a Sephadex LH-20 column and eluted with CH_2Cl_2-MeOH (1:1) to give **10** (9.2 mg) and **11** (12.8 mg). Fr. IVa3 was purified using RP-HPLC ($MeOH/H_2O$, 8:2, 3 mL/min), to give **12** (6.8 mg, t_R 12 min) and **13** (5.4 mg, t_R 13 min). Fr. IVb (1.0 g) was loaded onto a Sephadex LH-20 column and eluted with CH_2Cl_2-MeOH (1:1) to give Fr. IVb1– Fr. IVb3. Fr. IVb1 was purified using RP-HPLC (CH_3CN/H_2O, 6:4, 3 mL/min), to give **9** (20.7 mg, t_R 15 min) and **8** (9.4 mg, t_R 16 min). Fr. VI (8 g) was subjected to silica gel CC (PE/EtOAc, 8:1 → 1:2) to give Fr. VIa– Fr. VIe. Fr. VIa was loaded onto a Sephadex LH-20 and eluted with EtOH, leading to Fr. VIa 1– Fr. VIa5. Fr. VIa1 was purified using RP-HPLC (CH_3CN/H_2O, 5:5, 3 mL/min), to give **6** (6.0 mg, t_R 13 min), **7** (5.4 mg, t_R 14 min) and **1** (11.2 mg, t_R 15 min). Fr. VIa2 was purified using RP-HPLC (CH_3OH/H_2O, 6:4, 3 mL/min), to give **5** (20.6 mg, t_R 11.5 min) and **4** (9.5 mg, t_R 13 min). Fr. VIb was chromatographed over a C_{18} reversed-phase (RP-C_{18}) column eluted with $MeOH/H_2O$ (6:4 → 10:0)

to afford Fr. VIb1– Fr. VIb4. Fr. VIb2 was purified using RP-HPLC (CH_3OH/H_2O, 7:3, 3 mL/min), to give **2** (8.2 mg, t_R 10 min) and **3** (12.3 mg, t_R 12 min). The purity of compounds **1**−**21** was greater than 95% as determined by ^{1}H NMR spectra.

Jatrointelone A (1). colorless crystals, mp 176-178 °C; $[\alpha]^{25}_D$ −127.4 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.88), 275 (3.87) nm; CD (*c* 1.58 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (+0.15), 250 (–5.01), 275 (+0.97) nm; IR (KBr) ν_{max} 3174, 2934, 1682, 1657, 1634, 1273, 1090, 915, 748 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 355.1889 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1879).

X-ray Crystallographic Study of Jatrointelone A (**1**): $C_{20}H_{28}O_4$, M = 332.42, orthorhombic, 0.6 × 0.2 × 0.2 mm^3, space group $P3_1$ (no.144), *a* = 14.57764 (10) Å, *b* = 14.57764(10) Å, *c* = 7.54977(6) Å, $\alpha = \beta = 90°$, $\gamma = 120°$, *V* = 1389.436(17) $Å^3$, *Z* = 3, D_c = 1.192 g/cm^3, F_{000} = 540, Xcalibur, Onyx, Nova, Cu Kα radiation, λ = 1.54184 Å, *T* = 173 K, $2\theta_{max}$ = 143.5°, 17884 reflections collected, 3593 unique (R_{int} = 0.0351). Final *GooF* = 1.036, *R1* = 0.0262, *wR2* = 0.0689, *R* indices based on 3535 reflections with I >2sigma(I) (refinement on F^2), 224 parameters, 1 restraint. Lp and absorption corrections applied, μ = 0.655 mm^{-1}. Flack parameter = 0.07 (10). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1031826) (ESI†).

Jatrointelone B (2). an amorphous solid; $[\alpha]^{25}_D$ −128.3 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.98), 267 (3.78) nm; CD (*c* 1.32 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (–4.83), 260 (+1.32), 320 (–2.56) nm; IR (KBr) ν_{max} 2973, 1711, 1621, 1216,

1112, 906, 751 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 353.1728 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1723).

Jatrointelone C (3). an amorphous solid; $[\alpha]^{25}_D$ –22.1 (*c* 1.44, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.13), 267 (4.07) nm; CD (*c* 2.28 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 244 (–1.06), 270 (+0.40), 315 (+1.30), 360 (–6.65) nm; IR (KBr) ν_{max} 2962, 1712, 1614, 1257, 1110, 860, 753 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 339.1921 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1930).

Jatrointelone D (4). an amorphous solid; $[\alpha]^{25}_D$ –21.0 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.69), 270 (3.78) nm; CD (*c* 1.81 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 244 (–1.64), 270 (+1.81), 315 (+1.45), 360 (–2.23) nm; IR (KBr) ν_{max} 3480, 1642, 1529, 1090, 759 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 355.1877 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1879).

Jatrointelone E (5). an amorphous solid; $[\alpha]^{25}_D$ –54.8 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.12), 270 (3.25) nm; CD (*c* 3.01 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 244 (–3.02), 270 (+0.63), 315 (+1.48), 360 (–1.50) nm; IR (KBr) ν_{max} 3480, 1725, 1635, 1367, 1220, 790 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 355.1874 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1879).

Jatrointelone F (6). an amorphous solid; $[\alpha]^{25}_D$ –103.8 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.09), 280 (3.67) nm; CD (*c* 1.58 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 270 (–2.37), 340 (+0.65), 380 (–0.28) nm; IR (KBr) ν_{max} 2965, 2178, 1710, 1602, 1442, 1375, 1189, 742 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 353.1715 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1723).

Jatrointelone H (7). an amorphous solid; $[\alpha]^{25}_{D}$ −63.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.94), 280 (3.61) nm; CD (*c* 1.29 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 268 (−1.75), 340 (−0.42), 377 (−1.25) nm; IR (KBr) ν_{max} 2965, 1719, 1609, 1034, 771 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 353.1729 (calcd for $C_{20}H_{26}O_4$, 353.1723).

Jatrointelone G (8). white powder; $[\alpha]^{25}_{D}$ +18.3 (*c* 0.70, MeOH); UV (MeOH) λ_{max} (log ε) 273 (3.82), 320 (3.37) nm; IR (KBr) ν_{max} 2961, 1683, 1634, 1462, 1262, 1091, 870 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 377.1712 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_4Na$, 377.1723).

Jatrointelone I (9). white powder; $[\alpha]^{25}_{D}$ +15.8 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 273 (3.65), 320 (3.45) nm; IR (KBr) ν_{max} 2962, 1694, 1577, 1463, 1241, 1218, 1118, 752 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 355.1907 $[M + H]^+$ (calcd for $C_{22}H_{27}O_4$, 355.1909).

Determination of the absolute configuration of the secondary alcohol unit in 3

Following the reported procedure,[36] a 1:2 mixture of secondary alcohol-$Rh_2(OCOCF_3)_4$ for **3** was subjected to CD measurements at a concentration of 0.1 mg/mL in anhydrous CH_2Cl_2. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the band at around 350 nm in the induced CD spectrum was correlated to the absolute configuration of the secondary alcohol.

Chemical transformation of 3 to 3a

To a suspension of PCC (10 mg) in CH_2Cl_2 (2 mL) was added compound **3** (9 mg) at room temperature. The mixture was stirred for 3 h, the crude mixture was purified on a flash silica gel eluted with CH_2Cl_2 to afford the **3a**.

Alkaline hydrolysis of 8 and 9

Each compound (1 mg) was stirred with 1 mL of 0.1 M NaOH (MeOH/H_2O, 3:1) for 2 h at rt, then the mixture was subjected to Sephadex LH-20 using EtOH as eluent to afford the pure hydrolysis product, which was identified by the ^{1}H NMR spectrum, R_f, and MS.

The oxygenated product of **3** (**3a**), colorless oil; $[\alpha]^{25}_D$ –69.5 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.85), 280 (3.70) nm; CD (*c* 1.15 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 265 (–2.89), 340 (+1.04), 380 (–0.63) nm; IR (KBr) ν_{max} 2974, 1714, 1452, 1336, 1245, 708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.04 (1H, d, *J* = 11.5 Hz, H-12), 3.65 (1H, s, H-5), 3.40 (1H, dd, *J* = 18.9, 6.9 Hz, H-1α), 2.49 (1H, m, H-2), 2.34 (1H, m, H-7α), 2.22 (1H, d, *J* = 18.9 Hz, H-1β), 2.16 (1H, m, H-8α), 2.01 (3H, s, H-20), 1.64 (1H, dd, *J* = 11.5, 8.4 Hz, H-11), 1.38 (1H, m, H-9), 1.28 (3H, s, H-17), 1.24 (1H, m, H-8β), 1.20 (3H, m, H-18), 1.19 (1H, d, *J* = 7.0 Hz H-16), 1.15 (1H, m, H-7β), 1.11 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 209.9 (C, C-3), 193.2 (C, C-14), 165.4 (C, C-4), 146.2 (CH, C-12), 137.8 (C, C-13), 63.0 (C, C-6), 61.4 (CH, C-5), 40.5 (CH, C-2), 37.0 (CH_2, C-7), 36.9 (CH, C-9), 35.4 (CH_2, C-1), 29.2 (CH, C-11), 29.1 (CH_3, C-18), 26.9 (C, C-10), 19.2 (CH_2, C-8), 17.22 (CH_3, C-17), 17.17 (CH_3, C-16), 16.4 (CH_3, C-19) 12.7 (CH_3, C-20); ESIMS *m*/*z* 315.4 $[M + H]^+$, HRESIMS *m*/*z* 313.1790

$[M - H]^-$.

Evaluation of the TrxR inhibitory activities

For determining the TrxR inhibitory activity of the compounds, the DTNB reduction assay was employed. All assays were conducted at 25℃ 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. The range of inhibitor concentrations was 50, 25, 12.5, 6.25, and 3.125 μM. After 5 min pre-incubation, the reaction was initiated with the addition of 3.2 μL 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.

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† Electronic supplementary information (ESI) available: IR, HRESIMS, 1D and 2D NMR spectra of **1**−**9** and **3a**, ^{1}H and ^{13}C NMR spectra of known compounds **10**−**21**, CD spectra of **3**−**7**. CCDC 1031826.

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Notes and references

1 J. Lu and A. Holmgren, *Free Radic. Biol. Med.*, 2014, **66**, 75-87.

2 D. F. D. Mahmood, A. Abderrazak, K. El Hadri, T. Simmet and M. Rouis, *Antioxid. Redox Signaling*, 2013, **19**, 1266-1303.

3 J. D. Pennington, K. M. Jacobs, L. Sun, G. BarSela, M. Mishra and D. Gius, *Curr. Pharm. Des.*, 2007, **13**, 3368-3377.

4 A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini and L. Messori, *Coord. Chem. Rev.*, 2009, **253**, 1692-1707.

5 A. Baker, C. M. Payne, M. M. Briehl and G. Powis, *Cancer Res.*, 1997, **57**, 5162-5167.

6 J. Lu and A. Holmgren, *Antioxid. Redox Signaling*, 2012, **17**, 1738-1747.

7 D. Zhang, Z. Xu, J. Yuan, Y. X. Zhao, Z. Y. Qiao, Y. J. Gao, G. A. Yu, J. Li and H. Wang, *J. Med. Chem.*, 2014, **57**, 8132-8139.

8 F. Z. Avval and A. Holmgren, *J. Biol. Chem.*, 2009, **284**, 8233-8240.

9 J. Nordberg and E. S. Arnér, *Free Radic. Biol. Med.*, 2001, **31**, 1287-1312.

10 M. Sujatha, N. Sivaraj and M. S. Prasad, *Biol. Plant.*, 2000, **43**, 167-171.

11 S. Sutthivaiyakit, W. Mongkolvisut, P. Ponsitipiboon, S. Prabpai, P. Kongsaeree, S. Ruchirawat and C. Mahidol, *Tetrahedron Lett.*, 2003, **44**, 3637-3640.

12 S. Sutthivaiyakit, W. Mongkolvisut, S. Prabpai and P. Kongsaeree, *J. Nat. Prod.*,

2009, **72**, 2024-2027.

13 A. Wele, C. Baragueye, W. Ndiaye, D. Fall, I. Ndoye, Y. Diop, L. Dubosq and B. Bodo, *Dakar Med.*, 2007, **52**, 209-215.

14 A. O. Eshilokun, A. A. Kasali, I. A. Ogunwande, T. M. Walker and W. N. Setzer, *Nat. Prod. Commun.*, 2007, **2**, 853-855.

15 J. Y. Zhu, B. Cheng, Y. J. Zheng, Z. Dong, S. L. Lin, G. H. Tang, Q. Gu and S. Yin, *RSC Adv.*, 2015, **5**, 12202-12208.

16 R. L. Brum, A. J. Cavalheiro, F. D. Monache and I. Vencato, *J. Braz. Chem. Soc.*, 2001, **12**, 259-262.

17 N. Harada and K. Nakanishi, *J. Am. Chem. Soc.*, 1969, **91**, 3989-3991.

18 N. Harada and K. Nakanishi, *Acc.Chem. Res.*, 1972, **5**, 257-263.

19 Y. Tian, W. Xu, C. Zhu, S. Lin, Y. Guo and J. Shi, *J. Nat. Prod.*, 2013, **76**, 1039-1046.

20 M. Gerards and G. Snatzke, *Tetrahedron: Asymmetry*, 1990, **1**, 221-236.

21 W. Naengchomnong, Y. Thebtaranonth, P. Wiriyachitra, K. T. Okamoto and J. Clardy, *Tetrahedron Lett.*, 1986, **27**, 5675-5678.

22 G. Schmeda-Hirschmann, F. Tsichritzis and J. Jakupovic, *Phytochemistry*, 1992, **31**, 1731-1735.

23 X. Q. Zhang, F. Li, Z. G. Zhao, X. L. Liu, Y. X. Tang and M. K. Wang, *Phytochem. Lett.*, 2012, **5**, 721-724.

24 Y. F. Yang, J. Q. Liu, L. Shi, Z. R. Li and M. H. Qiu, *Nat. Prod. Bioprospect.*, 2013, **3**, 99-102.

25 G. Chianese, E. Fattorusso, O. O. Aiyelaagbe, P. Luciano, H. C. Schroder, W. E. Muller and O. Taglialatela-Scafati, *Org. Lett.*, 2011, **13**, 316-319.

26 K. K. Purushothaman, C. Sundaram, A. F. Cameron, J. D. Connolly, C. Labbé, A. Maltz and D. S. Rycroft, *Tetrahedron Lett.*, 1979, **20**, 979-980.

27 M. Chen, L. Hou and G. Zhang, *Zhiwu Xuebao*, 1988, **30**, 308-311.

28 J. Q. Liu, Y. F. Yang, X. Y. Li, E. Q. Liu, Z. R. Li, L. Zhou, Y. Li and M. H. Qiu, *Phytochemistry*, 2013, **96**, 265-272.

29 W. Naengchomnong, Y. Thebtaranonth, P. Wiriyachitra, K. T. Okamoto and J.

Clardy, *Tetrahedron Lett.*, 1986, **27**, 2439-2442.

30 J. Jakupovic, M. Grenz and G. Schmeda-Hirschmann, *Phytochemistry*, 1988, **27**, 2997-2998.

31 J. Q. Liu, Y. F. Yang, C. F. Wang, Y. Li and M. H. Qiu, *Tetrahedron*, 2012, **68**, 972-976.

32 X. Qiu, Z. Liu, W. Y. Shao, X. Liu, D. P. Jing, Y. J. Yu, L. K. An, S. L. Huang, X. Z. Bu, Z. S. Huang and L. Q. Gu, *Bioorg. Med. Chem.*, 2008, **16**, 8035-8041.

33 W. Cai, L. Zhang, Y. Song, B. Wang, B. Zhang, X. Cui, G. Hu, Y. Liu, J. Wu and J. Fang, *Free Radic. Biol. Med.*,2012, **52**, 257-265.

34 P. Nigro, F. Dal Piaz, D. Gallotta, N. De Tommasi and M. A. Belisario, *Free Radic. Biol. Med.*,2008, **45**, 875-884.

35 F. Saccoccia, F. Angelucci, G. Boumis, D. Carotti, G. Desiato, A. E. Miele and A. Bellelli, *Curr. Protein Pept. Sci.*, 2014, **15**, 621-646.

36 J. Frelek and W. J. Szczepek, *Tetrahedron: Asymmetry*, 1999, **10**, 1507-1520.

Table 1. ^{1}H NMR Data of jatrointelones A−G (**1**−**9**) in $CDCl_3$ (400 MHz, δ in ppm, J in Hz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1a	7.44, d (1.2)	6.91, d (1.4)	3.11, dd (15.6, 7.4)	3.21, dd (16.2, 7.8)	3.10, dd (15.6, 7.4)	3.15, d (18.5)	3.34, d (18.4)	3.26, dd (16.7, 7.4)	3.21, dd (16.6, 7.4)
1b			2.08, m	2.08, m	2.08, m	2.74, d (18.5)	2.71, d (18.4)	2.50, dd (16.7, 4.6)	2.54, dd (16.6, 3.9)
2			2.13, m	2.21, m	2.13, m			2.68, m	2.63, m
3			4.44, d (6.0)	4.49, d (6.0)	4.45, d (6.0)				
4	2.64, d (8.0)								
5a	2.46, d (15.9)	5.62, brs	3.79, s	3.93, s	3.79, s	3.67, s	3.66, s		
5b	1.30, (15.9, 8.0)								
7a	1.70, m	1.94, m	2.30, m	2.33, m	2.35, m	2.35, m	2.34, m	2.70, m	2.69, m
7b	1.26, m	1.41, m	1.20, m	1.22, m	1.20, m	1.16, m	1.15, m	2.67, m	2.62, m
8a	1.87, m	1.80, m	2.15, m	2.19, m	2.16, m	2.19, m	2.21, m	1.81, m	1.84, m
8b	0.96, m	0.92, m	1.20, m	1.26, m	1.24, m	1.24, m	1.31, m	0.96, m	0.96, m
9	1.40, m	1.52, m	1.22, m	1.31, m	1.30, m	1.38, m	1.42, m	1.14, m	1.12, m
11	1.55, dd (11.5, 8.0)	1.53, dd (11.5, 8.7)	1.53, dd (11.2, 8.0)	1.70, dd (11.6, 8.0)	1.69, dd (11.0, 8.7)	1.66, dd (11.3, 8.3)	1.67, dd (11.4, 8.3)	1.84, d (8.7)	1.85, d (8.7)
12	6.76, dd (11.5, 0.8)	6.39, d (11.5, 1.0)	5.61,dd (11.2, 1.4)	5.84,d (11.6)	5.59,d (11.0)	6.02, d (11.3, 0.9)	6.13, d (11.4, 1.2)		
16	1.83, (1.2)	1.87, d (1.4)	1.13, d (6.8)	1.14, d (6.8)	1.15, d (6.8)	1.34, s	1.37, s	1.28, d (7.3)	1.29, d (7.4)
17a	1.19, s	1.86, s	1.34, s	1.40, s	1.35, s	1.21, s	1.39, s	5.20, dd (2.0, 2.0)	5.23, dd (2.0, 2.0)
17b								4.67, d (2.0, 2.0)	4.68, dd (2.0, 2.0)
18a	1.19, s	1.13, s	1.14, s	1.18, s	3.45, s	1.21, s	1.22, s	4.10, d (11.2)	4.10, d (11.2)
18b					3.45, s			3.96, d (11.2)	3.96, d (11.2)
19	1.18, s	0.98, s	1.04, s	1.08, s	1.14, s	1.10, s	1.10, s	0.91, s	0.90, s
20a	1.82, d (0.8)	1.90, d (1.0)	1.95, d (1.4)	4.58, d (12.3)	1.98, s	2.01, d (0.9)	2.02, d (1.2)	2.31, s	2.31, s
20b				4.42, d (12.3)					
18-OAc								2.11, s	2.10, s

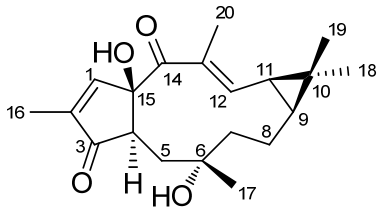
Table 2. ^{13}C NMR Data of jatrointelones A−G (**1**−**9**) in $CDCl_3$ (100 MHz, δ in ppm)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	160.2, CH	151.8, CH	38.6, CH_2	38.7, CH_2	38.5, CH_2	42.3, CH_2	42.5, CH_2	30.3, CH_2	30.1, CH_2
2	140.3, C	145.4, C	39.7, CH	39.6, CH	39.7, CH	75.5, C	75.2, C	42.5, CH	42.4, CH
3	213.2, C	195.7, C	87.5, CH	87.7, CH	87.5, CH	208.8, C	208.4, C	207.7, C	208.1, C
4	47.9, CH	139.9, C	148.8, C	149.1, C	148.8, C	162.2, C	164.8, C	136.9, C	137.5, C
5	33.4, CH_2	149.9, CH	63.6, CH	63.6, CH	63.6, CH	60.6, CH	61.6, CH	134.3, C	134.4, C
6	71.6, C	75.9, C	65.2, C	65.4, C	65.2, C	63.1, C	63.8, C	145.5, C	145.0, C
7	37.8, CH_2	41.4, CH_2	37.9, CH_2	37.6, CH_2	37.9, CH_2	36.9, CH_2	37.0, CH_2	33.4, CH_2	33.3, CH_2
8	20.9, CH_2	19.6, CH_2	19.1, CH_2	19.1, CH_2	18.8, CH_2	19.2, CH_2	19.3, CH_2	20.8, CH_2	20.8, CH_2
9	35.8, CH	38.1, CH	35.1, CH	36.2, CH	30.8, CH	36.9, CH	37.3, CH	23.1, CH	23.1, CH
10	28.8, C	26.9, C	24.6, C	26.3, C	30.1, C	26.8, C	27.6, C	23.0, C	23.0, C
11	28.6, CH	28.9, CH	27.7, CH	27.9, CH	23.9, CH	29.1, CH	29.2, CH	25.9, CH	25.8, CH
12	149.5, CH	153.5, CH	137.6, CH	142.8, CH	135.0, CH	145.8, CH	148.4, CH	135.4, C	135.3, C
13	132.5, C	128.4, C	137.9, C	140.8, C	138.8, C	137.8, C	137.7, C	130.6, C	131.0, C
14	199.3, C	199.0, C	195.0, C	194.4, C	195.2, C	192.5, C	192.1, C	150.1, C	150.3, C
15	81.8, C	82.6, C	141.5, C	141.9, C	141.4, C	136.2, C	135.2, C	132.7, C	131.8, C
16	10.1, CH_3	10.8, CH_3	18.2, CH_3	18.3, CH_3	18.2, CH_3	26.1, CH_3	25.0, CH_3	15.8, CH_3	17.0, CH_3
17	29.0, CH_3	25.5, CH_3	17.8, CH_3	17.9, CH_3	17.8, CH_3	17.4, CH_3	17.1, CH_3	115.2,CH_2	115.5,CH_2
18	29.1, CH_3	28.5, CH_3	29.0, CH_3	29.0, CH_3	71.9, CH_2	29.1, CH_3	29.9, CH_3	73.8, CH_2	73.8, CH_2
19	16.4, CH_3	15.5, CH_3	16.1, CH_3	16.2, CH_3	11.8, CH_3	16.4, CH_3	16.5, CH_3	12.1, CH_3	12.1, CH_3
20	11.7, CH_3	12.0, CH_3	13.6, CH_3	58.6, CH_2	13.8, CH_3	12.7, CH_3	12.5, CH_3	12.6, CH_3	12.6, CH_3
18-OAc								171.3, C	171.3, C
								20.9, CH_3	20.9, CH_3

Table 3. The TrxR inhibitory activity of some compounds (IC_{50}, μM)

compound	IC_{50} (μM)[a]	compound	IC_{50} (μM)[a]
1	23.7 ± 1.1	**16**	24.3 ± 1.3
2	34.2 ± 2.3	**17**	9.4 ± 0.6
3	16.3 ± 1.3	**18**	14.2 ± 0.9
4	39.1 ± 2.7	**19**	6.8 ± 0.5
5	25.2 ± 1.5	**20**	10.9 ± 1.0
6	12.9 ± 0.8	**21**	16.8 ± 1.3
7	14.5 ± 0.9	curcumin[b]	25.0 ± 2.2
15	21.2 ± 1.6		

[a] Values are represented as means ± SD based on three independent experiments. [b] Positive control.



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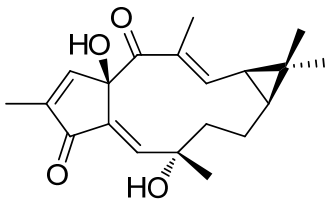

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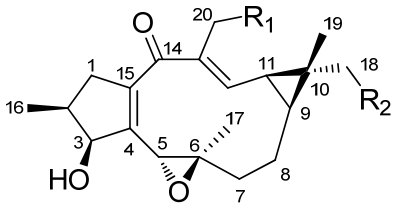
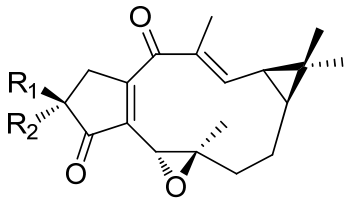
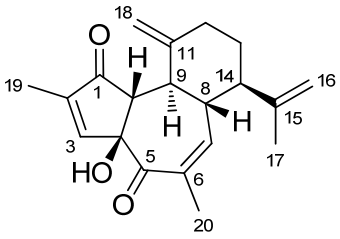


3 $R_1 = H$ $R_2 = H$
4 $R_1 = OH$ $R_2 = H$
5 $R_1 = H$ $R_2 = OH$



6 $R_1 = CH_3$ $R_2 = OH$
7 $R_1 = OH$ $R_2 = CH_3$
3a $R_1 = CH_3$ $R_2 = H$



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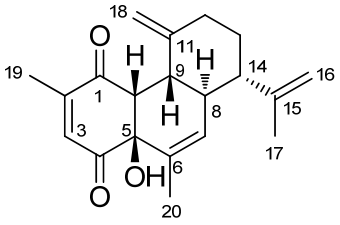

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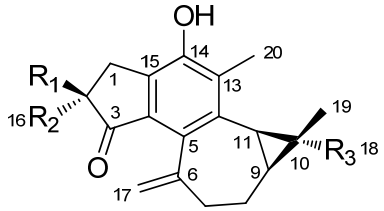
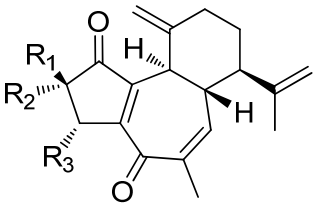


8 $R_1 = CH_3$ $R_2 = H$ $R_3 = CH_2OAc$
9 $R_1 = H$ $R_2 = CH_3$ $R_3 = CH_2OAc$
10 $R_1 = CH_3$ $R_2 = H$ $R_3 = CH_3$
11 $R_1 = H$ $R_2 = CH_3$ $R_3 = CH_3$
12 $R_1 = H$ $R_2 = CH_3$ $R_3 = CH_2OH$
13 $R_1 = H$ $R_2 = CH_3$ $R_3 = CHO$



15 $R_1 = CH_3$ $R_2 = H$ $R_3 = H$
16 $R_1 = H$ $R_2 = CH_3$ $R_3 = H$
17 $R_1 = CH_3$ $R_2 = OH$ $R_3 = H$
18 $R_1 = OH$ $R_2 = CH_3$ $R_3 = H$
19 $R_1 = CH_3$ $R_2 = H$ $R_3 = OH$
20 $R_1 = H$ $R_2 = CH_3$ $R_3 = OH$

Figure 1.Structures of compounds **1**–**21**

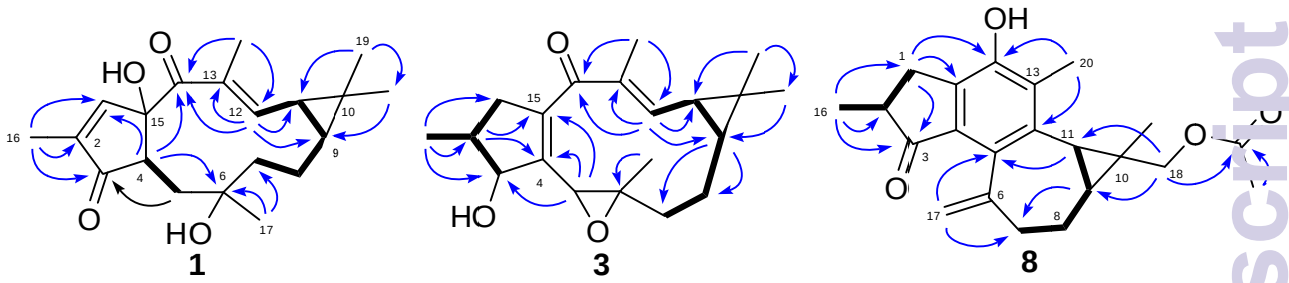


Figure2. Selected $^{1}H–^{1}H$ COSY (▬) and HMBC (→) correlations of **1**, **3**, and **8**.

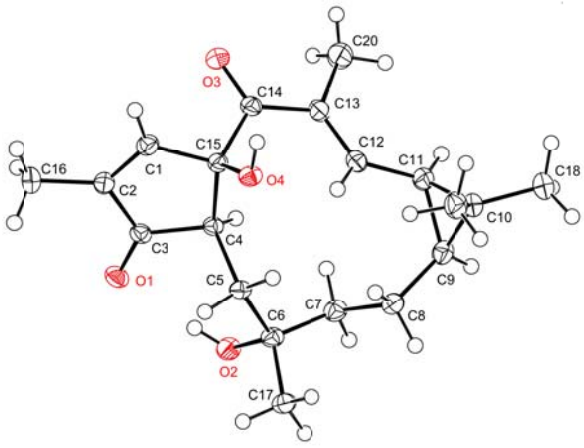


Figure 3. ORTEP diagram of compound **1**.

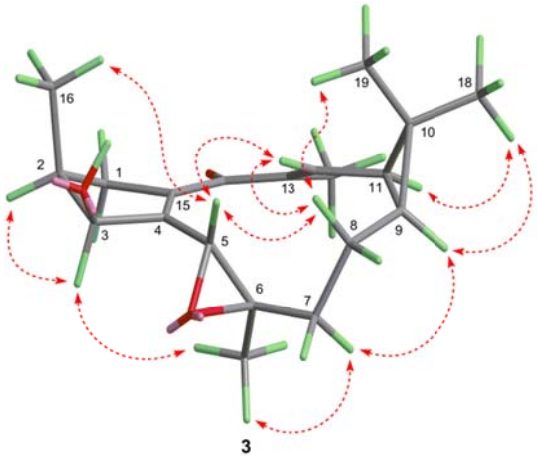
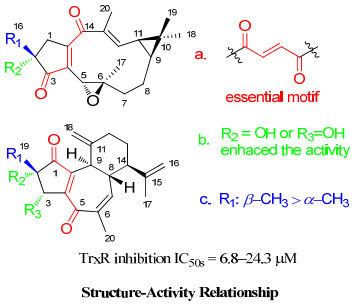


Figure 4. Key NOE correlations (⇢) of **3**.

TOC:



Nine new diterpenoids were isolated from *Jatropha integerrima*. The active diterpenoids represent the rare examples of non-aromatic TrxR inhibitors from nature.