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New Flavones, a 2-(2-Phenylethyl)-4*H*-chromen-4-one Derivative, and Anti-Inflammatory Constituents from the Stem Barks of *Aquilaria sinensis*

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Received: 14 October 2015 ; Accepted: 16 November 2015 ; Published: 24 November 2015
Academic Editors: Nancy D. Turner and Isabel C. F. R. Ferreira

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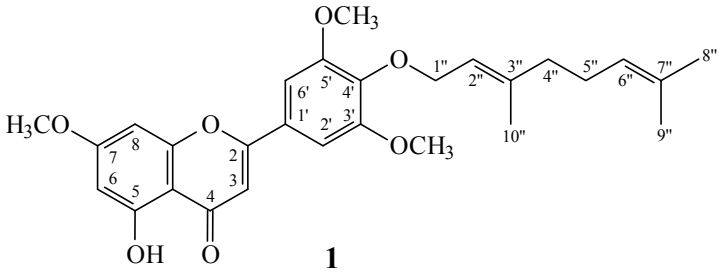
Abstract: In the current study, two new flavones, 4′-*O*-geranyltricin (**1**) and 3′-*O*-geranylpolloin (**2**), and a new 2-(2-phenylethyl)-4*H*-chromen-4-one derivative, 7-hydroxyl-6-methoxy-2-(2-phenylethyl)chromone (**3**), have been isolated from the stem barks of *A. sinensis*, together with 21 known compounds **4**–**24**. The structures of new compounds **1**–**3** were determined through spectroscopic and MS analyses. Compounds **2**, **3**, **5**, **6**, and **8**–**10** exhibited inhibition ($IC_{50} \leqslant 12.51$ μM) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds **3**, **6**, **8**, **10**, and **19** inhibited fMLP/CB-induced elastase release with IC_{50} values $\leqslant 15.25$ μM. This investigation reveals bioactive isolates (especially **2**, **3**, **5**, **6**, **8**, **9**, **10**, and **19**) could be further developed as potential candidates for the treatment or prevention of various inflammatory diseases.

Keywords: *Aquilaria sinensis*; Thymelaeaceae; structure elucidation; flavone; 2-(2-phenylethyl)-4*H*-chromen-4-one; anti-inflammatory activity

1. Introduction

Aquilaria sinensis (Lour.) Gilg. (Thymelaeaceae) is an evergreen tree, distributed in southern China [1]. *A. sinensis*, locally called "Chen Xiang", is used in China as a folk medicine for treatment of circulatory disorders, abdominal pain, vomiting, and dyspnea [1]. Its leaf has been commercially used as a functional tea with anti-diabetes activity [2]. Benzenoids [3–7], flavonoids [8–13], 2-(2-phenyl-ethyl)-4*H*-chromen-4-ones [4,10,14–19], steroids [19–22], sesquiterpenoids [21,23], triterpenoids [4], and their derivatives were isolated from this plant in previous studies.



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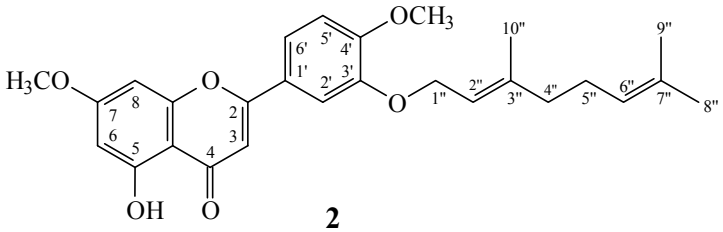

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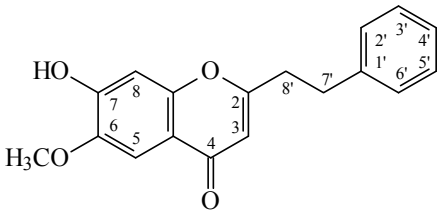

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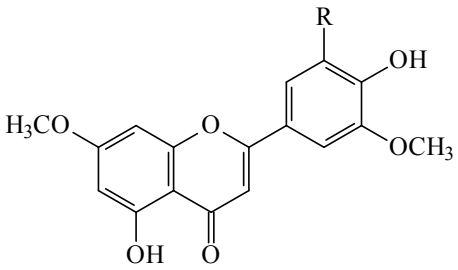

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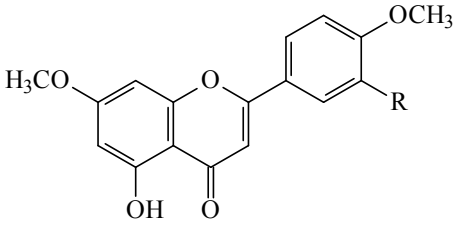

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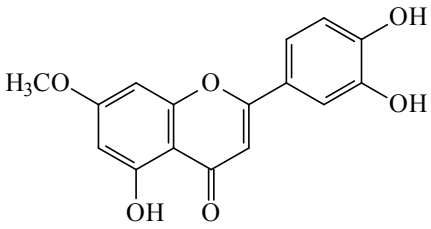

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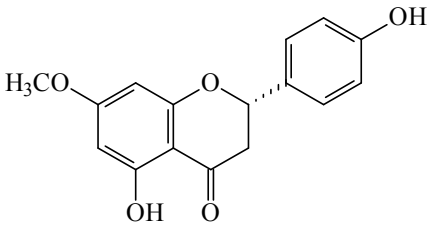

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Figure 1. *Cont.*

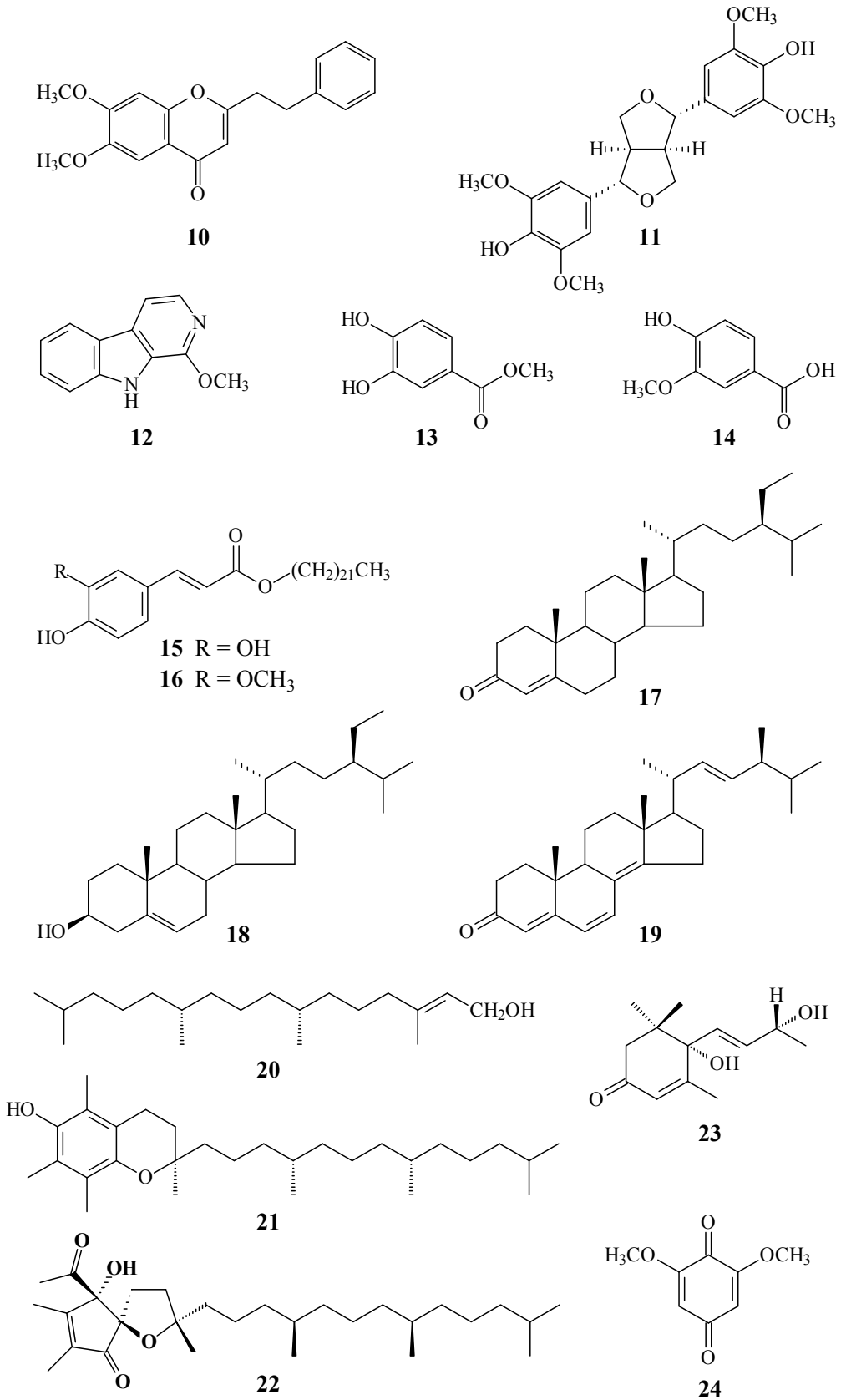

Figure 1. The chemical structures of new compounds **1**–**3** and known compounds **4**–**24** isolated from *A. sinensis*.

Many of these compounds were found to exhibit antitumor [5,18], nitrite scavenging [13], anti-acetylcholinesterase [23], and anti-inflammatory [12,19] activities. In our studies on the anti-inflammatory constituents of Formosan plants and Chinese herbal medicines, many species have been screened for *in vitro* inhibitory activity on neutrophil pro-inflammatory responses, and *A. sinensis* was found to be an active species. The MeOH extract of stem barks of *A. sinensis* showed potent inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Figure 1 illustrates the structures of two new flavones, 4′-*O*-geranyltricin (**1**) and 3′-*O*-geranylpolloin (**2**), and a new 2-(2-phenylethyl)-4*H*-chromen-4-one derivative, 7-hydroxyl-6-methoxy-2-(2-phenylethyl)-chromone (**3**) isolated from this extract. Twenty-one known compounds **4**–**24**, have also been isolated and identified from the stem barks of *A. sinensis* and their structures are depicted in Figure 1. This paper describes the structural elucidation of the compounds numbered **1** through **3**, and the inhibitory activities of all isolates on superoxide generation and elastase release by neutrophils.

2. Results

Chromatographic purification of the EtOAc-soluble fraction of a MeOH extract of stem barks of *A. sinensis* on a silica gel column and preparative thin-layer chromatography (TLC) afforded three new compounds **1**–**3** and twenty-one known compounds **4**–**24**.

4′-*O*-Geranyltricin (**1**) was isolated as yellowish needles. Its molecular formula, $C_{28}H_{32}O_7$, was determined on the basis of the positive HRESIMS peak at *m/z* 481.22185 $[M + H]^+$ (calcd 481.22208) and this was supported by the ^{1}H-, ^{13}C-, and DEPT-NMR data. The IR spectrum showed the presence of OH (3408 cm^{-1}) and carbonyl (1657 cm^{-1}) groups. Comparison of the ^{1}H-NMR data of **1** with those of tricin (**4**) [24,25] suggested that their structures were closely related, except that the 4′-geranyloxy group signals at δ 1.59 (3H, br s, H-9″), 1.67 (3H, br s, H-8″), 1.68 (3H, br s, H-10″), 2.03 (2H, m, H-4″), 2.08 (2H, m, H-5″), 4.64 (2H, d, *J* = 7.2 Hz, H-1″), 5.07 (1H, br t, *J* = 7.2 Hz, H-6″), and 5.56 (1H, br t, *J* = 7.2 Hz, H-2″) of **1** replaced the 4′-hydroxy group of **4**. This was supported by HMBC correlations observed between H-1″ (δ_H 4.64) and C-4′ (δ_C 140.4), C-2″ (δ_C 119.9), and C-3″ (δ_C 142.1), and by NOESY correlations observed between H-1″ (δ_H 4.64) and OMe-3′/5′ (δ_H 3.95), H-2″ (δ_H 5.56), and H-10″ (δ_H 1.68). Furthermore, the full assignment of ^{1}H- and ^{13}C-NMR resonances of **1** was confirmed by the ^{1}H-^{1}H COSY, NOESY (Figure 2), DEPT, HSQC, and HMBC (Figure 2) experiments. On the basis of the above data, the structure of **1** was elucidated as 4′-*O*-geranyltricin.

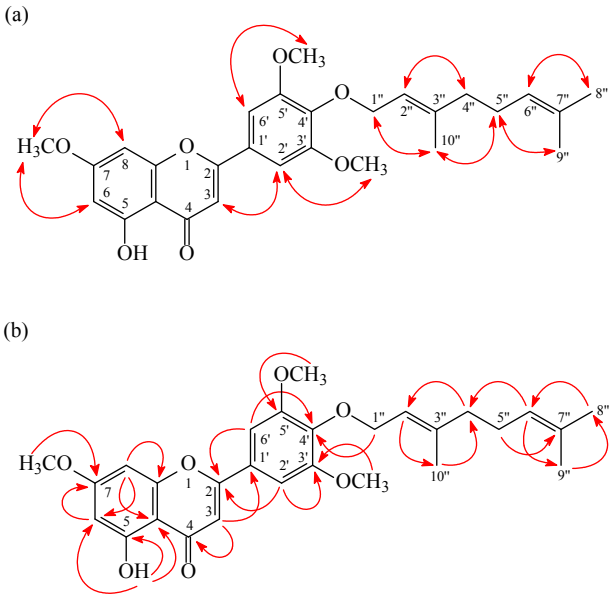

Figure 2. NOESY (**a**) and HMBC (**b**) correlations of **1**.

3′-*O*-Geranylpolloin (**2**) was obtained as yellowish needles. The molecular formula, $C_{27}H_{30}O_6$, was deduced from a proton adduct ion at *m/z* 451.21133 $[M + H]^+$ (calcd 451.21152) in the HRESI mass spectrum. IR absorptions for OH (3412 cm^{-1}) and carbonyl (1660 cm^{-1}) functions were observed. The ^{1}H-NMR data of **2** were similar to 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**) [26], except that the 3′-geranyloxy group [δ 1.58 (3H, br s, H-9″), 1.64 (3H, br s, H-8″), 1.81 (3H, br s, H-10″), 2.10 (2H, m, H-4″), 2.12 (2H, m, H-5″), 4.72 (2H, d, *J* = 6.6 Hz, H-1″), 5.07 (1H, br t, *J* = 6.6 Hz, H-6″), 5.53 (1H, br t, *J* = 6.6 Hz, H-2″)] of **2** replaced the OMe-3′ [δ3.99 (3H, s)] of **5**. This was supported by HMBC correlation observed between H-1″ (δ_H 4.72) and C-3′ (δ_C 148.5), C-2″ (δ_C 119.2), and C-3″ (δ_C 141.6), and by NOESY correlations observed between H-1″ (δ_H 4.72) and H-2′ (δ_H 7.36), H-2″ (δ_H 5.53), and H-10″ (δ_H 1.81). On the basis of the above data, the structure of **2** was elucidated as 3′-*O*-geranylpolloin, which was further confirmed by ^{1}H-^{1}H COSY and NOESY (Figure 3) experiments. The assignment of ^{13}C-NMR resonances was confirmed by DEPT, HSQC and HMBC (Figure 3) techniques.

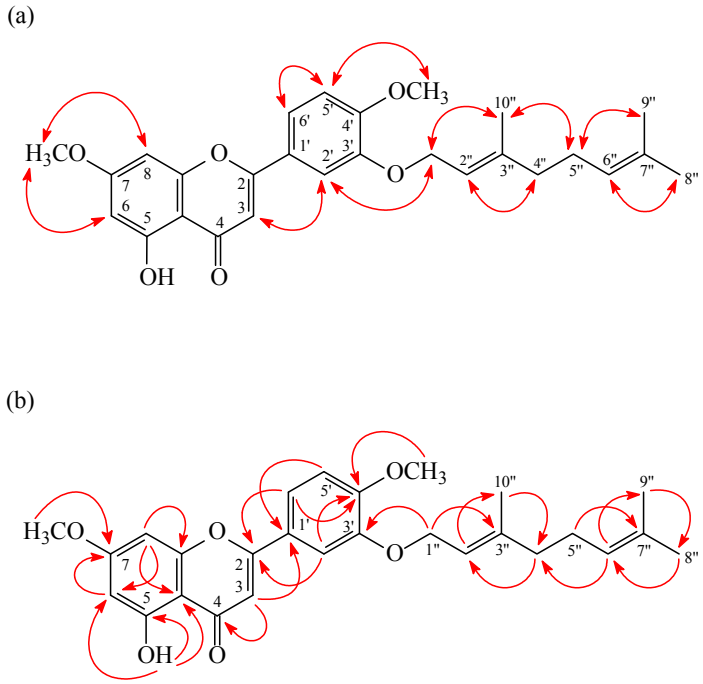

Figure 3. NOESY (**a**) and HMBC (**b**) correlations of **2**.

7-Hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**) was obtained as a colorless prisms. The molecular formula $C_{18}H_{16}O_4$ was deduced from a proton adduct ion peak at *m/z* 297.11209 $[M + H]^+$ (calcd 297.11214) in the HRESI mass spectrum. The presence of hydroxy and carbonyl groups was revealed by the bands at 3418 and 1634 cm^{-1}, respectively, in the IR spectrum. The ^{1}H-NMR spectrum indicated the presence of a 2-phenylethyl group [δ 2.91 (2H, t, *J* = 7.5 Hz, H-8′), 3.04 (2H, t, *J* = 7.5 Hz, H-7′), 7.20 (2H, br d, *J* = 7.5 Hz, H-2′ and H-6′), 7.21 (1H, br t, *J* = 7.5 Hz, H-4′), 7.29 (2H, br t, *J* = 7.5 Hz, H-3′ and H-5′)], a methoxy group [δ 4.00 (3H, s, OMe-6)], a hydroxy group [δ 6.24 (1H, s, D_2O exchangeable, OH-7)], and three singlet aromatic protons [δ 6.08 (1H, s, H-3), 6.94 (1H, s, H-8), 7.54 (1H, s, H-5)]. Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) [27] suggested that their structures were closely related, except that 7-hydroxy group [δ 6.24 (1H, s, D_2O exchangeable, OH-7)] of **3** replaced the OMe-7 [δ 3.97 (3H, s)] of **10**. This was supported by HMBC correlations between OH-7 (δ 6.24) and C-6 (δ 145.1), C-7 (δ 151.2), and C-8 (δ 102.7) and NOESY correlations between OH-7 (δ 6.24) and OMe-6 (δ 4.00). Furthermore, the full assignment of ^{1}H- and ^{13}C-NMR resonances was confirmed by the

^{1}H-^{1}H COSY, NOESY (Figure 4), DEPT, HSQC, and HMBC (Figure 4) experiments. According to the above data, the structure of **3** was elucidated as 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone.

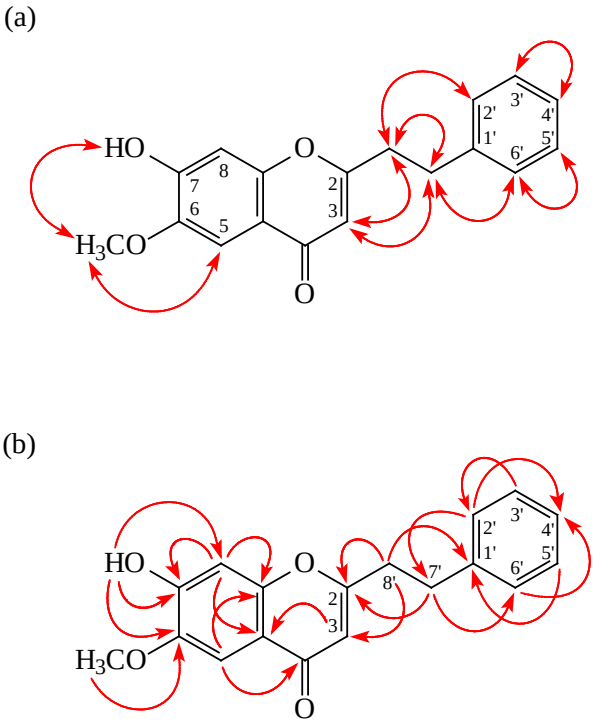

Figure 4. NOESY (**a**) and HMBC (**b**) correlations of **3**.

The known isolates were readily identified by a comparison of their physical and spectroscopic data (UV, IR, ^{1}H-NMR, $[\alpha]_D$, and MS) with the corresponding authentic samples or literature values, and included six flavones: tricin (**4**) [25], 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**) [26], velutin (**6**) [28], apigenin 7,4′-dimethyl ether (**7**) [29], 3′-hydroxygenkwanin (**8**) [30], and sakuranetin (**9**) [31], a 2-(2-phenylethyl)-4*H*-chromen-4-one, 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) [27], a lignan, (–)-syringaresinol (**11**) [32], a β-carboline, taraxacine A (**12**) [33], four benzenoids, methyl 3,4-dihydroxybenzoate (**13**) [34], vanillic acid (**14**) [35], docosyl caffeate (**15**) [36], and docosyl *trans*-ferulate (**16**) [37], three steroids, β-sitostenone (**17**) [38], β-sitosterol (**18**) [39], and ergosta-4,6,8(14),22-tetraen-3-one (**19**) [40], a diterpene, *trans*-phytol (**20**) [41], two α-tocopheroids, α-tocopherol (**21**) [42] and α-tocospiro A (**22**) [43], a cyclohex-2-en-1-one, blumenol A (**23**) [44], and a benzoquinone, 2,6-dimethoxy-*p*-benzoquinone (**24**) [45].

Human neutrophils are known to play an important role in the host defense against microorganisms and in the pathogenesis of various diseases such as rheumatoid arthritis, ischemia-reperfusion injury, asthma, and chronic obstructive pulmonary disease [46,47]. In response to different stimuli, activated neutrophils secrete a series of cytotoxins, such as superoxide anion ($O_2^{\bullet-}$), a precursor of other reactive oxygen species (ROS), granule proteases, bioactive lipids [46,48,49]. Suppression of the extensive or inappropriate activation of neutrophils by drugs has been proposed as a way to ameliorate inflammatory diseases. Reactive oxygen species (ROS) [e.g., superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide] and granule proteases (e.g., elastase, cathepsin G, and proteinase-3) produced by human neutrophils are involved in the pathogenesis of a variety of inflammatory diseases. The effects on neutrophil pro-inflammatory responses of compounds isolated from the stem barks of *A. sinensis* were evaluated by suppressing fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced superoxide anion ($O_2^{\bullet-}$) generation and elastase release by human neutrophils. The inhibitory activity data on neutrophil pro-inflammatory responses are summarized in Table 1.

Table 1. Inhibitory effects of compounds **1**–**24** from the stem barks of *A. sinensis* on superoxide radical anion generation and elastase release by human neutrophils in response to fMet-Leu-Phe/cytochalasin B.

Compound	IC_{50} (μM) [a] or (Inh %) [b]	
	Superoxide Anion Generation	Elastase Release
4′-*O*-Geranyltricin (**1**)	(13.23 ± 6.82)	(12.80 ± 6.84)
3′-*O*-Geranylpolloin (**2**)	12.51 ± 2.75 [e]	(17.34 ± 3.81) [c]
7-Hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**)	4.62 ± 1.48 [e]	3.91 ± 0.87 [e]
Tricin (**4**)	(3.61 ± 2.29)	(17.69 ± 1.71) [e]
5-Hydroxy-7,3′,4′-trimethoxyflavone (**5**)	4.69 ± 0.94 [e]	(9.32 ± 1.37) [e]
Velutin (**6**)	1.78 ± 0.35 [e]	4.26 ± 0.12 [d]
Apigenin 7,4′-dimethyl ether (**7**)	elicit superoxide anion generation and elastase release	
3′-Hydroxygenkwanin (**8**)	7.96 ± 0.76 [e]	4.56 ± 0.63 [e]
Sakuranetin (**9**)	1.74 ± 0.17 [e]	(23.84 ± 4.91) [d]
6,7-Dimethoxy-2-(2-phenylethyl)chromone (**10**)	11.54 ± 2.19 [e]	10.48 ± 1.35 [d]
(–)-Syringaresinol (**11**)	(30.23 ± 1.71) [e]	(25.12 ± 6.22) [d]
Taraxacine A (**12**)	(14.64 ± 2.95) [d]	(44.43 ± 1.90) [e]
Methyl 3,4-dihydroxybenzoate (**13**)	(1.32 ± 2.25)	(19.13 ± 5.85) [c]
Vanillic acid (**14**)	29.34 ± 6.01 [e]	29.92 ± 2.50 [e]
Docosyl caffeate (**15**)	(1.23 ± 5.08)	(25.57 ± 5.00) [d]
Docosyl *trans*-ferulate (**16**)	(27.83 ± 4.37)	(27.12 ± 6.23)
β-Sitostenone (**17**)	(2.74 ± 0.96) [c]	(3.92 ± 2.22)
β-Sitosterol (**18**)	(9.08 ± 6.13)	(2.43 ± 2.95)
Ergosta-4,6,8(14),22-tetraen-3-one (**19**)	(42.63 ± 1.82) [d]	15.25 ± 3.75 [c]
trans-Phytol (**20**)	(4.91 ± 5.52)	(22.65 ± 5.66) [d]
α-Tocopherol (**21**)	(0.55 ± 2.51)	(9.37 ± 4.92)
α-Tocospiro A (**22**)	(0.55 ± 2.51)	(9.37 ± 4.92)
Blumenol A (**23**)	(1.37 ± 1.38)	(10.72 ± 1.62) [d]
2,6-Dimethoxy-*p*-benzoquinone (**24**)	(47.09 ± 2.85) [e]	(16.00 ± 5.53) [c]
Diphenyleneiodonium	1.73 ± 0.72 [e]	–
Phenylmethylsulfonyl fluoride	–	199.6 ± 30.7 [e]

Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive control. Results are presented as averages ± SEM (n = 4). [a] Concentration necessary for 50% inhibition (IC_{50}); [b] Percentage of inhibition (Inh %) at 30 μM; [c] $p < 0.05$ compared with the control; [d] $p < 0.01$ compared with the control; [e] $p < 0.001$ compared with the control.

Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls for $O_2^{\bullet-}$ generation and elastase release, respectively. From the results of our biological tests, the following conclusions can be drawn: (a) 3′-*O*-Geranylpolloin (**2**), 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**), 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**), velutin (**6**), 3′-hydroxygenkwanin (**8**), sakuranetin (**9**), and 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) exhibited potent inhibition ($IC_{50} \leqslant 12.51$ μM) of superoxide anion ($O_2^{\bullet-}$) generation by human neutrophils in response to fMLP/CB; (b) 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**), velutin (**6**), sakuranetin (**9**), 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**), and ergosta-4,6,8(14)-22-tetraen-3-one (**19**) exhibited potent inhibition ($IC_{50} \leqslant 15.25$ μM) against fMLP-induced elastase release; (c) 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**) (with a 7-hydroxy group) exhibited more effective inhibition than its analogue, 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) (with a 7-methoxy substituent) against fMLP-induced $O_2^{\bullet-}$ generation and elastase release; (d) among the flavone analogues (**1**, **2**, and **4**–**8**), velutin (**6**) (with a 4′-hydroxy-3′-methoxyphenyl moiety) and 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**) (with a 3′,4′-dimethoxyphenyl moiety) exhibited more effective inhibition than their analogues, **1**, **2**, **4**, **7**, and **8**, against fMLP-induced $O_2^{\bullet-}$ generation; velutin (**6**) and 3′-hydroxygenkwanin (**8**) (with a 3′,4′-dihydroxyphenyl moiety) exhibited more effective inhibition than their analogues, **1**, **2**, **4**, **5**, and **7**, against fMLP-induced elastase release; (e) among the flavone analogues (**1**, **2**, and **4**–**8**), only apigenin 7,4′-dimethyl ether (**7**) (with a 4′-methoxyphenyl moiety) at 10 μg/mL alone elicited superoxide anion generation and elastase release by human neutrophils in the absence of fMLP/CB; (f) sakuranetin (**9**)

and velutin (**6**) were the most effective among the isolated compounds, with IC_{50} values of 1.74 ± 0.17 and 1.78 ± 0.35 μM, against fMLP-induced superoxide anion generation; (g) 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**), velutin (**6**), and 3′-hydroxygenkwanin (**8**) were the most effective among the isolated compounds, with IC_{50} value of 3.91 ± 0.87, 4.26 ± 0.12, and 4.56 ± 0.63 μM, against fMLP-induced elastase release.

3. Discussion

Twenty four compounds, including two new flavones, 4′-*O*-geranyltricin (**1**) and 3′-*O*-geranylpolloin (**2**), and a new 2-(2-phenylethyl)-4*H*-chromen-4-one derivative, 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**) were isolated from the stem barks of *A. sinensis*. Known compounds **9**, **12**–**16**, and **19**–**24** were obtained from this plant for the first time. The structures of these compounds were established on the basis of spectroscopic data. Discovery of the two new flavones and a new 2-(2-phenylethyl)-4*H*-chromen-4-one derivative from the genus *Aquilaria* may not only provide more structure-activity data of flavones and 2-(2-phenylethyl)-4*H*-chromen-4-one, but may also contribute to enhancing our understanding of the taxonomy and evolution of the genus *Aquilaria*.

Granule proteases (e.g., elastase, cathepsin G) and reactive oxygen species (ROS) e.g., superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide] and produced by human neutrophils contribute to the pathogenesis of inflammatory diseases. Inhibition of the inappropriate activation of neutrophils by drugs has been proposed as a way to ameliorate inflammatory diseases. Based on the results of our biological tests (Table 1), 3′-*O*-geranylpolloin (**2**), 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**), 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**), velutin (**6**), 3′-hydroxygenkwanin (**8**), sakuranetin (**9**), and 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) were the most effective among these compounds, with IC_{50} values of 12.51 ± 2.75, 4.62 ± 1.48, 4.69 ± 0.94, 1.78 ± 0.35, 7.96 ± 0.76, 1.74 ± 0.17, and 11.54 ± 2.19 μM, respectively, against fMLP-induced superoxide anion generation. 7-Hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**), velutin (**6**), 3′-hydroxygenkwanin (**8**), 6,7-di-methoxy-2-(2-phenylethyl)chromone (**10**), and ergosta-4,6,8(14),22-tetraen-3-one (**19**) exhibited the most effective among the isolates, with IC_{50} values of 3.91 ± 0.87, 4.26 ± 0.12, 4.56 ± 0.63, 10.48 ± 1.35, and 15.25 ± 3.75 μM, respectively, against fMLP-induced elastase release. Our study suggests *A. sinensis* and its isolates (especially **3**, **5**, **6**, and **8**–**10**) could be further developed as potential candidates for the treatment or prevention of various inflammatory diseases. More experiments should be performed to deduce the action modes of these compounds.

4. Experimental Section

4.1. Ethics Statement

Blood was taken from healthy human donors (20–30 years old) by venipuncture, using a protocol (No. 102-1595A3) approved by the Institutional Review Board at Chang Gung Memorial Hospital. All donors gave written consent. The Medical Ethics Committee of Chang Gung Memorial Hospital approved this consent procedure.

4.2. General Experimental Procedures

Melting points were determined on a Yanaco micro-melting point apparatus (Kyoto, Japan) and are uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter (Jasco, Tokyo, Japan) in $CHCl_3$. Ultraviolet (UV) spectra were obtained on a UV-240 spectrophotometer (Jasco). Infrared (IR) spectra (neat or KBr) were recorded on a 2000 FT-IR spectrometer (Perkin Elmer, Norwalk, CT, USA). The proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity Plus and Mercury 400 (Varian, Palo Alto, CA, USA), a Varian Inova 500 and a Varian Unity Plus 600 spectrometer operating at 400, 500 and 600 MHz, the carbon NMR spectra, including correlation spectroscopy (COSY), nuclear Overhauser effect spectrometry (NOESY), heteronuclear

multiple-bond correlation (HMBC), heteronuclear single-quantum coherence (HSQC) experiments, were recorded on a Varian Unity Plus 600 spectrometer operating at 600 MHz (^{1}H) and 150 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI), high-resolution electrospray ionization (HRESI) and electron ionization (EI)-mass spectra were recorded on an APEX II mass spectrometer (Bruker, Billerica, MA, USA) and Trace GC/Polaris Q MS (Thermo Finnigan, San Jose, CA, USA). Silica gel (70–230, 230–400 mesh) (Merck, Darmstadt, Germany) was used for column chromatography (CC). Silica gel 60 F-254 (Merck) was used for thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC).

4.3. Plant Material

The stem barks of *A. sinensis* was collected from Pingtung County, Taiwan, in August 2013 and identified by Prof. J.J. Chen. A voucher specimen (AS 201308) was deposited in the Department of Pharmacy, Tajen University, Pingtung, Taiwan.

4.4. Extraction and Isolation

The dried stem barks (4.1 kg) of *A. sinensis* were pulverized and extracted three times with MeOH (20 L each) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and the residue (390 g) was partitioned between *n*-hexane and H_2O (1:1). The *n*-hexane layer was concentrated to give a residue (fraction A, 93 g). The water layer was further extracted with EtOAc, and the EtOAc-soluble part (fraction B, 75 g) and the water-solubles (fraction C, 212 g) were separated. Fraction B (75 g) was chromatographed on silica gel (70–230 mesh, 3.2 kg), eluting with *n*-hexane, gradually increasing the polarity with acetone or MeOH to give 11 fractions: B1 (2 L, *n*-hexane/acetone, 20:1), B2 (2 L, *n*-hexane/acetone, 15:1), B3 (6.5 L, *n*-hexane/acetone, 10:1), B4 (5 L, *n*-hexane/acetone, 8:1), B5 (10.5 L, *n*-hexane/acetone, 5:1), B6 (2 L, *n*-hexane/acetone, 4:1), B7 (12 L, *n*-hexane/acetone, 3:1), B8 (5 L, *n*-hexane/acetone, 2:1), B9 (4 L, *n*-hexane/acetone, 1:1), B10 (3 L, acetone), and B11 (1 L, MeOH). Fraction B1 (5.1 g) was chromatographed further on silica gel (230–400 mesh, 230 g) eluting with CH_2Cl_2/EtOAc (100:1–0:1) to give 10 fractions (each 1.2 L, B1-1–B1-10). Fraction B1-2 (138 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 15:1) to obtain α-tocopherol (**21**) (5.8 mg) (R_f = 0.45). Fraction B1-5 (167 mg) was purified further by preparative TLC (silica gel, *n*-hexane/CH_2Cl_2, 1:5) to obtain α-tocospiro A (**22**) (4.6 mg) (R_f = 0.38). Fraction B1-6 (153 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 4:1) to yield β-sitostenone (**17**) (8.6 mg) (R_f = 0.76). Fraction B2 (5.7 g) was chromatographed further on silica gel (230–400 mesh, 260 g) eluting with CH_2Cl_2/EtOAc (80:1–0:1) to give 12 fractions (each 900 mL, B2-1–B2-12). Fraction B2-3 (405 mg) was purified by MPLC (silica column, CH_2Cl_2/EtOAc 60:1–0:1) to afford six subfractions (each 200 mL, B2-3-1–B2-3-6). Fraction B2-3-3 (38 mg) was purified by preparative TLC (silica gel, *n*-hexane/acetone, 8:1) to obtain *trans*-phytol (**20**) (3.9 mg) (R_f = 0.29). Fraction B2-6 (195 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 5:1) to give ergosta-4,6,8(14),22-tetraen-3-one (**19**) (6.3 mg) (R_f = 0.52). Fraction B3 (6.6 g) was chromatographed on silica gel (230–400 mesh, 300 g) eluting with CH_2Cl_2/MeOH (70:1–0:1) to give 10 fractions (each 1 L, B3-1–B3-10). Fraction B3-1 (210 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 3:1) to yield tetracosyl *trans*-ferulate (**16**) (6.3 mg) (R_f = 0.52). Fraction B3-4 (220 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 60:1) to afford β-sitosterol (**18**) (10.7 mg) (R_f = 0.52). Fraction B5 (10.9 g) was chromatographed on silica gel (230–400 mesh, 495 g) eluting with CH_2Cl_2/MeOH (50:1–0:1) to give 12 fractions (each 1.5 L, B5-1–B5-12). Fraction B5-2 (203 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/MeOH, 50:1) to yield 5-hydroxy-7,3′,4′-trimethoxyflavone (**5**) (8.3 mg) (R_f = 0.84). Fraction B5-3 (198 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 30:1) to yield 3′-*O*-geranylpolloin (**2**) (4.7 mg) (R_f = 0.83). Fraction B5-6 (216 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 11:10) to afford sakuranetin

(**9**) (5.2 mg) (R_f = 0.71), 6,7-dimethoxy-2-(2-phenylethyl)chromone (**10**) (4.6 mg) (R_f = 0.62), and taraxacine-A (**12**) (5.1 mg) (R_f = 0.45). Fraction B5-7 (187 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 1:1) to yield tetracosyl caffeate (**15**) (4.7 mg) (R_f = 0.76). Fraction B6 (5.6 g) was chromatographed further on silica gel (230–400 mesh, 255 g) eluting with CH_2Cl_2/acetone (25:1–0:1) to give 8 fractions (each 1.3 L, B6-1–B6-8). Fraction B6-1 (205 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 3:2) to obtain apigenin 7,4′-dimethyl ether (**7**) (8.7 mg) (R_f = 0.67). Fraction B6-3 (169 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 10:1) to obtain 2,6-dimethoxy-1,4-benzoquinone (**24**) (5.3 mg) (R_f = 0.64). Fraction B7 (8.3 g) was chromatographed on silica gel (230–400 mesh, 380 g) eluting with CH_2Cl_2/MeOH (20:1–0:1) to give 11 fractions (each 1.2 L, B7-1–B7-11). Fraction B7-1 (177 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/acetone, 25:1) to obtain velutin (6) (10.7 mg) (R_f = 0.57). Fraction B7-5 (215 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 30:1) to yield 3′-*O*-geranylpolloin (**1**) (4.1 mg) (R_f = 0.71). Fraction B7-7 (220 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 1:1) to obtain tricin (**4**) (9.4 mg) (R_f = 0.57). Fraction B7-9 (203 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 1:1) to obtain 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**) (5.2 mg) (R_f = 0.79). Fraction B8 (6.3 g) was chromatographed on silica gel (230–400 mesh, 285 g) eluting with CH_2Cl_2/MeOH (12:1–0:1) to give 10 fractions (each 1 L, B8-1–B8-10). Fraction B8-4 (188 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/acetone, 6:1) to yield methyl 3,4-dihydroxybenzoate (**13**) (5.4 mg) (R_f = 0.50). Fraction B8-5 (193 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 1:5) to obtain blumenol (**23**) (4.9 mg) (R_f = 0.69). Fraction B8-6 (213 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/acetone, 5:1) to yield 3′-hydroxygenkwanin (**8**) (6.2 mg) (R_f = 0.40). Fraction B8-7 (183 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 3:2) to obtain vanillic acid (**14**) (7.9 mg) (R_f = 0.23). Fraction B9 (5.3 g) was chromatographed on silica gel (230–400 mesh, 240 g) eluting with CH_2Cl_2/MeOH (10:1–0:1) to give 9 fractions (each 900 mL, B9-1–B9-9). Fraction B9-4 (189 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 20:1) to obtain (-)-syringaresinol (**11**) (6.8 mg) (R_f = 0.48).

4′-O-Geranyltricin (**1**). Yellowish needles (CH_2Cl_2-MeOH); m.p. 256–258 °C; UV (MeOH) λ_{max} (log ε) 207 (4.67), 268 (4.14), 331 (4.21) nm; IR (KBr) ν_{max} 3408 (OH), 1657 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 1.59 (3H, br s, H-9′′), 1.67 (3H, br s, H-8′′), 1.68 (3H, br s, H-10′′), 2.03 (2H, m, H-4′′), 2.08 (2H, m, H-5′′), 3.89 (3H, s, OMe-7), 3.95 (6H, s, OMe-3′ and OMe-5′), 4.64 (2H, d, *J* = 7.2 Hz, H-1′′), 5.07 (1H, br t, *J* = 7.2 Hz, H-6′′), 5.56 (1H, br t, *J* = 7.2 Hz, H-2′′), 6.39 (1H, d, *J* = 2.1 Hz, H-6), 6.50 (1H, d, *J* = 2.1 Hz, H-8), 6.60 (1H, s, H-3), 7.08 (2H, s, H-2′ and H-6′), 12.73 (1H, s, D_2O exchangeable, OH-5); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 16.4 (C-10′′), 17.6 (C-9′′), 25.6 (C-8′′), 26.4 (C-5′′), 39.6 (C-4′′), 55.8 (OMe-7), 56.4 (OMe-3′ and OMe-5′), 69.6 (C-1′′), 92.7 (C-8), 98.2 (C-6), 103.8 (C-2′ and C-6′), 105.6 (C-3), 105.6 (C-10), 119.9 (C-2′′), 123.9 (C-6′′), 126.4 (C-1′), 131.7 (C-7′′), 140.4 (C-4′), 142.1 (C-3′′), 154.1 (C-3′ and C-5′), 157.7 (C-9), 162.3 (C-5), 164.0 (C-2), 165.6 (C-7), 182.4 (C-4); ESIMS *m/z* 481 $[M + H]^+$; HRESIMS *m/z* 481.22185 $[M + H]^+$ (calcd for $C_{28}H_{33}O_7$, 481.22208).

3′-O-Geranylpolloin (**2**). Yellowish needles (CH_2Cl_2-MeOH); m.p. 246–248 °C; UV (MeOH) λ_{max} (log ε) 205 (4.59), 250 (4.20), 268 (4.13), 339 (4.24) nm; IR (KBr) ν_{max} 3412 (OH), 1660 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 1.58 (3H, s, H-9′′), 1.64 (3H, s, H-8′′), 1.81 (3H, s, H-10′′), 2.10 (2H, m, H-4′′), 2.12 (2H, m, H-5′′), 3.89 (3H, s, OMe-7), 3.95 (3H, s, OMe-4′), 4.72 (2H, d, *J* = 6.6 Hz, H-1′′), 5.07 (1H, br t, *J* = 6.6 Hz, H-6′′), 5.53 (1H, br t, *J* = 6.6 Hz, H-2′′), 6.37 (1H, d, *J* = 2.4 Hz, H-6), 6.48 (1H, d, *J* = 2.4 Hz, H-8), 6.56 (1H, s, H-3), 6.97 (1H, d, *J* = 8.4 Hz, H-5′), 7.36 (1H, d, *J* = 2.4 Hz, H-2′), 7.51 (1H, dd, *J* = 8.4, 2.4 Hz, H-6′), 12.80 (1H, s, D_2O exchangeable, OH-5); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 16.8 (C-10′′), 17.7 (C-9′′), 25.6 (C-8′′), 26.3 (C-5′′), 39.6 (C-4′′), 55.8 (OMe-7), 56.1 (OMe-4′), 66.2 (C-1′′), 92.7 (C-8), 98.0 (C-6), 104.6 (C-3), 105.6 (C-10), 111.0 (C-2′), 111.4 (C-5′), 119.2 (C-2′′), 120.1 (C-6′), 123.7 (C-1′), 123.7 (C-6′′), 131.9 (C-7′′), 141.6 (C-3′′), 152.9 (C-4′), 148.5 (C-3′), 157.7 (C-9), 164.1 (C-2), 165.5 (C-7), 182.4 (C-4); ESIMS *m/z* 451 $[M + H]^+$; HRESIMS *m/z* 451.21133 $[M + H]^+$ (calcd for $C_{27}H_{31}O_6$, 451.21152).

7-Hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**). Colorless prism (CH_2Cl_2); m.p. 181–183 °C; UV (MeOH) λ_{max} (log ε) 207 (4.69), 226 (4.56), 282 (4.11), 319 (4.15) nm; IR (KBr) ν_{max} 3418 (OH), 1634 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 2.91 (2H, t, *J* = 7.5 Hz, H-8′), 3.04 (2H, t, *J* = 7.5 Hz, H-7′), 4.00 (3H, s, OMe-6), 6.08 (1H, s, H-3), 6.24 (1H, s, D_2O exchangeable, OH-7), 6.94 (1H, s, H-8), 7.20 (2H, br d, *J* = 7.5 Hz, H-2′ and H-6′), 7.21 (1H, br t, *J* = 7.5 Hz, H-4′),7.29 (2H, br t, *J* = 7.5 Hz, H-3′ and H-5′), 7.54 (1H, s, H-5) ; ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 33.0 (C-7′), 36.0 (C-8′), 56.5 (OMe-6), 102.7 (C-8), 104.4 (C-5), 109.4 (C-3), 117.0 (C-10), 126.5 (C-4′), 128.3 (C-2′ and C-6′), 128.6 (C-3′ and C-5′), 139.8 (C-1′), 145.1 (C-6), 151.2 (C-7), 152.7 (C-9), 167.7 (C-2), 177.6 (C-4); ESIMS *m/z* 297 $[M + H]^+$; HRESIMS *m/z* 297.11209 $[M + H]^+$ (calcd for $C_{18}H_{17}O_4$, 297.11214).

4.5. Biological Assay

The effect of the isolated compounds on neutrophil pro-inflammatory response was evaluated by monitoring the inhibition of superoxide anion generation and elastase release in fMLP/CB-activated human neutrophils in a concentration-dependent manner. The purity of the tested compounds was >98% as identified by NMR and MS.

4.5.1. Preparation of Human Neutrophils

Human neutrophils from venous blood of healthy, adult volunteers (20–28 years old) were isolated using a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes [50]. Purified neutrophils containing >98% viable cells, as determined by the trypan blue exclusion method [51], were re-suspended in a calcium (Ca^{2+})-free HBSS buffer at pH 7.4 and were maintained at 4 °C prior to use.

4.5.2. Measurement of Superoxide Anion Generation

The assay for measurement of superoxide anion generation was based on the SOD-inhibitable reduction of ferricytochrome *c* [52,53]. In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with different concentrations (10–0.01 μg/mL) of compounds or DMSO (as control) for 5 min. Cells were incubated with cytochalasin B (1 μg/mL) for 3 min prior to the activation with 100 nM formyl-L-methionyl-L-leucyl-L-phenylalanine for 10 min. Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ε = 21.1/mM/10 mm).

4.5.3. Measurement of Elastase Release

Degranulation of azurophilic granules was determined by measuring elastase release as described previously [53,54]. Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with compounds for 5 min. Cells were stimulated with fMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously in order to assay elastase release. The results were expressed as the percent of elastase release in the fMLP/CB-activated, drug-free control system.

4.5.4. Statistical Analysis

Results are expressed as the mean ± SEM, and comparisons were made using Student's *t*-test. A probability of 0.05 or less was considered significant. The software SigmaPlot was used for the statistical analysis.

5. Conclusions

Twenty four compounds, including two new flavones, 4′-*O*-geranyltricin (**1**) and 3′-*O*-geranylpolloin (**2**), and a new 2-(2-phenylethyl)-4*H*-chromen-4-one derivative, 7-hydroxyl-6-methoxy-2-(2-phenylethyl)chromone (**3**) were isolated from the stem barks of *A. sinensis*. The structures of these compounds were established on the basis of spectroscopic data. Reactive oxygen species (ROS) e.g., superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide] and granule proteases (e.g., elastase, cathepsin G) produced by human neutrophils contribute to the pathogenesis of inflammatory diseases. The effects on neutrophil pro-inflammatory responses of isolates were evaluated by suppressing fMLP/CB-induced $O_2^{\bullet -}$ generation and elastase release by human neutrophils. The results of anti-inflammatory experiments indicate that compounds **2**, **3**, **5**, **6**, **8**–**10**, and **19** can significantly inhibit fMLP-induced $O_2^{\bullet -}$ generation and/or elastase release. Sakuranetin (**9**) and 7-hydroxy-6-methoxy-2-(2-phenylethyl)chromone (**3**) were the most effective among the isolated compounds, with IC_{50} values of 1.74 ± 0.17 and 3.91 ± 0.87 μM, respectively, against fMLP-induced $O_2^{\bullet -}$ generation and elastase release. Our study suggests *A. sinensis* and its isolates (especially **3**, **5**, **6**, and **8**–**10**) are worthy of further biomedical investigation and could be expectantly developed as potential candidates for the treatment or prevention of various inflammatory diseases.

Supplementary Materials: ESI-MS, HR-ESI-MS, ^{1}H-NMR, and ^{13}C-NMR spectra of three new compounds **1**–**3** are available as Supplementary Information. Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/11/19736/s1.

Acknowledgments: This research was supported by a grant from the National Science Council of the Republic of China (No. NSC 101-2320-B-127-001-MY3), awarded to J.-J. Chen.

Author Contributions: Jih-Jung Chen designed the research; Sin-Ling Wang, Tsong-Long Hwang, and Mei-Ing Chung, performed the experiments; Jih-Jung Chen, Sin-Ling Wang, Ping-Jyun Sung, Chih-Wen Shu, and Ming-Jen Cheng contributed to the analysis of the data; Jih-Jung Chen wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are available from the authors.