Journal of Natural Medicines (2018) 72:632–640
https://doi.org/10.1007/s11418-018-1192-z

ORIGINAL PAPER

New geranyl flavonoids from the leaves of *Artocarpus communis*

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Received: 22 January 2018 / Accepted: 16 February 2018 / Published online: 24 February 2018

Abstract
Four new geranyl flavonoids **1**–**4** and four known flavonoids **5**–**8** were obtained from the leaves of *Artocarpus communis* collected in Indonesia. The planar structures of flavonoids were elucidated by analyses of MS and NMR spectroscopic data. Absolute configurations of **1** and **2** were determined by ECD spectroscopy. Analyses by HPLC with a chiral-phase column and ECD spectra confirmed that **3** and **4** were stereoisomeric mixtures and **7** and **8** were racemic mixtures. The compounds obtained in this study inhibited the enzymatic activities of ubiquitin-specific protease 7 (USP7) and the chymotrypsin-like activity of the proteasome. Among the geranyl flavonoids tested in this experiment, the USP7 inhibitory activity of **6** (IC_{50} value, 0.094 μM) was 55 times more potent than the commercially available positive control, P5091 (IC_{50} value, 5.2 μM).

Keywords *Artocarpus communis* · Moraceae · Geranyl flavonoid · Chiral resolution · USP7 · Proteasome

Introduction

Medicinal plants have been used as folk medicines in Indonesia, and the bioactive metabolites of these traditional medicines have attracted much interest as new drug candidates [1, 2]. The genus *Artocarpus* is distributed in the tropical and subtropical areas of Asia and used as traditional medicines to treat inflammation, diabetes, malarial fever, abscesses, and diarrhea [3] as well as a food in South-East Asia [4]. The constituents of *Artocarpus* species are well-studied and a variety of phenolic compounds including flavonoids, arylbenzofurans, and stilbenoids were reported as the major bioactive components [5]. Flavonoids isolated from *Artocarpus* species show a wide range of biological activities such as cytotoxicity [6, 7], antibacterial and antifungal [8], antimalarial [9], anti-inflammatory [10, 11], and tyrosinase inhibitory activities [9]. Continuing research on the constituents of the traditional medicinal plants is necessary in order to utilize the traditional ethnopharmacological knowledge for the development of new drugs. In this study, we investigated the biologically active constituents of the leaves of *A. communis* (family Moraceae) collected in Indonesia and obtained four new geranyl flavonoids along with four known congeners.

Results and Discussion

Separation of eight geranyl flavonoids (1–8)

The leaves of *A. communis* were extracted with EtOH. The extract was concentrated and partitioned between EtOAc and H_2O, and then the organic layer was concentrated and partitioned between *n*-haxane and 90% MeOH–H_2O. The concentrated aqueous MeOH fraction was subjected to silica gel column chromatography followed by HPLC to afford eight compounds. The structures of four new geranyl flavonoids were determined to be (*S*,*E*)-2-(3,4-dihydroxyphenyl)-8-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxychroman-4-one (**1**), (*S*)-5,7-dihydroxy-8-((2*E*,5*E*)-7-hydroxy-3,7-dimethylocta-2,5-dien-1-yl)-2-(4-hydroxyphenyl)chroman-4-one (**2**), (2*S*)-5,7-dihydroxy-8-((*E*)-6-hydroxy-3,7-dimethylocta-2,7-dien-1-yl)-2-(4-hydroxyphenyl)chroman-4-one (**3**), and 1-(2,4-dihydroxyphenyl)-3-(8-hydroxy-2-(3-hydroxy-4-methylpent-4-en-1-yl)-2-methyl-2*H*-chromen-5-yl)propan-1-one (**4**) (Fig. 1) by detailed analyses of the spectroscopic data. In addition to **1**–**4**, four known flavonoids sophoraflavanone A [12, 13], 2-geranyl-2′,3,4,4′-tetrahydroxydihydrochalcone [14], 1-(2,4-dihydroxyphenyl)-

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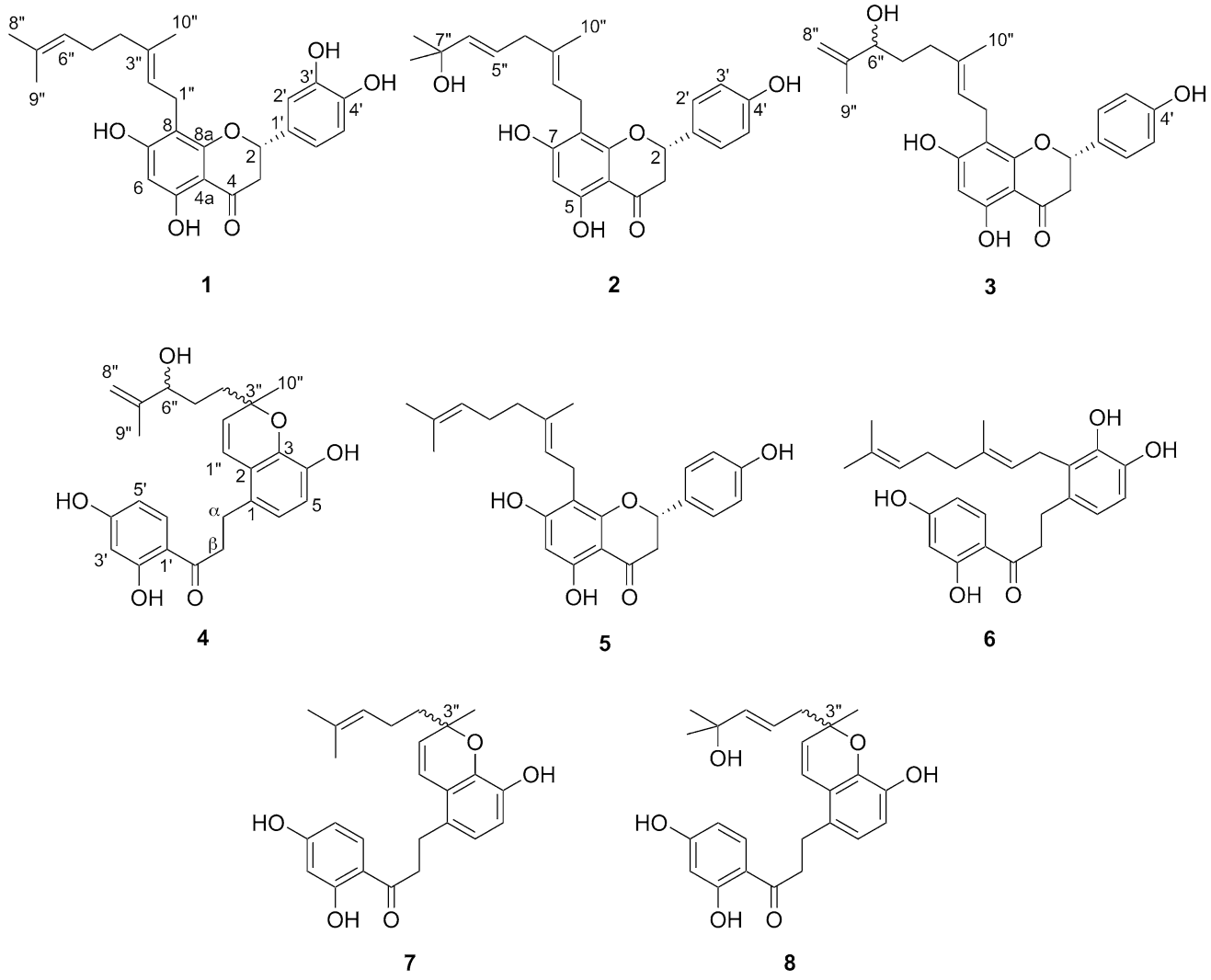

Fig. 1 Chemical structures of geranyl flavonoids **1–8** from the leaves of *A. communis*

3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyranyl]-1-propanone [14], and 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2*H*-1-benzopyran-5-yl]-1-propanone (**8**) [15] were identified (Fig. 1).

Structure elucidation of four new geranyl flavonoids (1–4)

Compound **1** was obtained as pale yellow solid. The molecular formula of $C_{25}H_{28}O_6$ was confirmed by HRESIMS. Interpretation of ^{1}H and ^{13}C NMR spectra together with HSQC spectrum revealed the presence of a carbonyl carbon at δ_C 196.6 (C-4), five oxygenated aromatic carbons at δ_C 161.1 (C-5), 164.4 (C-7), 159.7 (C-8a), 145.2 (C-3′), and 145.5 (C-4′), eleven sp^2 carbons at δ_C 101.8 (C, C-4a), 95.2 (CH, C-6), 107.0 (C, C-8), 129.8 (C, C-1′), 114.2 (CH, C-2′), 115.3 (CH, C-5′), 117.6 (CH, C-6′), 122.4 (CH, C-2″), 133.8 (C, C-3″), 124.1 (CH, C-6″), and 130.6 (C, C-7″), four methylenes at δ_C 42.1 (C-3), 21.2 (C-1″), 39.3 (C-4″), and 26.2 (C-5″), one methine at δ_C 78.2 (C-2), and three methyls at δ_C 25.5 (C-8″), 17.5 (C-9″), and 15.8 (C-10″) (Table 1). The COSY correlations indicated the presence of three spin systems, H-2 (δ_H 5.33)/H-3 (δ_H 3.10), H-1″ (δ_H 3.08)/H-2″ (δ_H 5.09), and H-4″ (δ_H 1.86)/H-5″ (δ_H 1.96)/H-6″ (δ_H 5.01) (Fig. 2a). The HMBC correlations from two methyls at δ_H 1.58 (Me-8″) and δ_H 1.51 (Me-9″) to C-7″ and C-6″, and from a methyl proton at δ_H 1.55 (H-10″) to C-2″, C-3″, and C-4″, together with a NOE correlation between H-1″ (δ_H 3.08) and H-10″ assigned the structure of a geranyl moiety (Fig. 2a). The flavanone skeleton was established by the HMBC correlations from an aromatic proton H-6 (δ_H 5.95) to C-4a, C-5, C-7, and C-8, from hydroxyl proton 5-OH (δ_H 12.09) to C-4a, C-5, and C-6, from H-2′ to C-2, C-4′, and

Table 1 ^{13}C NMR data for **1**–**3** (150 MHz)

No.	**1**[a]	**2**[b]	**3**[b]
2	78.2	78.6	78.7
3	42.1	43.0	43.1
4	196.6	196.3	196.4
4a	101.8	103.1	103.2
5	161.1	162.2	162.2
6	95.2	96.7	96.7
7	164.4	163.7	163.6
8	107.0	106.5	106.4
8a	159.7	159.8	159.7
1′	129.8	130.6	130.8
2′	114.2	127.7	127.8
3′	145.2	115.6	115.6
4′	145.5	156.3	156.1
5′	115.3	115.6	115.6
6′	117.6	127.7	127.8
1″	21.2	21.7	21.7
2″	122.4	122.8	122.2
3″	133.8	135.9	137.5
4″	39.3	42.4	35.82/35.87
5″	26.2	124.8	32.8
6″	124.1	139.5	75.76/75.80
7″	130.6	70.9	147.2
8″	25.5	29.7	111.1
9″	17.5	29.7	17.7
10″	15.8	16.2	16.1

[a]Measured in DMSO-d_6

[b]Measured in $CDCl_3$

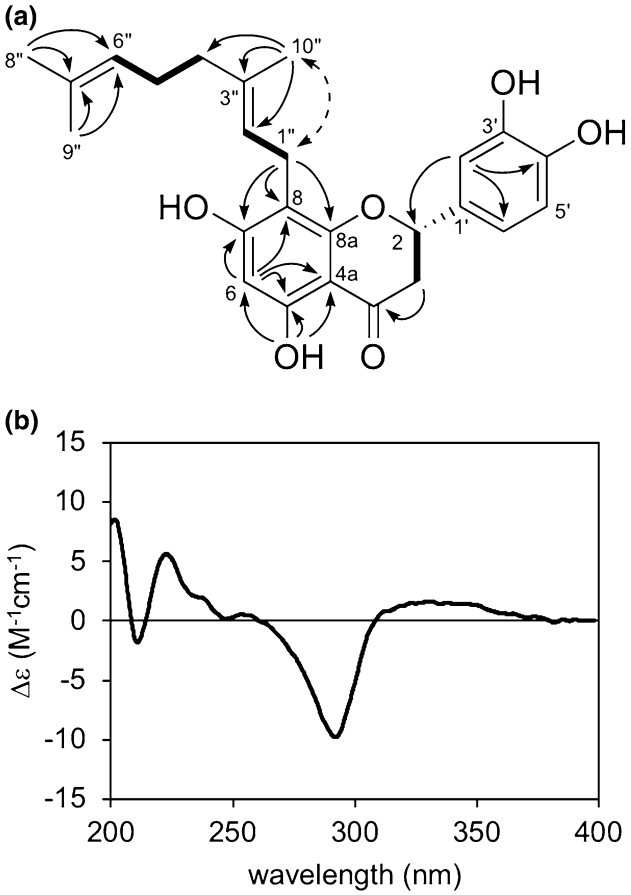

Fig. 2 **a** COSY (bold line), key HMBC (solid arrow), and key NOE (dashed arrow) correlations of **1**. **b** ECD spectrum of **1** in MeOH

C-6′, and from H-3 (δ_H 2.69) to C-4. The geranyl side chain was attached to C-8, which was established by the HMBC correlations from H-1″ to C-7, C-8, and C-8a. The structure of ring B was determined as 3′,4′-dihydroxyphenyl on the basis of the comparison of the ^{13}C NMR chemical shifts reported in the literature [16]. Although the proton signals for H-5′ (δ_H 6.72) and H-6′ (δ_H 6.72) were completely overlapped in DMSO-d_6, they were slightly differentiated in CD_3OD, and the *ortho* coupling constants (8.3 Hz) of H-5′ (δ_H 6.78)/H-6′ (δ_H 6.80) and the *meta* coupling constants (1.5 Hz) of H-2′ (δ_H 6.92)/H-6′ confirmed the 3′,4′-dihydroxyphenyl structure. The absolute configuration at C-2 was unambiguously determined to be *S* by the negative Cotton effect around 290 nm [17] (Fig. 2b). Yang et al. [16] previously pointed out that the assignment of the substitution pattern in the dihydroxy aromatic ring requires careful analyses of ^{1}H and ^{13}C NMR data and revised the structures of 28 compounds with 3,5-dioxygenated aromatic rings. During this study, we found that the reported 3′,5′-dihydroxyphenyl structures of three flavanones, 5,7,3′,5′-tetrahydroxyflavanone [18], schizolaenone C [19], and xeractinol [20], should be corrected to the 3′,4′-dihydroxyphenyl structures (Fig. 3), which were ultimately identified to be (–)-eriodictyol [21], diplacone [22], and taxifolin 6-*C*-glucoside [23], respectively.

The molecular formula of **2** was determined to be $C_{25}H_{28}O_6$. Comparison of the ^{1}H NMR spectra of **1** and **2** indicated their structural similarity (Table 2). The ^{1}H NMR spectrum of **2** showed mutually coupled aromatic protons at δ_H 7.28 (H-2′ and H-6′) and δ_H 6.86 (H-3′ and H-5′), indicating the presence of a *p*-substituted phenyl group. The HMBC correlations from H-2′/H-6′ to a methine carbon at δ_C 78.6 (C-2) and an oxygenated aromatic carbon at δ_C 156.3 (C-4′) and from H-3′/H-5′ to an aromatic carbon at δ_C 130.6 (C-1′) assigned a hydroxyl group substituted at C-4′ in the aromatic ring B of the flavanone. Detailed analysis of the 1D and 2D NMR spectra of **2** indicated the 5,7,4′-trihydroxy flavanone with a modified geranyl side chain bound to C-8. The ^{1}H NMR spectrum showed the presence of an *E*-olefin ($J_{H\text{-}5'',H\text{-}6''}$ 15.0 Hz) at C-5″ (δ_H 5.54, δ_C 124.8)/C-6″ (δ_H 5.60, δ_C 139.5). The HMBC correlation from H-8″/H-9″ (δ_H 1.28) to C-6″ and C-7″ (δ_C 70.9) confirmed the position of a hydroxyl group at C-7″. Finally, the planar structure of

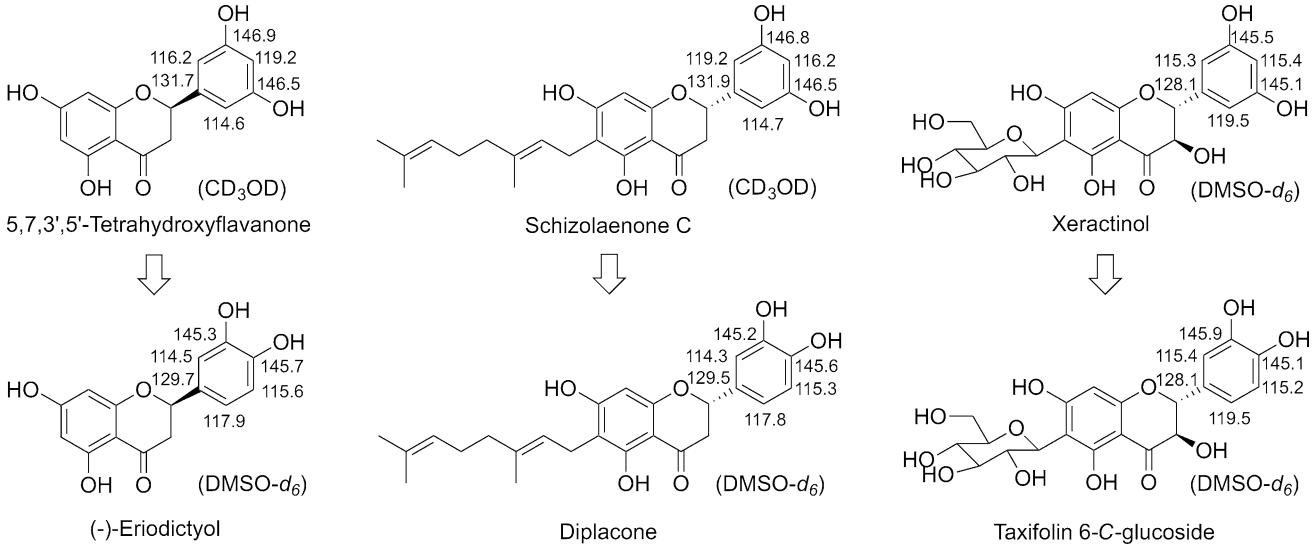

Fig. 3 Proposed (upper) and corrected (lower) structures of three previously reported flavanones

Table 2 ^{1}H NMR data for **1**–**3** (600 MHz)

No.	**1**[a]	**2**[b]	**3**[b]
2	5.33, dd (2.8, 12.5)	5.33, dd (2.9, 12.5)	5.33, dd (3.0, 12.7)
3	2.69, dd (2.8, 17.0)	2.79, dd (2.9, 17.6)	2.78, dd (3.0, 17.0)
	3.10, dd (12.5, 17.0)	3.00, dd (12.5, 17.6)	3.02, dd (12.7, 17.0)
6	5.95, s	6.00, s	5.98, s
2′	6.86, brs	7.28, d (8.7)	7.30, d (8.5)
3′		6.86, d (8.7)	6.86, d (8.5)
5′	6.72, brs	6.86, d (8.7)	6.86, d (8.5)
6′	6.72, brs	7.28, d (8.7)	7.30, d (8.5)
1″	3.08, d (6.5)	3.27, d (6.8)	3.28, d (7.1)
2″	5.09, t (6.5)	5.20, t (6.8)	5.20, t (7.1)
4″	1.86, t (7.0)	2.66, d (6.4)	2.03, m
5″	1.96, m	5.54, dd (6.4, 15.0)	1.60, m
6″	5.01, t (6.4)	5.60, d (15.0)	4.00, t (6.5)
8″	1.58, s	1.28, s	4.80, s
			4.88, s
9″	1.51, s	1.28, s	1.69, s
10″	1.55, s	1.64, s	1.69, s
5-OH	12.09, s	11.98, s	11.98, s

[a]Measured in DMSO-d_6

[b]Measured in $CDCl_3$

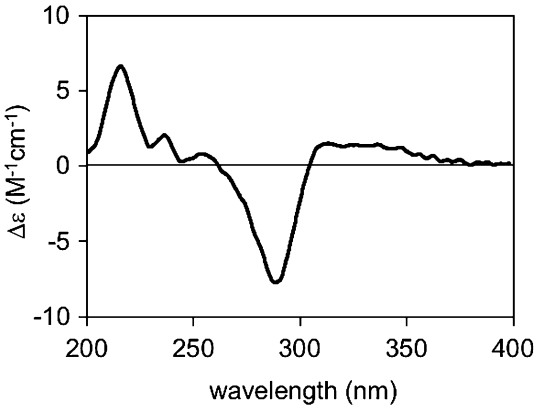

Fig. 4 ECD spectrum of **2** in MeOH

2 was determined and the absolute configuration at C-2 was determined to be *S* by the negative Cotton effect at 290 nm in ECD spectroscopy (Fig. 4) [17].

Compound **3** had the molecular formula $C_{25}H_{28}O_6$. The ^{1}H and ^{13}C NMR spectra of **2** and **3** implied a difference in the modified geranyl side chain. HMBC correlations from a methyl at δ_H 1.69 (Me-9″) to an oxymethine carbon at δ_C 75.8 (C-6″) and olefin carbons at δ_C 147.2 (C-7″) and δ_C 111.1 (C-8″) assigned the planar structure of **3**. The 2*S* configuration of **3** was determined by the ECD spectrum (Fig. 5a) [17]. Detailed analysis of the ^{13}C NMR spectrum of **3** showed two sets of slightly shifted signals at δ_C 35.82/35.87 (C-4″) and δ_C 75.76/75.80 (C-6″), indicating that **3** was an epimeric mixture at C-6″. Purification by HPLC with a chiral-phase column furnished two equivalent peaks (Fig. 5b), and thereby **3** was determined to be an epimeric mixture of 6″*R*- and 6″*S*-**3** in the ratio 1:1.

The molecular formula of **4** was determined to be $C_{25}H_{28}O_6$ from the HRESIMS. In the ^{13}C NMR spectrum of **4**, most carbons showed sets of slightly shifted chemical shifts in the ratio 1:1 (Table 3), and **4** was indicated to be a mixture of stereoisomers. 1D and 2D NMR spectroscopic data showed one carbonyl carbon at δ_C 203.74/203.78, four oxygenated aromatic carbons at δ_C 139.40/139.45 (C-3), δ_C

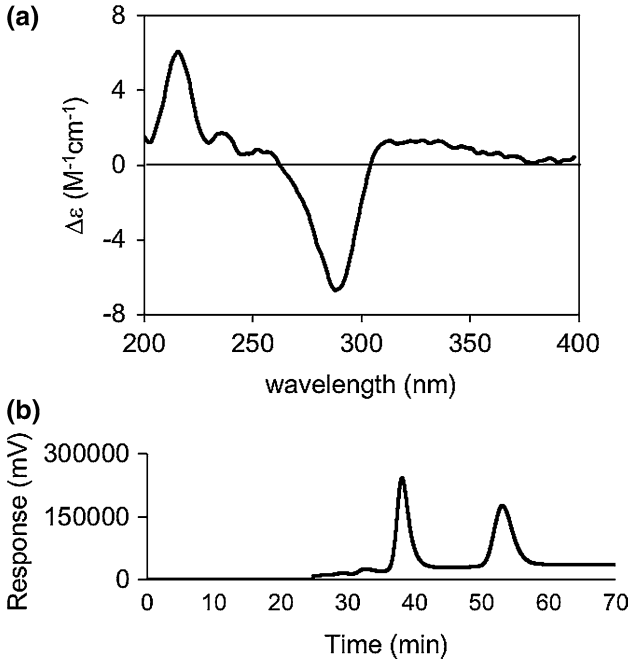

Fig. 5 **a** ECD spectrum of **3** in MeOH. **b** HPLC chromatogram of **3** (0.8 mg) with a chiral-phase column

143.06/143.07 (C-4), δ_C 165.3 (C-2′), and δ_C 163.27/163.28 (C-4′), 12 sp^2 carbons at δ_C 127.96/127.98 (C, C-1), δ_C 118.88/118.93 (CH, C-2), δ_C 114.69/114.71 (CH, C-5), δ_C 121.26/121.29 (CH, C-6), δ_C 113.62/113.64 (C, C-1′), δ_C 103.6 (CH, C-3′), δ_C 107.9 (CH, C-5′), δ_C 132.21/132.25 (CH, C-6′), δ_C 119.7 (CH, C-1″), δ_C 129.64/129.71 (CH, C-2″), δ_C 146.99/147.07 (C, C-7″), and δ_C 111.44/111.57 (CH_2, C-8″), two methyls at δ_C 29.15/29.29 (C-9″) and δ_C 26.25/26.41 (C-10″), four methylenes at δ_C 39.64/39.69 (C-α), 26.58/26.63 (C-β), δ_C 36.68/36.88 (C-4″), and δ_C 17.51/17.59 (C-5″), one oxymethine at δ_C 75.87/75.90 (C-6″), and one quaternary carbon at δ_C 78.51/78.63 (C-3″). COSY correlations revealed five spin systems H-5 (δ_H 6.71)/H-6 (δ_H 6.60), H-α (δ_H 3.07)/H-β (δ_H 2.96), H-5′ (δ_H 6.30)/H-6′ (δ_H 7.48), H-1″ (δ_H 6.50)/H-2″ (δ_H 5.58), and H-5″ (δ_H 1.61)/H-6″ (δ_H 4.05) (Fig. 6a). A dihydrochalcone skeleton was confirmed by the HMBC correlations from H-5 to C-1 and C-3, from H-6 to C-2 and C-4, from H-5′ to C-1′ and C-3′, from H-6′ to C-2′, C-4′, and a carbonyl carbon, from H-β to C-1, C-2, and C-6, from H-α to a carbonyl carbon (Fig. 6a). The remaining structure was elucidated from the 1D and 2D NMR spectroscopic data. A coupling constant of 10.0 Hz between olefin protons H-1″ and H-2″ confirmed the *Z*-olefin configuration. HMBC correlations from H-1″ to C-1, C-3, and C-3″, from H-2″ to C-2 and C-3″, from H-6″ to C-4″, C-5″, C-7″, and C-8″, from Me-10″ (δ_H 1.36) to C-2″, C-3″, and C-4″, from Me-9″ (δ_H 1.68) to C-6″, C-7″, and C-8″ established the planar structure of **4**. As explained above, the ^{13}C NMR spectrum showed **4** to be a mixture of two stereoisomers. In addition, no Cotton effect in the ECD spectroscopy indicated that **4** had a racemic nature. Taken together, **4** appeared to be a mixture of four stereoisomers. HPLC analysis with a chiral-phase column showed four peaks with nearly equal peak areas. Thus, **4** was confirmed to be a mixture of four stereoisomers at C-3″ and C-6″ (Fig. 6b).

Table 3 ^{1}H and ^{13}C NMR data for **4** in $CDCl_3$

No.	δ_C	δ_H, mult. (*J* in Hz)
1	127.96/127.98	
2	118.88/118.93	
3	139.40/139.45	
4	143.06/143.07	
5	114.69/114.71	6.71, d (8.2)
6	121.26/121.29	6.60, d (8.2)
α	39.64/39.69	3.07, m
β	26.58/26.63	2.96, m
C=O	203.74/203.78	
1′	113.62/113.64	
2′	165.3	
3′	103.6	6.35, brd (2.4)
4′	163.27/163.28	
5′	107.9	6.30, dd (2.4, 8.8)
6′	132.21/132.25	7.48, d (8.8)
1″	119.7	6.50, d (10.0)
2″	129.64/129.71	5.58, d (10.0)
3″	78.51/78.63	
4″	36.68/36.88	1.61, m
		1.74, m
5″	29.15/29.29	1.61, m
		1.70, m
6″	75.87/75.90	4.05, m
7″	146.99/147.07	
8″	111.44/111.57	4.83, m
		4.92, brs
9″	17.51/17.59	1.68, brs
10″	26.25/26.41	1.36, s
2′-OH		12.74, s

Measured at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR

Study on the absolute configurations of four known geranyl flavonoids (5–8)

Four known geranyl flavonoids **5**–**8** were obtained from *A. communis* in this study. However, as there were no previous descriptions of the stereochemistries of **7** and **8** [14, 15], we investigated their absolute configurations. The specific rotations of **7** and **8** were nearly zero (**7**, − 0.7; **8**, + 0.4), and no obvious Cotton effects were observed in the ECD spectra. Resolution of **7** by HPLC with a chiral-phase

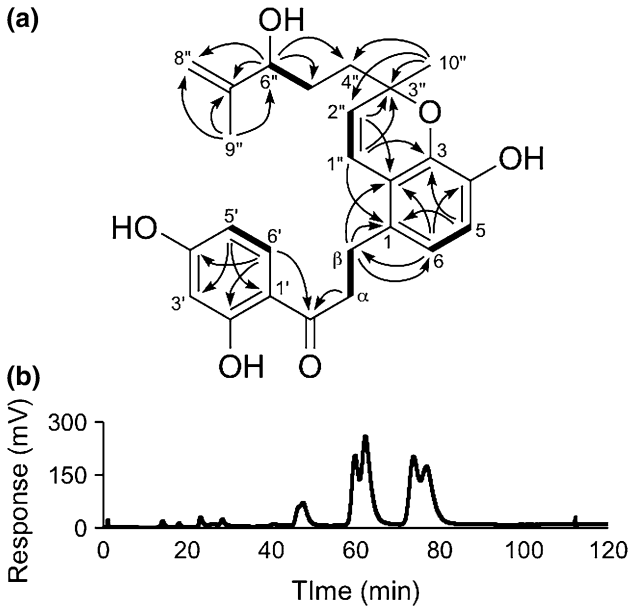

Fig. 6 **a** COSY (bold line) and key HMBC (arrow) correlations of **4**. **b** HPLC chromatogram of **4** (10 µg) with a chiral-phase column

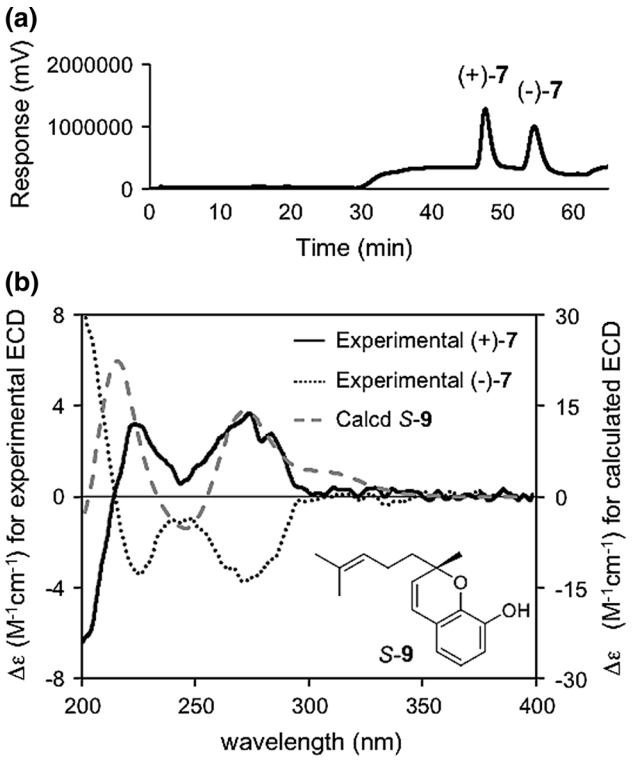

Fig. 7 **a** HPLC chromatogram of **7** (1.2 mg) with a chiral-phase column. **b** Experimental ECD spectra of (+)- and (–)-**7** and calculated ECD spectrum of a model compound *S*-**9**

column successfully afforded equivalent amounts of (+)-**7** ($[\alpha]_D^{20}$ + 40) and (–)-**7** ($[\alpha]_D^{20}$ − 45), and thus **7** was concluded to be a racemic mixture (Fig. 7a). The ECD spectrum of (+)-**7** showed positive Cotton effects around 220 and 275 nm, and (−)-**7** showed opposite Cotton effects to (+)-isomer (Fig. 7b). A Cotton effect due to the styrene chromophore is expected to be observed around 270 nm [24, 25]. Thus, the positive Cotton effect around 270 nm of (+)-**7** indicated a 3″*S* configuration. To confirm this result, the ECD spectrum of *S*-chromenone (**9**) was calculated and a positive Cotton effect was observed at 270 nm (Fig. 7b). Compound **8** was separated by HPLC with a chiral-phase column to afford (+)- and (–)-isomers in the ratio 1:1 (Fig. 8a). The absolute configurations of (+)-and (−)-isomers were determined to be 3″*S* and 3″*R*, respectively, from their ECD spectra (Fig. 8b).

Biological activities of 1–8

The biological activities of **1**–**6**, (+)- and (–)-**7**, and (+)- and (–)-**8** were evaluated with our in-house screening including cytotoxicity, antimicrobial activities, inhibitory activity of the cholesterol ester accumulation in macrophages, inhibitory activity of the RANKL-induced formation of multinuclear osteoclasts, and inhibitory activities of the ubiquitin–proteasome system (proteasome, E1, Ubc13 (E2)–Uev1A interaction, p53–Mdm2 (E3) interaction, and USP7). Among them, **1**, **4**, **5**, and **6** inhibited USP7 activity with 76, 92, 42, and 94% inhibition, respectively, at 1 µM, and the IC_{50} values of **1**, **5**, and **6** were 0.26, 1.2, and 0.094 µM, respectively. In addition, **1**, **5**, and **6** inhibited the

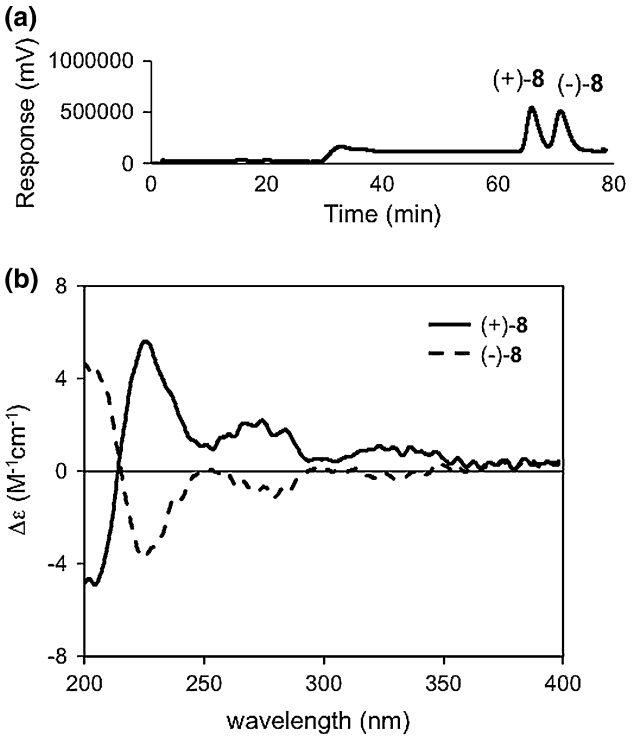

Fig. 8 **a** HPLC chromatogram of **8** (1.5 mg) with a chiral-phase column. **b** Experimental ECD spectra of (+)- and (−)-**8**

chymotrypsin-like activity of the proteasome by 77, 24, and 67%, respectively, at 1 μM. This study showed that these geranyl flavonoids inhibited the enzymatic activity of USP7 and the proteasome at relatively low concentrations. Flavonoids are highly reactive and are known to inhibit various enzymatic activities including the proteasome [26, 27]. Further study is needed to reveal the relationship between structures of geranyl flavonoids and inhibitory activities of USP7 and the proteasome.

Conclusion

Four new geranyl flavonoids were obtained from the leaves of *A. communis* collected in Indonesia along with four known congeners. The chemical structures were elucidated by analyses of NMR and MS spectroscopic data together with the calculated ECD spectra. During the structure elucidation, we found that the reported structures for 5,7,3′,5′-tetrahydroxyflavanone, schizolaenone C, and xeractinol were incorrect and the same as those of (–)-eriodictyol, diplacone, and taxifolin 6-*C*-glucoside, respectively. The geranyl flavonoids obtained in this study inhibited the activities of USP7 and the chymotrypsin-like activity of the proteasome. Although an inhibitory effect of flavonoids on the proteasome has already been reported, this is the first report of geranyl flavonoids that inhibited USP7 and the proteasome. Among the geranyl flavonoids tested in this experiment, the USP7 inhibitory activity of **6** (IC_{50} value, 0.094 μM) was 55 times more potent than the commercially available positive control, P5091 (IC_{50} value, 5.2 μM). Ubiquitin-dependent protein degradation is a promising target for cancer drug discovery, and our study showed the potential of geranyl flavonoids as inhibitors of the ubiquitin–proteasome system.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter in MeOH. UV spectra were measured on a JASCO V-550 spectrophotometer in MeOH. ECD spectra were measured on a JASCO J-820 spectropolarimeter in MeOH. IR spectra were recorded on a Perkin Elmer Frontier FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 NMR spectrometer. HRESIMS spectra were measured on a Bruker BioTOF and impact II mass spectrometer. The preparative HPLC system comprised a Waters 515 HPLC pump, Waters 2489 UV/visible detector, and Pantos Unicorder U-228. Silica gel-precoated plates (TLC Silica gel $60F_{254}$, Merck) were used for TLC detection, and spots were visualized using UV light (254 and/or 365 nm) and *p*-anisaldehyde reagent.

Plant material

Leaves of *A. communis* (15P099, fresh weight 169 g) were collected at Sam Ratulangi University, Kampus Bahu, Indonesia, in 2015 and soaked in EtOH immediately.

Extraction and separation

Leaves of *A. communis* (fresh weight, 169 g) were extracted with EtOH. Half of the extract was partitioned between EtOAc and H_2O. The EtOAc fraction was partitioned between *n*-hexane and 90% MeOH–H_2O. The 90% MeOH–H_2O layer (1.1 g) was concentrated in vacuo and subjected to silica gel column chromatography with a stepwise gradient elution using *n*-hexane/EtOAc (2:1), CH_2Cl_2/MeOH (19:1 and 9:1), and CH_2Cl_2/MeOH/H_2O (6:4:1) to afford 10 fractions. A portion (30 mg) of Fr. 2 (130 mg), eluted with *n*-hexane/EtOAc (2:1), was applied to gel filtration HPLC (Asahipak GS-310P, Asahi Chemical Industry Co., Ltd., 21.5 × 500 mm) with *n*-hexane/EtOAc (2:1) and HPLC (Inertsil Diol, GL Sciences, 20 × 250 mm) with *n*-hexane/EtOAc (2:1) to afford **7** (6.9 mg). A portion (30 mg) of Fr. 3 (90 mg), eluted with *n*-hexane/EtOAc (2:1), was applied to gel filtration HPLC (Asahipak GS-310P, 21.5 × 500 mm) with CH_2Cl_2/MeOH (10:1) to yield **5** (10.4 mg). Fr. 4 (64 mg), eluted with CH_2Cl_2/MeOH (19:1), was subjected to gel filtration HPLC (Asahipak GS-310P, 21.5 × 500 mm) with CH_2Cl_2/MeOH (4:1) followed by HPLC (Inertsil Diol, 20 × 250 mm) with *n*-hexane/EtOAc (1:1) to afford **1** (2.5 mg) and **6** (2.2 mg). The remaining half of the EtOH extract was subjected to solvent partitioning followed by silica gel column chromatography in a similar manner to that described above and afforded 10 fractions. Fr. 1 (140 mg), eluted with CH_2Cl_2/MeOH (19:1), was applied to gel filtration HPLC (Asahipak GS-310P, 21.5 × 500 mm) with MeOH to afford nine fractions. Frs. 6 (13 mg) and 7 (14 mg) were combined and subjected to normal phase HPLC (COSMOSIL 5SL-II, Nacalai Tesque Inc., 10 × 250 mm) with *n*-hexane/CH_2Cl_2/MeOH (20:19:1) followed by ODS HPLC (COSMOSIL 5C18-AR-II, Nacalai Tesque Inc., 10 × 250 mm) eluted with 70% MeOH–H_2O to afford **2** (0.5 mg), **3** (0.8 mg), and **4** (1.7 mg). Fr. 5 (12 mg) was purified by normal phase HPLC (COSMOSIL 5SL-II, 10 × 250 mm) with *n*-hexane/CH_2Cl_2/MeOH (20:19:1) and ODS HPLC (COSMOSIL π-Nap, Nacalai Tesque Inc., 10 × 250 mm) with 70% MeOH–H_2O to afford **8** (1.5 mg). Separation of **3** (0.8 mg) by HPLC using a chiral-phase column (CHIRALCEL OJ-H, Daicel, 4.6 × 250 mm) with *n*-hexane/EtOH (4:1) afforded two equivalent amounts of diastereomers (0.4 mg each). Analysis of **4** (10 μg) under

the same conditions revealed four peaks. Resolution of **7** (2.4 mg) and **8** (1.5 mg) was carried out by the same column with *n*-hexane/2-propanol (4:1) to yield (+)- (1.1 mg)/(–)-**7** (1.1 mg) and (+)- (0.4 mg)/(–)-**8** (0.6 mg), respectively.

Compound **1**: a yellow amorphous solid. $[\alpha]_D^{21}-19$ (c = 2.1, MeOH). UV λ_{max} (MeOH) nm (log ε): 292 (3.9) nm. IR (film) υ_{max} 3198, 2923, 2854, 1634, 1602, 1520, 1436, 1378, 1342, 1264, 1180, 1072, 1114, 1072, 1022, 1000, 820, 781, 550 cm^{-1}. ^1H and ^{13}C NMR data (DMSO-d_6), see Tables 1 and 2. HRESIMS *m/z* 423.1837 $[M–H]^-$ (calcd for $C_{25}H_{27}O_6$, 423.1813).

Compound **2**: a yellow amorphous solid. $[\alpha]_D^{25}-20$ (c = 0.4, MeOH). UV λ_{max} (MeOH) nm (log ε): 292 (4.1) nm. IR (film) υ_{max} 3210, 2925, 1635, 1600, 1519, 1436, 1343, 1267, 1170, 1073, 1023, 834 cm^{-1}. ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 1 and 2. HRESIMS *m/z* 447.1773 $[M + Na]^+$ (calcd for $C_{25}H_{28}NaO_6$, 447.1778).

Compound **3**: a yellow amorphous solid. $[\alpha]_D^{25}-14$ (c = 0.4, MeOH). UV λ_{max} (MeOH) nm (log ε): 294 (3.9) nm. IR (film) υ_{max} 3210, 2924, 1634, 1600, 1518, 1435, 1342, 1267, 1170, 1073, 1023, 833 cm^{-1}. ^1H and ^{13}C NMR data ($CDCl_3$), see Tables 1 and 2. HRESIMS *m/z* 447.1774 $[M + Na]^+$ (calcd for $C_{25}H_{28}NaO_6$, 447.1778).

Compound **4**: a yellow amorphous solid. $[\alpha]_D^{21}+7.2$ (c = 1.9, MeOH). UV λ_{max} (MeOH) nm (log ε): 326 (3.6), 276 (4.1), 218 (4.3) nm. IR (film) υ_{max} 3252, 2926, 1719, 1627, 1494, 1443, 1365, 1239, 1207, 1133, 1022, 987, 902, 802, 724, 614, 563 cm^{-1}. ^1H and ^{13}C NMR data ($CDCl_3$), see Table 3. HRESIMS *m/z* 447.1777 $[M + Na]^+$ (calcd for $C_{25}H_{28}NaO_6$, 447.1778).

Conformational analysis and ECD calculation of *S*-9

This experiment was conducted as previously reported [28]. Conformational analysis was performed with Spartan'16 (Ver. 1.0.0; Wavefunction Inc., Irvine, CA), and ECD calculation was performed with Gaussian09 (Revision D.01; Gaussian, Wallingford, CT, USA) [29] at the B3LYP/TZVP level. No wavelength correction was needed.

USP7 inhibition assay

This assay was performed as previously reported [30]. P5091 (Abcam, Inc.) was used as positive control and its IC_{50} value was 5.2 μM.

Proteasome inhibition assay

This experiment was performed as previously reported [31]. MG132 (Peptide Institute, Inc.) was used as positive control and its IC_{50} value was 0.037 μM.

Acknowledgements This work was supported by JSPS KAKENHI Grants 26305005 (S.T.) and The Program for Promoting the Enhancement of Research Universities, MEXT (H.K.). We thank V. H. Bahihi and S. Puasa of Sam Ratulangi University for collection and identification of the plant.

References

1. Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14:111–129
2. Rates SM (2001) Plants as source of drugs. Toxicon 39:603–613
3. Jagtap UB, Bapat VA (2010) *Artocarpus*: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 129:142–166
4. Baliga MS, Shivashankara AR, Haniadka R, Dsouza J, Bhat HP (2011) Phytochemistry, nutritional and pharmacological properties of *Artocarpus heterophyllus* Lam (jackfruit): a review. Food Res Int 44:1800–1811
5. Hakim A (2010) Diversity of secondary metabolites from genus *Artocarpus* (Moraceae). Nusant Biosci 2:146–156
6. Hsu CL, Shyu MH, Lin JA, Yen GC, Fang SC (2011) Cytotoxic effects of geranyl flavonoid derivatives from the fruit of *Artocarpus communis* in SK-Hep-1 human hepatocellular carcinoma cells. Food Chem 127:127–134
7. Suhartati T, Achmad SA, Aimi N, Hakim EH, Kitajima M, Takayama H, Takeya K (2001) Artoindonesianin L, a new prenylated flavone with cytotoxic activity from *Artocarpus rotunda*. Fitoterapia 72:912–918
8. Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HM, Ambassa P, Ngadjui BT (2011) Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl (Moraceae). BMC Complement Altern Med 11:6
9. Bourjot M, Apel C, Martin MT, Grellier P, Nguyen V, Guéritte F, Litaudon M (2010) Antiplasmodial, antitrypanosomal, and cytotoxic activities of prenylated favonoids isolated from the stem bark of *Artocarpus styracifolius*. Planta Med 76:1600–1604
10. Hsu CL, Chang FR, Tseng PY, Chen YF, El-Shazly M, Du YC, Fang SC (2012) Geranyl flavonoid derivatives from the fresh leaves of *Artocarpus communis* and their anti-inflammatory activity. Planta Med 78:995–1001
11. Wei BL, Weng JR, Chiu PH, Hung CF, Wang JP, Lin CN (2005) Antiinflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. J Agric Food Chem 53:3867–3871
12. Shirataki Y, Endo M, Yokoe I, Komatsu M (1983) Studies on the constituents of *Sophora* species. XVIII. Constituents of the root of *Sophora tomentosa* L. Chem Pharm Bull 31:2859–2863
13. Shirataki Y, Yokoe I, Endo M, Komatsu M (1985) Determination of C-6 or C-8 substituted flavanone using ^{13}C-^1H long range coupling and the revised structures of some flavanones. Chem Pharm Bull 33:444–447
14. McLean S, Reynolds WF, Tinto WF, Chan WR, Shepherd V (1996) Complete ^{13}C and ^1H spectral assignments of prenylated flavonoids and a hydroxy fatty acid from the leaves of caribbean *Artocarpus communis*. Magn Reson Chem 34:719–722
15. Wang Y, Xu K, Lin L, Pan Y, Zheng X (2007) Geranyl flavonoids from the leaves of *Artocarpus altilis*. Phytochemistry 68:1300–1306
16. Yang YN, Zhu H, Chen Z, Liu F, An YW, Feng ZM, Jiang JS, Zhang PC (2015) NMR spectroscopic method for the assignment of 3,5-dioxygenated aromatic rings in natural products. J Nat Prod 78:705–711

17. Slade D, Ferreira D, Marais JPJ (2005) Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66:2177–2215
18. Hashmi MA, Khan A, Ayub K, Farooq U (2014) Spectroscopic and density functional theory studies of 5,7,3′,5′-tetrahydroxyflavanone from the leaves of *Olea ferruginea*. Spectrochim Acta Part A Mol Biomol Spectrosc 128:225–230
19. Murphy B, Cao S, Norris A, Miller J, Ratovoson F, Andriantsiferana R, Rasamison V, Kingston D (2006) Cytotoxic compounds of *Schizolaena hystrix* from the madagascar rainforest. Planta Med 72:1235–1238
20. Dokkedal AL, Lavarda F, Santos LC, Vilegas W (2007) Xeractinol: a new flavanonol *C*-glucoside from *Paepalanthus argenteus* var. *argenteus* (Bongard) Hensold (Eriocaulaceae). J Braz Chem Soc 18:437–439
21. Hori K, Satake T, Saiki Y, Tanaka N, Murakami T, Chen CM (1988) Chemical and chemotaxonomical studies of filices. LXXIV. The novel flavanone glycosides of *Pyrrosia linearfolia* (HOOK.) CHING. Yakugaku Zasshi 108:417–421
22. Phillips WR, Baj NJ, Gunatilaka AAL, Kingston DGI (1996) *C*-Geranyl compounds from *Mimulus clevelandii*. J Nat Prod 59:495–497
23. Mbafor JT, Fomum ZT, Promsattha R, Sanson DR, Tempesta MS (1989) Isolation and characterization of taxifolin 6-*C*-glucoside from *Garcinia epunctata*. J Nat Prod 52:417–419
24. Choi H, Hwang H, Chin J, Kim E, Lee J, Nam SJ, Lee BC, Rho BJ, Kang H (2011) Tuberatolides, potent FXR antagonists from the Korean marine tunicate *Botryllus tuberatus*. J Nat Prod 74:90–94
25. Kikuchi T, Mori Y, Yokoi T, Nakazawa S, Kuroda H, Masada Y, Kitamura K, Kuriyama K (1983) Structure and absolute configuration of sargatriol, a new isoprenoid chromenol from a brown alga, *Sargassum tortile* C. Agardh. Chem Pharm Bull 31:106–113
26. Chang TL (2009) Inhibitory effect of flavonoids on 26S proteasome activity. J Agric Food Chem 57:9706–9715
27. Chen D, Landis-Piwowar KR, Chen MS, Dou QP (2007) Inhibition of proteasome activity by the dietary flavonoid apigenin is associated with growth inhibition in cultured breast cancer cells and xenografts. Breast Cancer Res 9:R80
28. Kagiyama I, Kato H, Nehira T, Frisvad JC, Sherman DH, Williams RM, Tsukamoto S (2016) Taichunamides: prenylated indole alkaloids from *Aspergillus taichungensis* (IBT 19404). Angew Chem Int Ed 55:1128–1132
29. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA Jr, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Keith T, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ (2013) Gaussian 09, Revision D.01. Gaussian Inc., Wallingford
30. Tanokashira N, Kukita S, Kato H, Nehira T, Angkouw ED, Mangindaan REP, Voogd NJD, Tsukamoto S (2016) Petroquinones: trimeric and dimeric xestoquinone derivatives isolated from the marine sponge *Petrosia alfiani*. Tetrahedron 72:5530–5540
31. Noda A, Sakai E, Kato H, Losung F, Mangindaan REP, Voogd NJD, Yokosawa H, Tsukamoto S (2015) Strongylophorines, meroditerpenoids from the marine sponge *Petrosia corticata*, function as proteasome inhibitors. Bioorg Med Chem Lett 25:2650–2653