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Quiquelignan A–H, eight new lignoids from the rattan palm *Calamus quiquesetinervius* and their antiradical, anti-inflammatory and antiplatelet aggregation activities

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ABSTRACT

Eight new lignin derivatives, termed quiquelignan A–H (**1**–**8**), comprising three tricin-type flavonolignans (**1**–**3**) and five 8-*O*-4′ neolignans (**4**–**8**), were isolated from the ethanol extract of *Calamus quiquesetinervius* stems. Structural elucidation of the new isolates was accomplished on the basis of spectroscopic data. Compounds **1**–**8** showed strong-to-moderate antioxidant activity against the hydroxy radical (·OH). Among them, compound **5** showed significantly higher hydroxy radical scavenging activity (IC_{50} 4.4 μg/mL). Compounds **2**–**4** and **6**–**8** dose-dependently suppressed the LPS-stimulated production of nitric oxide (NO) in RAW 264.7 cells. The anti-inflammatory potency of **4** and **6** was 2.7–4.5-fold higher compared with quercetin. Compounds **2**–**4**, **6** and **8** also exhibited mild collagen-antagonistic activity, but were inactive with respect to thrombin-induced platelet aggregation.

1. Introduction

The genus *Calamus* is a spiny climbing vine belonging to the subfamily Calamoideae, family Arecaceae. It is widely distributed in the tropical and subtropical regions of Southeast Asia and the western Pacific.[1] In plant taxonomy, molecular and morphological evidences indicate that its molecular phylogenetics are non-monophyletic compared with the related rattan genera *Daemonorops*, *Ceratolobus*, *Calospatha*, *Pogonotium* and *Retispatha*.[2] Plants belonging to this genus are used to treat ulcers, dysentery, dyspepsia or pyrexia in alternative medicine. The shoots of some *Calamus* spp., for instance *Calamus ornatus* in the Philippines and *Calamus tenuis* in Laos and Thailand, are consumed as foodstuffs.[3,4] Although the genus encompasses approximately 370 species in the world, few reports are available on the pharmacognosy or pharmacology of these plants. Only steroidal saponins from this genus having anti-inflammatory activities and tumor-cell proliferation inhibitory activities have been reported.[3,5] The biosynthetic pathway of the secondary metabolites of these plants and their physiological benefits are unknown.

There are four *Calamus* spp. endemic to Taiwan: *Calamus quiquesetinervius* Burret, *Calamus Formosanus* Becc., *Calamus Siphonospathus* Mart. Var. sublaevis Becc., and *Calamus beccarii* Henderson. Among them, *C. quiquesetinervius* (the most abundant species) is a clustered prickly rattan palm that grows at low and medium elevations throughout the island.[6–8] In addition to being used as a raw material in industry, the tender shoots of *C. quiquesetinervius* are consumed in salads or soups by native people of Taiwan. In traditional herbal medicine, the stems and roots are primarily used for treating hypertension, hepatitis and skin diseases.[7]

To investigate the pharmacological effects of this plant, we found that an EtOAc extract of the stem of *C. quiquesetinervius* exhibited notable antioxidant, anti-inflammatory and antiplatelet aggregation activities in vitro. Intrigued by these bioactivities, we investigated the chemical constituents of *C. quiquesetinervius* and tested their pharmacological activities.

Bioactivity-directed fractionations derived from that the EtOAc extract of *C. quiquesetinervius* yielded eight new lignoids (Fig. 1). These comprised three tricin-type flavonolignans (**1**–**3**) and five

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8-*O*-4′ neolignans (**4**–**8**) categorized as β-*O*-4 inter-unit ether linkage lignins which resulted from the oxidative polymerization of guaiacyl, syringyl and *para*-hydroxybenzoyl subunits. To investigate the pharmacological efficacy on cardiovascular disease (CVD), these isolates were evaluated in vitro for the free-radical scavenging activities against hydroxy radical (·OH) and superoxide anion radical ($O_2^{\cdot-}$), inhibitory effects on the production of nitric oxide (NO) by a macrophage-mediated inflammatory platform, as well as collagen- or thrombin-induced platelet aggregation.

2. Results and discussion

2.1. Structure elucidation of new compounds

Dried stems (15.4 kg) of *C. quiquesetinervius* Burret were extracted with 95% EtOH. The extract was evaporated under reduced pressure to give a brown syrup. The resulting brown syrup was resuspended in 90% aqueous-EtOH and partitioned with *n*-hexane, ethyl acetate and *n*-butanol successively to give the corresponding organic extracts. All extracts were dried under vacuum and tested for bioactivities. The most bioactive EtOAc extract was repeatedly subjected to column chromatography followed by reverse-phase high-performance liquid chromatography (RP-HPLC) and preparative thin-layer chromatography (PTLC) purification to yield eight new lignoids **1**–**8**.

Quiquelignan A (**1**) was isolated as an orange–yellow amorphous powder. The HRESIMS spectrum showed an ion at *m*/*z* 579.1895 $[M+Na]^+$. This indicated a molecular formula of $C_{29}H_{32}O_{11}$ with 14 of unsaturation. The UV absorption maxima (λ_{max} 212, 230, 286 and 322 nm) indicated a flavanone moiety in **1**.[9] The IR absorption bands of **1** were characteristic of hydroxy (ν_{max} 3366 cm^{-1}), carbonyl (ν_{max} 1707 cm^{-1}) and aromatic ring (ν_{max} 1600 and 1457 cm^{-1}). The ^1H NMR spectrum of **1** revealed seven aromatic, three methoxy (δ_H 3.81 and 3.78 × 2), three methylene (δ_H 3.91/3.80, 3.42 and 3.08/2.73), three methine (δ_H 5.35, 4.58 and 4.29) and one methyl triplet signal (δ_H 1.17). Its ^{13}C NMR spectrum exhibited signals for one phenolic ketone, eighteen aromatic, three methoxy, three oxygenated methine, and two oxygenated methylene carbon signals. Detailed inspection of the ^1H and ^{13}C NMR spectra of **1** showed that the signals for the flavanone moiety were similar to those of dihydrotricin.[10] Three aromatic signals (δ_H 6.88, br s, H-2″, δ_H 6.76, d, *J* = 7.8 Hz, H-6″ and δ_H 6.73, d, *J* = 8.4 Hz, H-5″) suggested a 1,3,4-trisubstituted benzyl moiety. HMBC correlations as –O*Me*/C-3″ (δ_C 148.7); H-2″/C-1″ (δ_C 132.1), C-4″ (δ_C 147.1), C-6″ (δ_C 121.9), C-7″ (δ_C 81.8); H-7″/C-1″ (δ_C 132.1), C-9″ (δ_C 61.7), C-10″ (δ_C 65.5); H-10″/C-11″ (δ_C 15.6) together with COSY cross peaks (H-7″/H-8″/H-9″ and H-10″/H-11″) confirmed a 7-*O*-ethylguaiacylglyceryl moiety in **1**. The guaiacyl type arylpropanoid moiety with an ABX coupling was also reported in the guaiacylglycerol derivative, 7-*O*-ethylguaiacylglycerol.[11] On the basis of the evidences described above, **1** was proposed to have a known dihydrotricin connected to an arylpropanoid moiety via an ether linkage. The long-range HMBC correlation (H-8″/C-4′), along with NOESY correlations (H-8″/H-2′/H-6′), confirmed that the ether linkage was between C-4′ and C-8″. Thus, **1** was identified as an 4′-*O*-8″ flavonolignan. The key NMR correlations (^1H–^1H COSY, HMBC, and NOESY) are shown in Figure 2.

Most of the relative configurations of **1** were confirmed by 2D NOE correlations as shown in Figure 2. However, the relative configuration of H-7″ and H-8″ was assigned by their coupling constants. The ^1H NMR spectrum of **1** was re-measured in acetone-d_6 because of the overlapping of H-7″ signal in CD_3OD. The larger coupling constant of H-7″/H-8″ ($J_{7'',8''}$ = 6.0 Hz) inferred their exposition as a *threo* form.[12,13] The absolute configuration

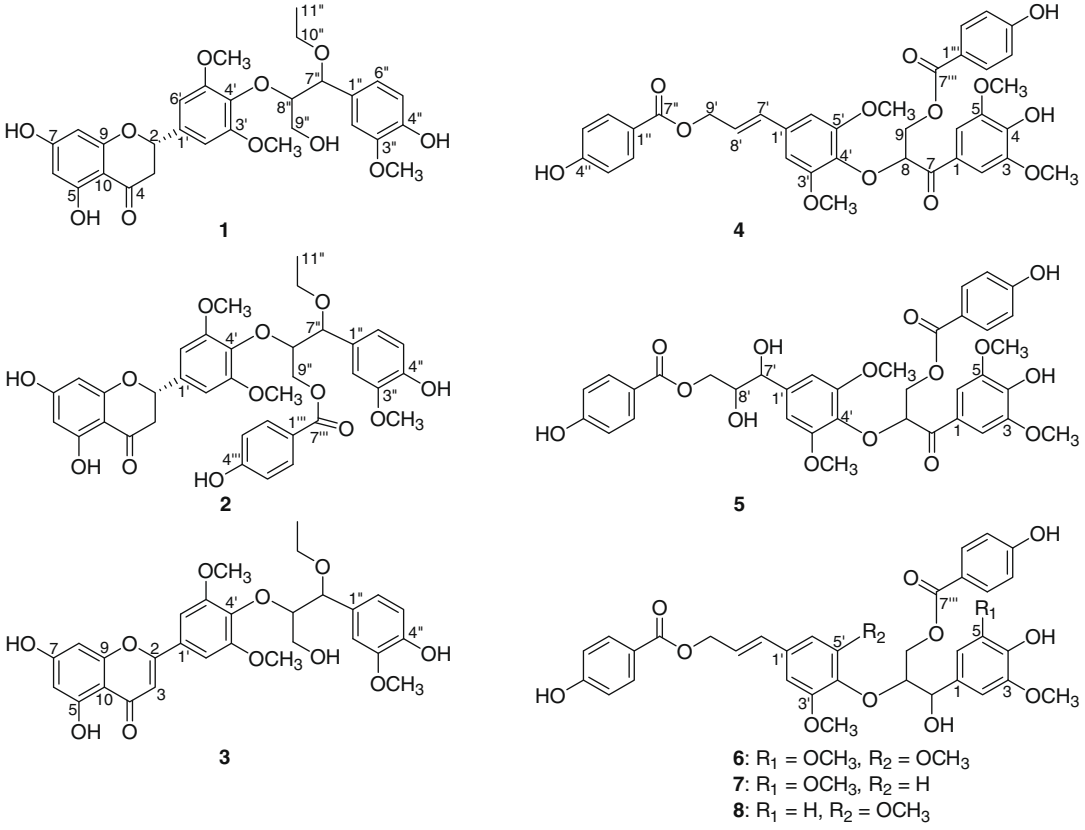

Figure 1. The chemical structures of compounds **1**–**8** from *Calamus quiquesetinervius*.

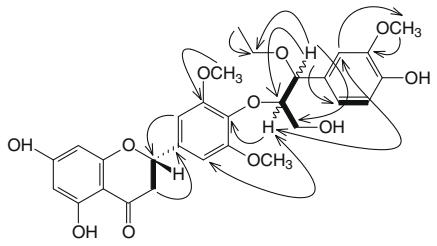

Figure 2. Key COSY (▬), HMBC (→) and NOESY (↔) correlations for compound **1**.

at C-2 was determined as *S* in accordance with a positive Cotton effect at 328 nm and a negative Cotton effect at 288 nm in its CD spectrum.[14] Therefore, **1** was assigned as (2*S*)-dihydrotricin 4′-*O*-[*threo*-β-(7″-*O*-ethylguaiacyl)-glyceryl] ether.

Quiquelignan B (**2**) was obtained as a pale–yellow amorphous powder. It had the molecular formula $C_{36}H_{36}O_{13}$ with 19 of unsaturation, as deduced by HRESIMS *m*/*z* 699.2054 $[M+Na]^+$. Characteristic UV and IR absorptions and NMR signals of **2** suggested that **2** also had a flavanone moiety as in **1**. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the presence of a benzoyl group in **2**. These spectral features suggested that **2** was also a flavonolignan, and contained the same basic skeleton as **1**. Characteristic proton signals (δ_H 7.67, d, J = 8.8 Hz, H-2‴, H-6‴ and δ_H 6.79, d, J = 8.4 Hz, H-3‴, H-5‴) for an A_2B_2 spin system and HMBC correlations [H-2‴/C-4‴ (δ_C 163.5), C-6‴ (δ_C 132.9), C-7‴ (δ_C 167.9); H-3‴/C-1‴ (δ_C 122.1), C-4‴ (δ_C 163.5), C-5‴ (δ_C 116.1)] permitted construction of a *para*-hydroxybenzoyl group. Compared with those of **1**, downshifted signals for H_2-9″ (δ_H 4.30/4.10, δ_C 66.2) in **2** and HMBC cross peak [H_2-9″/C-7‴ (δ_C 167.9)] revealed the connectivity between the 7″-*O*-ethylguaiacylglyceryl ether and the *para*-hydroxybenzoyl by an ester linkage.

Like **1**, the overlapping H-7″ signal of **2** in CD_3OD was resolved in acetone-d_6 and the relative configuration of H-7″ and H-8″ ($J_{7'',8''}$ = 4.8 Hz) was assumed to be an *erythro* form.[13,15] Therefore, **2** was determined to be (2*S*)-dihydrotricin 4′-*O*-{*erythro*-β-[7″-*O*-ethyl-(9″-*O*-4-hydroxybenzoyl)-guaiacyl]-glyceryl} ether.

Quiquelignan C (**3**) was obtained as a yellow amorphous powder. Its molecular formula was deduced to be $C_{29}H_{30}O_{11}$ with 15 of unsaturation by HRESIMS *m*/*z* 553.1727 $[M-H]^-$. The characteristic UV and IR absorption bands, as well as the ^1H and ^{13}C NMR spectra of **1** and **3**, indicated that both had similar structural features[16] except for an oxygenated double bond (δ_H 6.59, s, H-3; δ_C 164.6, 105.3, C-2, C-3) in **3** instead of an oxygenated single bond in **1**. These findings, along with the HMQC and HMBC correlations, suggested that **3** had a partial structure of the tricin (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone) moiety. On the basis of the above-mentioned evidences, **3** was identified as tricin 4′-*O*-[*threo*-β-(7″-*O*-ethylguaiacyl)-glycerol] ether.

Quiquelignan D (**4**) was obtained as a yellow amorphous powder. The positive HRESIMS *m*/*z* 697.1935 $[M+Na]^+$ indicated the molecular formula to be $C_{36}H_{34}O_{13}$ with 20 of unsaturation. The UV absorption maxima (λ_{max} 261, 275 sh and 312 nm) and IR absorbance bands (ν_{max} 3346, 1699, 1603, 1508, 1453, 1275, 1168 and 1116 cm^{-1}) indicated arylphenyl ketone and ester moieties in **4**.[17,18] The ^1H NMR spectrum of **4** exhibited two sets of symmetrical phenyl signals (δ_H 7.57, s, H-2, H-6 and δ_H 6.80, s, H-2′, H-6′), two sets of *para*-substituted aromatic moieties (A_2B_2 system; δ_H 7.92, d, J = 8.8 Hz, H-2″, H-6″; δ_H 7.65, d, J = 8.8 Hz, H-2‴, H-6‴; δ_H 6.92, d, J = 8.8 Hz, H-3″, H-5″; δ_H 6.82, d, J = 8.8 Hz, H-3‴, H-5‴), an aliphatic oxygenated methine (δ_H 5.76 m, H-8), one *trans*-olefinic methine (δ_H 6.70, d, J = 15.6 Hz, H-7′ and δ_H 6.43 m, H-8′), and two sets of oxygenated methylenes (δ_H 4.62, dd, J = 11.6, 4.0 Hz, H_a-9; δ_H 4.59, dd, J = 11.6, 6.8 Hz, H_b-9; δ_H 4.90, br d, J = 6.0 Hz, H_2-9′). Its ^{13}C and DEPT spectra exhibited four methoxy, 24 phenolic, two olefinic, three aliphatic and three carbonyl carbon signals. Detailed analysis of 2D NMR (^1H–^1H COSY, HMQC, HMBC) data revealed two *para*-hydroxybenzoyls, one 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone and one 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propene moiety. Further HMBC correlations confirmed their interconnections. Two arylpropanoids were connected through an ether linkage C8–*O*–C4′ (HMBC cross peak H-8/C-4′). One *para*-hydroxybenzoyl group was attached at C-9 (HMBC cross peak H-9/C-7‴) and the other connected at C-9′ (HMBC cross peak H-9′/C-7″) via ester bonds. Thus, **4** was an 8-*O*-4′ neolignan derivative comprising arylpropanoids[19] and sinapyl ether.[18] Key correlations for ^1H–^1H COSY, HMBC, and NOESY are shown in Figure 3.

A large coupling constant $J_{7',8'}$ = 15.6 Hz indicated an *E* geometry of the double bond at H-7′ and H-8′. However, configurations at C-8 and C-9 remained to be solved. Therefore, **4** was elucidated as 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-{2,6-dimethoxy-4[(1*E*)-3-(4-hydroxybenzoyl)-1-propenyl]phenoxy}-3-(4-hydroxybenzoyl)propan-1-one.

Quiquelignan E (**5**) was obtained as yellowish powder. Its molecular formula $C_{36}H_{36}O_{15}$ (19 of unsaturation) was derived by HRESIMS *m*/*z* 731.1978 $[M+Na]^+$. The similarity of UV and IR absorption bands in **4** and **5** indicated that both shared common structural features. The ^1H and ^{13}C NMR spectra of **5** also showed similarity with those of **4**, except for that the *trans*-olefinic signals in **4** were replaced by a 1,2-diol (δ_H 4.65, H-7′; δ_C 75.9, C-7′and δ_H 3.95, H-8′; δ_C 74.9, C-8′) moiety in **5**. Thus, **5** was an analog of 8-*O*-4′ neolignan with 1-oxo functionality.[19]

The relative configuration at H-7′ and H-8′ of **5** was established on the basis of their coupling constants. A relatively large coupling constant ($J_{7',8'}$ = 6.0 Hz) indicated a *threo* relative configuration of H-7′ and H-8′ in **5**.[12,20,21] Accordingly, **5** was elucidated as 1-(4-hydroxy-3,5-dimethoxy-phenyl)-2-{2,6-dimethoxy-4-[*threo*-3-(4-hydroxyl-benzoyl)-1,2-propanediol]phenoxy}-3-(4-hydroxybenzoyl) propan-1-one.

Quiquelignan F (**6**) was isolated as a yellow amorphous powder. Its molecular formula was assigned as $C_{36}H_{36}O_{13}$ (19 of unsaturation) by HRESIMS *m*/*z* 699.2144 $[M+Na]^+$. The UV and IR absorption bands in **6**, like **4**, showed an 8-*O*-4′ neolignan derivative. The ^1H and ^{13}C NMR spectra of **6** also showed similarity with those of **4**, except for the absence of the ketone group, which was replaced by a hydroxyl group (δ_H 5.01, d, J = 4.0 Hz, H-7; δ_C 74.5, C-7) in **6**. The ^1H–^1H COSY correlations (H-7/H-8/H_2-9) and HMBC correlations (H-7/C-1, C-2, C-6, C-8 and C-9) confirmed the position of the hydroxy group at C-7. Thus, **6** was assigned as *erythro*-1-(4-hydroxy-3,5-dimethoxy-phenyl)-2-{2,6-dimethoxy-4-[(1*E*)-3-(4-hydroxybenzoyl)-1-propenyl]phenoxy}-3-(4-hydroxybenzoyl) propan-1-ol.

Quiquelignan G (**7**) and Quiquelignan H (**8**) had the same molecular formula $C_{35}H_{34}O_{12}$ as determined by HRESIMS (*m*/*z* 669.1964 $[M+Na]^+$ for **7** and 669.1967 $[M+Na]^+$ for **8**). They also showed spectral similarity with those of **6**. The only difference was observed in the 1,2,3,5-tretrasubstituted phenyl moieties in **6**. A methoxy group (C5′–OMe) was replaced by a proton in **7**, whereas another methoxy group (C5–OMe) was replaced by a pro-

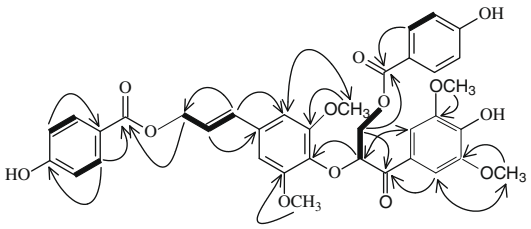

Figure 3. Key COSY (▬), HMBC (→) and NOESY (↔) correlations for compound **4**.

ton in **8**. These minor structural changes were further supported by 2D NMR correlations (C5–O*Me*/C-3, C-5, C-3′ in **7** and C5′–O*Me*/C-3, C-3′, C-5′ in **8**). Both of them belonged to guaiacyl-type lignins with the (*β*)8-*O*-4 interunit ether linkage.[22]

In **7** and **8**, a *threo* relative configuration of H-7 and H-8 was concluded by the relatively large coupling constant ($J_{7,8}$ = 6.0 Hz, in CD_3OD for **7** and $J_{7,8}$ = 7.2 Hz, in acetone-d_6 for **8**).[19,20] Consequently, the gross structures of **7** and **8** were designated as *threo*-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-{4-hydroxy-3-methoxyphenyl-4-[(1*E*)-3-(4-hydroxybenzoyl)-1-propenyl]phenoxy}-3-(4-hydroxybenzoyl) propan-1-ol and *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-{2,6-dimethoxy-4-[(1*E*)-3-(4-hydroxybenzoyl)-1-propenyl]phenoxy}-3-(4-hydroxybenzoyl)propan-1-ol, respectively.

Lignoids are natural products serving as precursors in the formation of lignins. These compounds have distinctive optically active configurations and remarkable biological activities: antioxidant, anti-inflammatory, antihypertensive, antifungal and neuroprotective.[23–26] In the view of their biosynthesis in *planta*, lignoids comprising guaicayl, syringyl or *para*-hydroxyphenyl moieties can be synthesized by non-regiospecific enzyme catalytic coupling of the monolignol pathways in *C. quiquesetinervius*.[27]

2.2. Evaluation of biological activity

Free-radicals participate in numerous inflammatory diseases in organisms, such as thrombosis, atherosclerosis and arthritis.[28] Overexpression of ROS or RNS not only destroys cells and tissue to expand the inflammatory status but also stimulates the secretion of inflammatory mediators (such as NO and TNF-α) from macrophages, particularly for the damaged cardiovascular wall.[29] Enhanced generation of free-radicals induced by chemokines also advances the activation and adhesion of platelets in the vessel lumen and activates the extrinsic coagulation cascade. Furthermore, in the inflammatory processes, platelets can also be activated by endogenous agonists (e.g., thrombin or collagen). Recent findings have further confirmed cross-talk between free-radicals, inflammation and platelet aggregation in the pathogenesis of CVD.[28,30,31] Some flavonolignans and 8-*O*-4′ neolignans with antioxidant activities also have been established to exhibit cardioprotection.[32–34] Therefore, for unequivocally exposed the structure-activity relationship, compounds **1**–**8** were further subjected to evaluate for antioxidant, anti-inflammatory and antiplatelet aggregation activities. Those exhibited strong-to-moderate biological activities in vitro (Table 3).

Lignoids **1**–**8** showed antioxidant activities against ·OH. All compounds showed very weak $O_2^{-\cdot}$ scavenging activity compared with ·OH scavenging activity. The relatively strong free-radical quenching ability of **3** may be due to the double bond at the C2–C3 position for tricin-type flavonolignans **1**–**3**. Among the neolignan ester derivatives **4**–**8**, compound **5** was the strong scavenger of ·OH, perhaps due to the diol group at C-7′ and C-8′. It also showed strong $O_2^{-\cdot}$ scavenging activity compared with other derivatives. Two-fold scavenging ability against ·OH of **6** compared with that of **4**, inferring that the reduction at C-7 of the arylpropanone group may enhance the efficiency of radical quenching. The missing methoxy group adjacent to a *para*-hydroxy group in the aromatic moiety in **7** and **8** may partly reduced their free-radical scavenging capacity. For structurally related guaiacyl or syringyl lignoids **6**–**8**, it was demonstrated that the *ortho*-methoxy substitution(s) could enhance resonance stabilization throughout the π-polyconjugation systems, making these lignoids good radical scavengers.[35,36] These radical-scavenging results further demonstrated that a diol in the arylglyceroloxy unit, hydroxy and methoxy groups in lignoids had a synergistic effect in antioxidant activity.

Compounds **2**–**4** and **6**–**8** were assessed a cytotoxic effect against Raw 264.7 cells after 24-h incubation. The MTS assay exhibited high cell viability (>85%) in the absence or presence of LPS in the culture medium at various concentrations. These compounds dose-dependently suppressed the LPS-stimulated NO production in RAW 264.7 cells. All compounds except **3**, **7** and **8** strongly reduced LPS-stimulated NO production than a positive control, quercetin (Table 3).[37] Compared with **4** and **6**, a missing methoxy group in **7** and **8** significantly reduced their anti-inflammatory activity (P <0.05). The reduction at C-7 of the arylpropanone group in **6** increased two-fold activities against NO production compared that of **4**.

Lignoids showed selective inhibitory effects toward collagen. No activity was observed for these lignoids in thrombin-induced platelet aggregation (IC_{50} >100 μM. Lignoids **2–4**, **6** and **8** showed comparable collagen-antagonistic activities with the clinical antiplatelet agent aspirin (IC_{50} 75.48 μM) (Table 3).[38] For tricin-type flavonolignans, the efficiency in **2** was notably evoked due to the additional *para*-hydroxybenzoyl moiety at C-9″ in contrast to **1**. The result also showed that **3** possesses higher activity than **1**, implying that the double bond, C2–C3, in the C-ring of tricin-type derivatives increases antiplatelet aggregation activity toward collagen. Furthermore, compound **6** showed better potency than **4, 7** and **8**. Supporting the redox form at C-7, the presence of methoxyl group, as well as anti-inflammatory activity, could obviously affect their collagen-induced platelet aggregation.

Our results showed the broad spectrum of biological activities of lignoids isolated from *C. quiquesetinervius*, and these bioactive lignoids may act on multiple targets to show their cardiovascular effects. The presence of oxygenated functional groups in lignoids has a pivotal role in controlling these activities. Compounds **2**–**4**, **6** and **8** could lower the pathogenic condition by reacting with ·OH directly or disturbing the formation of NO in the initial inflammatory stage. By regulating the release of radicals, those of compounds also may affect the antiplatelet aggregation activity.[28] Because of the specificity of antiplatelet aggregation induced by collagen, we speculated that potent isolates may also inhibit the signaling pathway of platelet activation via kinas SyK and phospholipase Cγ2.[38]

In summary, on the basis of their promising antioxidant activities, inhibitory effects on NO production and platelet aggregation, the flavonolignans (**2**, **3**) and 8-*O*-4′ neolignans (**4**–**6** and **8**) from *C. quiquesetinervius* may serve as new natural nutraceutical supplements and pharmaceutical products in the cardiovascular system in accordance with traditional herbal medications. Further in vivo study will clarify their effectiveness in CVDs.

3. Experimental

3.1. General experimental procedures

Melting points were measured with a Fisher-Johns melting point apparatus and reported without correction. Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were measured with a Hitachi U-3200 spectrophotometer. IR was recorded with KBr disks using a Nicolet Avatar 320 FT-IR spectrometer. Circular Dichroism spectra were measured with a JASCO J-715 spectropolarimeter. Mass was measured with a Finnigan MAT95S mass spectrometer. NMR spectra were measured on a Bruker NMR spectrometer (Unity Plus 400 MHz) and a Varian Unity Plus 600 (600 MHz, Varian Inc., Palo Alto, CA) spectrometer. Chromatographic materials were Celite 535 (Macherey-Nagel GmbH & Co. KG, Germany), Sephadex™ LH-20 (GE Healthcare Bio-Sciences AB), Silica Gel 60 (70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), Silica Gel 60 GF_{254} (Merck, Darmstadt, Germany). Silica Gel 60 F_{254} plates (Merck, Darmstadt, Germany) were used

for TLC. The chromatographic substances on TLC plates were visualized by anisaldehyde-sulfuric acid as a spray reagent followed by heating and UV-lamp (254 and 365 nm). The MPLC was performed using a Buchi column and pump (B-688). A semi-preparative recycle HPLC was performed on a Shimadzu LC-6AD series apparatus equipped with a SPD-6AV UV-VIS spectrophotometric detector and a preparative Cosmosil 5C^{18}-AR-II column, 250 × 20 mm i.d.). The BJL ultra-weak chemiluminescence (CL) analyzer with a high-sensitivity detector (3.3×10^{-15} W/cm^2 count) from Jye Horn Co. (Taipei, Taiwan) was used for assessing the free-radical scavenging activities. A Lumi-aggregometer (Chrono-Log Corp., Havertown, PA, USA) was used for the antiplatelet aggregation assay. All reagents used were purchased from Sigma–Aldrich Inc.

3.2. Plant material

The stems of *C. quiquesetinervius* were collected at the mountains of Yunlin County, Taiwan, in September 2005. The plant was authenticated by Professor Muh-Tsuen Kao, National Institute of Chinese Medicine. A voucher specimen of *C. quiquesetinervius* (No. NRICM20050916A) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

3.3. Biological activity evaluation procedures

3.3.1. Hydroxy radical and super oxide anion scavenging ability[39,40]

The reaction mixture used in the ·OH scavenging activity assay contained EDTA (0.05 mL, 10 mM), indoxyl-β-glucuronide (IBG, 1.0 mL, 3 μM), H_2O_2 (1.6 mL, 3%) and $FeSO_4$ (0.1 mL, 1.0 mM). On the other hand, the reaction mixture containing lucugenin (1.0 mL, 2.0 mM), methylglyoxal (0.05 mL, 1.4 μM), arginine (0.05 mL, 1.0 M) and PBS (1.0 mL, pH 7.4) was used for the O_2^{-} scavenging activity assay. The ultra-weak photons were measured using a BJL-ultra-weak CL analyzer with a high-sensitivity detector (3.3×10^{-15} W/cm^2 count). When the chemiluminescence absorbance of the reaction mixture in the CL analyzer reached the plateau, various concentrations of test samples were added into the reaction mixture. As a result, varying degrees of sudden drops in CL counts could be observed. These data represent the different degrees of ·OH or O_2^{-} scavenging abilities. The inhibitory concentration (IC_{50}) was defined as the concentration of tested sample that inhibited 50% of the generated ·OH or O_2^{-}. Trolox® was used as a positive control in these ROS assay system.

3.3.2. Measurement of nitric oxide in RAW 264.7 macrophage cells[37,41]

Murine RAW 264.7 macrophage cells obtained from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan) were maintained in 96-well plates (1×10^5 cells/well) containing Dulbecco's modified Eagle's medium (DMEM) (Sigma–Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS) and $NaHCO_3$ (3.7 g/L) under an atmosphere of 5% CO_2 at 37 °C. After overnight pre-incubation, cells were incubated with vehicle or compounds **2**–**4** and **6**–**8** (20.0, 10.0, 5.0, 2.50, 1.25, 0.62, 0.31 μg/mL) in the absence or presence of 1 μg/mL lipopolysaccharide (LPS) from *Escherichia coli* serotype 0127:B8 (Sigma–Aldrich) for 24 h. All tested compounds were dissolved in dimethyl sulfoxide (DMSO) and the final concentration was adjusted to 0.2% (v/v). Nitric oxide (NO) concentration was determined by measuring the accumulation of nitrite (NO_2^{-}) in the RAW 264.7 cell culture supernatant. The aforementioned supernatant medium (100 μL) was aliquoted and mixed with 100 μL Griess reagent (1:1, v/v, 1% sulfanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% H_3PO_4) in the 96-well plates. After 5-min incubation, the optical density was measured at 540 nm with an ELISA reader (Molecular Dynamics Spectra Max, GMI Inc., Minnesota, USA).

For further evaluation of cell viability, the MTS assay was also carried out in the presence or absence of LPS and tested compounds. MTS solution (Promega Corporation, Madison, WI, USA) (16.7% MTS in RPMI 1640) was added to each well and incubated for 10 min. Absorbance was measured at 490 nm with an ELISA reader.

3.3.3. Antiplatelet aggregation assay

Platelet-rich plasma preparation and antiplatelet aggregation assay were carried out in accordance with the methods described by Chia et al.[42] Briefly, platelet pellets collected from human blood was resuspended in Tyrode's solution consisting of NaCl (136.8 mM), KCl (2.8 mM), $NaHCO_3$ (11.9 mM), $MgCl_2$ (2.1 mM), NaH_2PO_4 (0.33 mM), $CaCl_2$ (1.0 mM), glucose (11.2 mM) and bovine serum albumin (0.35%). These platelet suspensions were incubated with vehicle or compounds **1**–**4** and **6**–**8** at 37 °C for 3 min under stirring (1200 rpm) before the addition of inducers (thrombin and collagen). By using a Lumi-aggregometer, the extent of platelet aggregation was measured as the increase of light transmission at 5 min after the addition of aggregation inducer.

3.3.4. Statistical analysis

The statistical analysis was calculated by Student's unpaired *t*-test and presented as mean ± SE and all experiments were performed in triplicate. *P* values less than 0.05 were considered to be significant.

3.4. Extraction and isolation

Dried stems (15.4 kg) of *C. quiquesetinervius* Burret were sectioned and extracted with 95% EtOH (100 L, 50 °C, 72 h). The EtOH extract was dried under reduced pressure at 45 °C to yield a brown syrup (1.0 kg). The concentrated brown syrup was resuspended in 10% aqueous-EtOH and partitioned with *n*-hexane (3 L × 5), ethyl acetate (4 L × 5) and *n*-butanol (4 L × 3) successively to afford 90, 85 and 462 g of dried organic extracts, respectively.

Extracts were tested for bioactivities, and a bioactivity-guided fractionation method was used for isolation work. On the basis of the bioactivities of the extracts, the most active EtOAc extract was fractionated over a Celite 535 column (35 × 8.0 cm) eluting stepwise with *n*-hexane (3 L), EtOAc (6 L), EtOAc/MeOH (1:1; 6 L) and MeOH (1.5 L) to give four fractions: A (5.2 g), B (52.0 g), C (25.8 g) and D (2.1 g). Fraction B was subjected to MPLC over Silica Gel 60 using an increasing amount of MeOH in CH_2Cl_2 to obtain six fractions (Fr B_1–B_6). Fraction B_3 (22.9 g) was passed through a Sephadex LH-20 column and eluted with CH_2Cl_2/MeOH (1:1) to afford six fractions (Fr B_{3-1}–B_{3-6}). Fraction B_{3-2} underwent further chromatography on a Silica Gel 60 column using a gradient of CH_2Cl_2/MeOH (25:1–4:1, v/v) to give six fractions (Fr B_{3-2-1}–B_{3-2-6}). Subsequent separation of Fr B_{3-2-5} (4.87 g) over a Sephadex LH-20 column using $CHCl_3$/MeOH (2:1) yielded eight fractions (Fr $B_{3-2-5-1}$–$B_{3-2-5-8}$). Fraction $B_{3-2-5-5}$ (2.68 g) was further separated over a Sephadex LH-20 using $CHCl_3$/MeOH (1:3) to furnish five fractions (Fr $B_{3-2-5-5-1}$–$B_{3-2-5-5-5}$). Finally, fraction $B_{3-2-5-5-4}$ (250 mg) was purified by preparative recycle HPLC (Cosmosil 5C^{18}-AR-II, 250 × 20 mm i.d., 50% aqueous-acetonitrile) to give compounds **1** (1.2 mg), **2** (4.9 mg), **3** (2.2 mg), **4** (3.1 mg), **6** (7.9 mg), **7** (1.3 mg) and **8** (5.6 mg).

Fraction B_4 (19.0 g) was fractionated over a Sephadex LH-20 column using CH_2Cl_2/MeOH (1:1) to give four subfractions (Fr B_{4-1}–B_{4-4}). Fraction B_{4-2} (15.45 g) was separated over a Sephadex LH-20 column using $CHCl_3$/MeOH (1:3) to give fractions B_{4-2-1}–B_{4-2-3}. Fraction B_{4-2-3} (3.72 g) was re-chromatographed over a Sephadex LH-20 column using MeOH to give three subfractions (Fr

$B_{4-2-3-1}$–$B_{4-2-3-3}$). Fraction $B_{4-2-3-1}$ (1.02 g) was fractionated over a reverse-phase semi-preparative HPLC (35% aqueous-MeOH, 10 mL/min) to afford Fr $B_{4-2-3-1-5}$–$B_{4-2-3-1-8}$. Fraction $B_{4-2-3-1-5}$ (516 mg) eluted at 13.5 min was further separated by HPLC (Cosmosil $5C^{18}$-AR-II column, 250 × 20 mm i.d., 2.5 mL/min, gradient of acetonitrile [A] and water [B], 26 to 31% A for 50 min and 31 to 80% A for 80 min) and eight subfractions (Fr $B_{4-2-3-1-5-1}$–$B_{4-2-3-1-8}$) were collected. Finally, compound **5** (1.4 mg) was purified from the fraction $B_{4-2-3-1-5-3}$ by PTLC using CH_2Cl_2/acetone/MeOH (8:1:0.4).

3.5. Quiquelignan A (1)

Orange yellow amorphous powder; mp 145 °C; $[\alpha]_D^{25}$ −14.29 (*c* 0.28, MeOH); UV (MeOH) λ_{max} 212, 230, 286, 322 nm; IR (KBr) ν_{max} 3366, 2934, 1707, 1632, 1600, 1457, 1275, 1121, 1029 cm^{-1}; CD ($c = 1.99 \times 10^{-5}$ M, MeOH) λ_{max} ($\Delta\varepsilon$) 328 (+1.77), 287 (−6.76) nm; ESIMS *m/z* 579.2 $[M+Na]^+$; HRESIMS *m/z* 579.1895 $[M+Na]^+$, calcd for $C_{29}H_{32}O_{11}Na$, 579.1842; For ^1H NMR (CD_3OD, 600 MHz), ^1H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1.

3.6. Quiquelignan B (2)

Pale yellow amorphous powder; mp 116 °C; $[\alpha]_D^{25}$ −9.76 (*c* 0.41, MeOH); UV (MeOH) λ_{max} 216, 260, 275, 325 nm; IR (KBr) ν_{max} 3370, 2943, 1703, 1639, 1603, 1461, 1271, 1124, 1025 cm^{-1}; CD ($c = 1.18 \times 10^{-4}$ M, MeOH) λ_{max} ($\Delta\varepsilon$) 326 (+2.13), 290 (−5.92) nm; ESIMS *m/z* 699.2 $[M+Na]^+$; HRESIMS *m/z* 699.2150 $[M+Na]^+$, calcd for $C_{36}H_{36}O_{13}Na$, 699.2054; For ^1H NMR (CD_3OD, 400 MHz), ^1H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1.

3.7. Quiquelignan C (3)

Yellow amorphous powder; mp 194 °C; $[\alpha]_D^{25}$ +7.14 (*c* 0.28, MeOH); UV (MeOH) λ_{max} 204, 272, 335 nm; IR (KBr) ν_{max} 3417, 2922, 1734, 1647, 1588, 1497, 1421, 1259, 1125, 1029 cm^{-1}; ESIMS m/z 553.0 $[M-H]^-$; HRESIMS *m/z* 553.1727 $[M-H]^-$, calcd for $C_{29}H_{30}O_{11}$, 553.1710; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 1.

3.8. Quiquelignan D (4)

Yellow amorphous powder; mp 109 °C; $[\alpha]_D^{25}$ +2.67 (*c* 0.75, MeOH); UV (MeOH) λ_{max} 209, 261, 275 (sh), 312 nm; IR (KBr) ν_{max} 3346, 2942, 1699, 1603, 1508, 1453, 1275, 1168, 1116, 1021, 851, 764 cm^{-1}; ESIMS *m/z* 697.1 $[M+Na]^+$; HRESIMS *m/z* 697.1935 $[M+Na]^+$, calcd for $C_{36}H_{34}O_{13}Na$, 697.1897; For ^1H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) spectroscopic data, see Table 2.

Table 1
^1H and ^{13}C NMR data of the compounds **1**–**3**[a,d]

	1[b]			**2**[c]			**3**[1c]	
	δ^1H^{1b}	δ^1H^{2b}	$\delta^{13}C^{1b}$	δ^1H^{1c}	δ^1H^{2c}	$\delta^{13}C^{1c}$	δ^1H	$\delta^{13}C$
2	5.35 dd (13.0,2.7)	5.44 br d (12.6)	80.6	5.35 dd (12.8, 2.8)	5.43 br d (12.0)	80.6		164.6
3	3.08 dd (17.1, 13.0)	3.08 dd (17.4, 12.6)	44.3	3.08 dd (17.2, 12.8)	3.18 dd (16.8, 12.0)	44.4	6.59 s	105.3
	2.73 dd (17.1, 2.7)	2.72 br d (17.4)		2.72 dd (17.2, 2.8)	2.72 br d (16.8)			
4			197.3			197.4		183.2
5			165.5			165.5		163.0
6	5.88 d (1.8)	5.94 br s	97.4	5.88 d (2.4)	6.03 br s	97.3	6.10 d (1.6)	102.0
7			169.1			168.7		171.6
8	5.91 br s	5.97 br s	96.4	5.94 br s	6.06 br s	96.4	6.33 d (1.6)	96.5
9			64.6			164.7		159.8
10			103.2			103.3		104.0
1′			136.2			135.9		127.9
2′,6′	6.75 s	6.84 s	104.8	6.73 s	6.84 s	104.6	7.14 s	104.9
3′,5′			154.5			154.5		154.6
4′			136.9			138.0		140.4
1″			132.1			131.3		131.9
2″	6.88 br s	6.88 br s	112.0	6.98 d (1.6)	7.08 br s	112.2	6.89 br s	112.2
3″			148.7			148.9		148.7
4″			147.1			147.4		147.2
5″	6.73 d (8.4)	6.79 d (7.8)	115.6	6.77 d (8.0)	6.82 d (7.8)	115.9	6.73 d (8.4)	115.6
6″	6.76 d (7.8)	6.85 d (7.2)	121.9	6.85 dd (8.0, 1.6)	6.94 d (7.8)	121.3	6.76 dd (8.0, 1.2)	122.0
7″	4.58 ov	4.70 d (6.0)	81.8	4.70 ov	4.77 d (4.8)	83.5	4.56 d (6.4)	81.9
8″	4.29 m	4.17 m	86.5	4.69 m	4.68 m	83.7	4.47 m	86.6
9″	3.91 dd (12.0, 4.2)	3.92 ov	61.7	4.30 dd (11.8, 2.2)	4.36 br d (12.0)	66.2	3.95 dd (12.0, 2.8)	62.0
	3.80 ov	3.78 ov		4.10 dd (11.8, 5.4)	4.13 dd (12.0, 6.0)		3.75 dd (12.0, 4.8)	
10″	3.42 m	3.44 m	65.5	3.42 m	3.46 m	65.6	3.40 m	65.5
11″	1.17 t (7.2)	1.15 t (6.9)	15.6	1.10 t (7.2)	1.09 t (6.9)	15.6	1.17 t (7.2)	15.6
1‴						122.1		
2‴, 6‴				7.67 d (8.8)	7.67 d (8.4)	132.9		
3‴, 5‴				6.79 d (8.8)	6.87 d (7.8)	116.1		
4‴						163.5		
7‴						167.9		
OMe at								
3′, 5′	3.78 s	3.82 s	56.7	3.72 s	3.74 s	56.5	3.86 s	56.8
OMe at								
3″	3.81 s	3.85 s	56.4	3.76 s	3.79 s	56.3	3.80 s	56.4

[b1b]Data were measured in CD_3OD at 600 MHz for ^1H NNR and 125 MHz for ^{13}C NNR, respectively; [2b]Data were measured in acetone-d_6 at 600 MHz for ^1H NNR.
[c1c]Data were measured in CD_3OD at 400 MHz for ^1H NNR and 100 MHz for ^{13}C NNR, respectively; [2c]Data were measured in acetone-d_6 at 400 MHz for ^1H NNR.
[a] Data based on ^1H–^1H COSY, HMQC, HMBC and NOESY spectra.
[d] br s: broad singlet, br d: broad doublet, d: doublet, dd: doublet of doublets, m: multiple, ov: overlapped, s: singlet, t: triplet; the *J* values were shown in parentheses.

Table 2
^{1}H and ^{13}C NMR data of the compounds **4**–**8**[a,d]

	4[b]		**5**[c]		**6**[c]		**7**[c]		**8**[b]	
	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C
1		127.4		127.0		132.7		132.5		132.9
2	7.57 s	108.0	7.47 s	108.3	6.73 s	105.0	6.75 s	105.0	7.05 d (1.6)	111.5
3		148.4		149.3		149.0		149.2		148.0
4		142.4		143.9		137.2		139.3		147.0
5		148.4		149.3		149.0		149.2	6.75 d (8.4)	115.3
6	7.57 s	108.0	7.47 s	108.3	6.73 s	105.0	6.75 s	105.0	6.91 d (8.4)	120.6
7		194.2		196.5	5.01 d (4.0)	74.5	4.95 d (6.0)	75.0	5.04 d (7.2)	74.6
8	5.76 m	81.3	5.60 m	82.6	4.62 m	84.8	4.70 m	84.0	4.36 m	86.7
9_a	4.62 dd (11.6, 4.0)	65.4	4.69 dd (12.0, 4.0)	65.9	4.53 dd (11.8, 7.0)	65.0	4.38 dd (12.0, 3.6)	65.2	4.50 dd (12.0, 3.2)	65.1
9_b	4.59 dd (11.6, 6.8)		4.59 dd (12.0, 6.8)		4.41 dd (11.8, 3.0)		4.22 dd (12.0, 6.4)		4.10 dd (12.0, 4.6)	
1′		133.4		139.3		133.9		132.9		133.3
2′	6.80 s	104.7	6.60 s	104.9	6.70 s	104.9	7.05 d (1.6)	111.5	6.82 s	104.8
3′		153.9		153.8		154.5		152.0		153.9
4′		136.7		136.5		135.8		149.5		137.6
5′		153.9		153.8		154.5	7.02 d (8.4)	119.0		153.9
6′	6.80 s	104.7	6.60 s	104.9	6.70 s	104.9	7.27 dd (8.4, 2.0)	121.0	6.82 s	104.8
7′	6.70 d (15.6)	134.3	4.65 d (6.0)	75.9	6.66 d (16.0)	134.9	6.68 d (16.0)	134.9	6.72 d (15.6)	134.3
8′	6.43 m	124.4	3.95 m	74.9	6.36 m	124.3	6.33 dt (16.0, 3.2)	123.3	6.45 m	124.4
$9'_a$	4.90 br d (6.0)	65.5	4.20 dd (11.6, 3.6)	66.1	4.92 ov	66.2	4.89 ov	66.4	4.91 br d (6.0)	65.5
$9'_b$			4.00 dd (11.6, 5.2)							
1″		121.9		122.0		122.2		121.7		122.0
2″	7.92 d (8.8)	132.5	7.87 d (8.8)	133.0	7.91 d (8.8)	132.9	7.91 d (8.8)	132.9	7.91 d (8.4)	132.5
3″	6.92 d (8.8)	116.1	6.81 d (8.8)	116.3	6.82 d (8.8)	116.2	6.83 d (8.8)	116.3	6.91 d (8.4)	116.1
4″		162.8		163.4		163.5		163.9		162.1
5″	6.92 d (8.8)	116.1	6.81 d (8.8)	116.3	6.82 d (8.8)	116.2	6.83 d (8.8)	116.3	6.91 d (8.4)	116.1
6″	7.92 d (8.8)	132.5	7.87 d (8.8)	133.0	7.91 d (8.8)	132.9	7.91 d (8.8)	132.9	7.91 d (8.4)	132.5
7″		166.3		168.1		168.0		168.1		166.4
1‴		122.2		121.7		122.1		122.2		122.0
2‴	7.65 d (8.8)	132.5	7.70 d (8.8)	132.9	7.55 d (8.8)	132.9	7.65 d (8.8)	132.9	7.71 d (8.8)	132.5
3‴	6.82 d (8.8)	115.9	6.75 d (8.8)	116.2	6.71 d (8.8)	116.0	6.74 d (8.8)	116.2	6.85 d (8.8)	115.9
4‴		162.9		163.6		163.8		163.9		162.9
5‴	6.82 d (8.8)	115.9	6.75 d (8.8)	116.2	6.71 d (8.8)	116.0	6.74 d (8.8)	116.2	6.85 d (8.8)	115.9
6‴	7.65 d (8.8)	132.5	7.70 d (8.8)	132.9	7.55 d (8.8)	132.9	7.65 d (8.8)	132.9	7.71 d (8.8)	132.5
7‴		166.3		167.8		168.0		167.8		166.3
OMe at 3	3.71 s	56.1	3.85 s	56.4	3.71 s	56.5	3.77 s	56.3	3.73	56.7
OMe at 5	3.71 s	56.1	3.85 s	56.4	3.71 s	56.5	3.77 s	56.3		
OMe at 3′	3.86 s	56.3	3.58 s	56.7	3.81 s	56.7	3.76 s	56.9	3.81 s	56.3
OMe at 5′	3.86 s	56.3	3.58 s	56.7	3.81 s	56.7			3.81 s	56.3

[d]br d: broad doublet, d: doublet, dd: doublet of doublets, dt: doublet of triplets, m: multiple, ov: overlapped, s: singlet; the *J* values were shown in parentheses.
[a] Data based on ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra.
[b] Data were measured in acetone-d_6 at 600 MHz for ^{1}H NNR and 125 MHz for ^{13}C NNR.
[c] Data were measured in CD_3OD at 400 MHz for ^{1}H NNR and 100 MHz for ^{13}C NNR.

Table 3
Free radical scavenging, anti-inflammatory and antiplatelet aggregation activities of isolates **1**–**8** from *C. quiquesetinervius*.

Compound	IC_{50} (μM)			
	·OH	$O_2^{-\cdot}$[a]	NO[b]	Collagen (10 μg/mL)[c]
1	27.4 ± 3.4	>200	ND[d]	>100[a]
2	19.9 ± 1.3	>200	36.0 ± 4.4	43.3
3	15.3 ± 1.4	>200	63.4 ± 4.4	49.4
4	27.5 ± 1.6	>200	20.0 ± 2.5	67.5
5	6.2 ± 1.2	53.8 ± 2.9	ND[d]	ND[d]
6	12.4 ± 1.7	>200	11.80 ± 2.5	26.9
7	19.0 ± 2.5	>200	100.6 ± 3.1	>100
8	20.4 ± 2.9	>200	52.4 ± 4.3	55.2
Trolox®[e]	4.4 ± 0.3	32.8 ± 0.1		
Quercetin[e]			54.3 ± 1.3	
Aspirin[e]				75.4

[a] A compound was regarded as inactive when the IC_{50} >200 μM for $O_2^{-\cdot}$ and >100 μM for antiplatelet aggregation.
[b] Effects of **2**–**4** and **6**–**8** were measured by LPS-stimulated NO production in RAW 264.7 macrophage cells.
[c] Effects of **1**–**4** and **6**–**8** were measured by collagen-induced platelet aggregation.
[d] ND, not measured.
[e] Trolox®, quercetin and aspirin were used as positive controls for free-radical scavenging, anti-inflammatory and antiplatelet aggregation activities, respectively.

3.9. Quiquelignan E (5)

Pale yellow amorphous powder; mp 214 °C; $[\alpha]_D^{25}$ −42.42 (*c* 0.33, MeOH); UV (MeOH) λ_{max} 204, 235, 258, 275 (sh), 310 nm; IR (KBr) ν_{max} 3359, 2959, 1700, 1608, 1513, 1463, 1272, 1168, 1123, 1027, 855, 771 cm^{-1}; ESIMS *m/z* 731.1 [M+Na]$^+$; HRESIMS *m/z* 731.1978 [M+Na]$^+$, calcd for $C_{36}H_{36}O_{15}Na$, 731.1952; For ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2 .

3.10. Quiquelignan F (6)

Yellow amorphous powder; mp 128 °C; $[\alpha]_D^{25}$ +2.13 (*c* 0.94, MeOH); UV (MeOH) λ_{max} 206, 260, 275 (sh) nm; IR (KBr) ν_{max} 3362, 2942, 1699, 1607, 1512, 1453, 1279, 1164, 1116, 1021, 851, 768 cm^{-1}; ESIMS *m/z* 699.1 [M+Na]$^+$; HRESIMS *m/z* 699.2144 [M+Na]$^+$, calcd for $C_{36}H_{36}O_{13}Na$, 699.2054; For ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2.

3.11. Quiquelignan G (7)

Pale yellow amorphous powder; mp 134 °C, $[\alpha]_D^{25}$ +1.89 (*c* 0.53, MeOH); UV (MeOH) λ_{max} 206, 261, 277 (sh) nm; IR (KBr) ν_{max} 3417,

2942, 2356, 1691, 1600, 1508, 1267, 1116, 1025, 851, 768 cm^{-1}; ESIMS *m/z* 669.1 $[M+Na]^+$; HRESIMS *m/z* 669.1964, calcd for $C_{35}H_{34}O_{12}Na$, 669.1948; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2.

3.12. Quiquelignan H (8)

Yellow amorphous powder; mp 140 °C; $[\alpha]_D^{25}$ +10.42 (*c* 0.48, MeOH); UV (MeOH) λ_{max} 202, 260, 276 (sh) nm; IR (KBr) ν_{max} 3429, 2962, 1695, 1607, 1512, 1263, 1116, 1025, 803 cm^{-1}; ESIMS m/*z* 669.1 $[M+Na]^+$; HRESIMS *m/z* 669.1967 $[M+Na]^+$, calcd for $C_{35}H_{34}O_{12}Na$, 669.1948; For ^1H NMR (acetone-d_6, 400 MHz,) and ^{13}C NMR (acetone-d_6, 100 MHz,) spectroscopic data, see Table 2.

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