

Article

2-(2-Phenylethyl)chromone-Sesquiterpene Hybrids from Agarwood of *Aquilaria sinensis*: Characterization and Biological Activity Evaluation

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Abstract: Aquisinenins G–I (1–3), three new 2-(2-phenylethyl)chromone-sesquiterpene hybrids, were isolated from the ethanol extract of Hainan agarwood derived from *Aquilaria sinensis*. Spectroscopic techniques, such as ¹D and ²D NMR and HRESIMS, were used to determine their structures. Experimental and computed ECD data were compared to confirm their absolute configurations. Compounds 1–3 are uncommon dimeric derivatives of 2-(2-phenylethyl)chromone-sesquiterpene, characterized by the fusion of 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone with agarofuran or agarospirane-type sesquiterpene units by an ester linkage. Compound 1 inhibited nitric oxide production in lipopolysaccharide-stimulated RAW264.7 cells, showing an IC₅₀ value of 22.31 ± 0.42 μM. The neuroprotective effects of compounds 1 and 3 against H₂O₂-induced apoptosis were assessed in human neuroblastoma SH-SY5Y cells. Compound 1 demonstrated cytotoxicity with IC₅₀ values of 72.37 ± 0.20 μM against K562 and 61.47 ± 0.22 μM against BEL-7402, while compounds 2 and 3 showed cytotoxicity across all five tested human cancer cell lines.

Keywords: Hainan agarwood; 2-(2-phenylethyl)chromone-sesquiterpene hybrids; structure elucidation; anti-inflammatory activity; neuroprotective activity; cytotoxic activity



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1. Introduction

Agarwood is the aromatic resinous heartwood obtained from the *Aquilaria* and *Gyrinops* genus of the Thymelaeaceae family [1,2]. It is a natural spice and traditional medicine commonly used in incense and pharmaceuticals. In traditional medicine, agarwood has been employed to address various health problems, including gastric disorders, cough, and asthma, due to its sedative, analgesic, carminative, and antiemetic effects [1,2]. Hainan agarwood is the resinous wood derived from *Aquilaria sinensis* (Lour.) Spreng. It demonstrates diverse pharmacological activities, including anti-inflammatory [3], cytotoxic [4], antifibrotic [5], antimalarial [6], neuroprotective [7], and gastric mucosal protective properties [8].

Hainan agarwood has yielded various compounds, including monosomic sesquiterpenes [4,9–13], flindersia-type 2-(2-phenylethyl)chromones [3,14–18], 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromones [3,19–21], sesquiterpene polymers [6,22], and 2-(2-phenylethyl)chromone-sesquiterpene hybrids [5,8]. In the pursuit of novel structurally intriguing and bioactive compounds from Hainan agarwood, Aquisinenins G–I (1–3) were found to comprise either agarofuran or agarospirane-type sesquiterpene moieties and 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone bound via an ester bond (Figure 1). This study outlines this isolation process and includes a structural analysis and bioactivity assessment of these compounds.

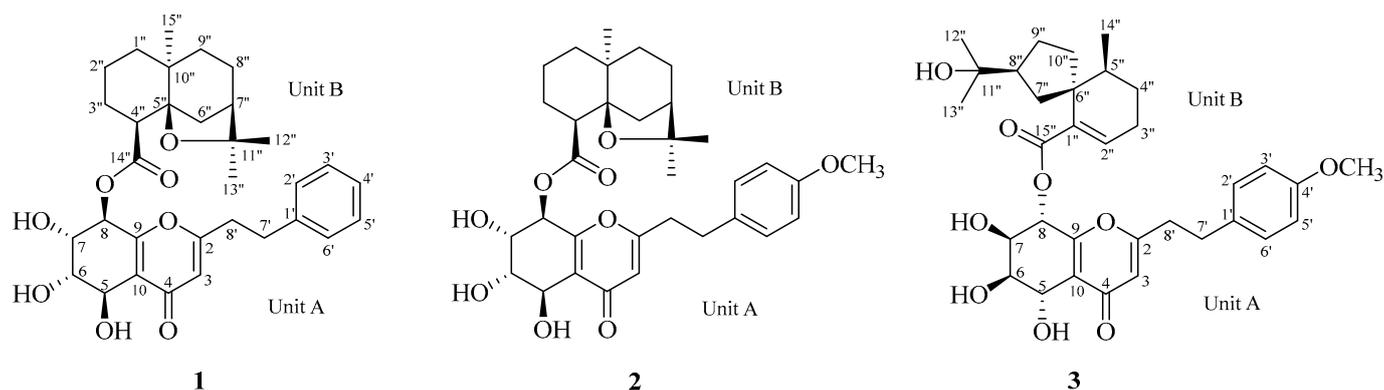


Figure 1. Chemical structures of compounds 1–3.

2. Results

2.1. Structure Elucidation

Compound **1** was obtained as a viscous yellow oil. HRESIMS analysis determined the molecular formula $C_{32}H_{40}O_8$, revealing a pseudomolecular ion peak at m/z 575.2613 $[M + Na]^+$ (calcd. 575.2615 for $C_{32}H_{40}NaO_8$), and indicating 13 degrees of unsaturation, as depicted in Figure S1. The 1H NMR data (Table 1) revealed a monosubstituted benzene with signals at δ_H 7.16 (2H, m, H-2', 6'), 7.25 (2H, t, $J = 7.7$ Hz, CH-3', 5'), and 7.16 (1H, m, H-4'), alongside an ethane-1,2-diyl group on the benzene ring at δ_H 2.93 (2H, t, $J = 7.4$ Hz, H-7'), 2.87 (2H, m, H-8'). Additionally, four consecutive hydroxylated methines were observed at δ_H 4.78 (1H, d, $J = 3.8$ Hz, H-5), 4.04 (1H, dd, $J = 3.8, 2.2$ Hz, H-6), 4.25 (1H, dd, $J = 8.0, 2.3$ Hz, H-7), and 6.05 (1H, d, $J = 8.1$ Hz, H-8). The spectrum also included three methines at δ_H 6.15 (1H, s, H-3), 2.66 (1H, d, $J = 3.9$ Hz, H-4''), and 1.94 (1H, m, H-7''), as well as three methyls at δ_H 1.36 (3H, s, H-12''), 1.20 (3H, s, H-13''), and 1.08 (3H, s, H-15''). The ^{13}C NMR data of **1** (Table 1) showed 32 carbon resonances comprising nine quaternary (two carbonyl at δ_C 181.3 and 174.6, four oxygenated at δ_C 170.9, 161.6, 83.0, and 87.6), twelve methine (four oxygenated at δ_C 66.3, 74.6, 70.0, and 70.8), eight methylene, and three methyl carbons. The 1H – 1H COSY spectrum of **1** identified two spin-coupling systems: one involving H-5, H-6, H-7, and H-8, and comprising H-2', H-3', H-4', H-5', and H-6' (Figure 2). The HMBC spectrum revealed correlations from: H-2', 6' (δ_H 7.16) to C-7' (δ_C 33.6); H-3' (δ_H 7.25) to C-1' (δ_C 140.8); H-6' (δ_H 7.16) to C-4' (δ_C 127.6); H-3 (δ_H 6.15) to C-8' (δ_C 36.1), C-4 (δ_C 181.3); H-5 (δ_H 4.78) to C-9 (δ_C 161.6), C-4 (δ_C 181.3); and H-8 (δ_H 6.05) to C-10 (δ_C 123.1) (Figure 3). Analysis of the data in relation to the known 2-(2-phenylethyl)chromone [2] indicated that compound **1** included a 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone (unit A). The 1H – 1H COSY spectrum of **1** identified two spin-coupling systems: one involving H-1''/H-2''/H-3''/H-4'' and another comprising H-6''/H-7''/H-8''/H-9'' (Figure 2). Analysis of the HMBC correlations (Figure 3), with the exception of unit A, from H-4'' (δ_H 2.66) to C-2'' (δ_C 19.7), C-5'' (δ_C 87.6), and C-10'' (δ_C 40.2); H-6'' (δ_H 2.33) to C-4'' (δ_C 51.4), C-8'' (δ_C 25.7), C-10'' (δ_C 40.2),

and C-11'' (δ_C 83.0); H-15'' (δ_H 1.08) to C-1'' (δ_C 38.5), C-5'' (δ_C 87.6), and C-9'' (δ_C 38.8); H-12'' (δ_H 1.36) and H-13'' (δ_H 1.20) to C-7'' (δ_C 45.4), indicated that unit B of compound **1** included two hexatomic rings and one tetrahydrofuran ring, which likens it structurally to previously reported analogues. The structure displays a close resemblance to reported agarofuran-type sesquiterpene, except for the C-14'' carboxyl group when compared to the ester (δ_C 174.6) in compound **1**. The ester bond formation between units A and B (C-8/O/C-14'') was verified by the significant deshielding of H-8 (δ_H 6.04, d, J = 8.1 Hz) and the crucial HMBC correlation linking H-8 to C-14''. As depicted in Figure 1, the planar structure of compound **1** comprises a 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone (unit A) and an agarofuran-type sesquiterpene moiety (unit B) connected via an ester linkage.

Table 1. ^1H NMR and ^{13}C NMR data for compounds **1–3**.

Position	1		2		Position	3	
	δ_H mult. (J in Hz)	δ_C , type	δ_H mult. (J in Hz)	δ_C , type		δ_H mult. (J in Hz)	δ_C , type
2		170.9, C		171.0, C	2		171.2, C
3	6.15, s	114.6, CH	6.17, s	114.6, CH	3	6.12, s	114.6, CH
4		181.3, C		181.3, C	4		181.7, C
5	4.78, d (3.8)	66.3, CH	4.80, dd (3.9)	66.4, CH	5	4.90, m	66.2, CH
6	4.04, dd (3.8, 2.2)	74.6, CH	4.04, m	74.6, CH	6	4.06, d (2.6)	70.4, CH
7	4.25, dd (8.0, 2.3)	70.0, CH	4.25, d (7.7)	70.2, CH	7	4.05, d (2.4)	72.7, CH
8	6.05, d (8.1)	70.8, CH	6.01, d (7.7)	71.2, CH	8	6.03, d (5.5)	71.5, CH
9		161.6, C		161.5, C	9		160.5, C
10		123.1, C		123.3, C	10		123.6, C
1'		140.9, C		132.8, C	1'		132.8, C
2'	7.16, m	129.3, CH	7.11, d (8.3)	130.3, CH	2'	7.05, d (8.5)	130.3, CH
3'	7.25, t (7.7)	129.6, CH	6.84, d (8.4)	115.0, CH	3'	6.79, d (8.6)	115.0, CH
4'	7.16, m	127.6, CH		159.8, C	4'		159.8, C
5'	7.25, t (7.7)	129.6, CH	6.84, d (8.4)	115, CH	5'	6.79, d (8.6)	130.3, CH
6'	7.16, m	129.3, CH	7.11, d (8.3)	130.3, CH	6'	7.05, d (8.5)	115.0, CH
7'	2.93, t (7.4)	33.6, CH ₂	2.91, m	32.7, CH ₂	7'	2.88, m	32.8, CH ₂
8'	2.87, m	36.1, CH ₂	2.85, m	36.5, CH ₂	8'	2.85, m	36.5, CH ₂
OMe-4'			3.76, s	55.7, CH ₃	OMe-4'	3.74, s	55.7, CH ₃
1''	1.15, m	38.5, CH ₂	1.16, m	38.4, CH ₂	1''		139.9, C
2''	1.64, Overlapped ^a		1.68, Overlapped ^a		2''	6.86, t (3.8)	140.7, CH
3''	1.89, m	19.7, CH ₂	1.90, m	19.7, CH ₂	3''	1.48, td (12.4, 6.8)	28.9, CH ₂
4''	1.41, d (12.9)		1.43, m		4''	2.00, td (12.7, 7.0)	40.4, CH ₂
5''	2.08, d (11.4)	26.3, CH ₂	2.10, m	26.2, CH ₂	5''	1.68, m	
6''	1.94, Overlapped ^a		1.90, Overlapped ^a		6''	1.70, Overlapped ^a	39.9, CH
7''	2.66, d (3.9)	51.4, CH	2.70, m	51.6, CH	7''		48.1, C
8''	2.34, dd (13.3, 4.0)	87.6, C		87.3, C	8''	1.76, m	27.5, CH ₂
9''	2.29, d (12.7)	39.6, CH ₂	2.33, m	39.9, CH ₂	9''	2.45, m	53.3, CH
10''	1.94, Overlapped ^a	45.4, CH	2.15, d (12.7)		10''	2.18, m	24.5, CH ₂
11''	1.71, Overlapped ^a	25.7, CH ₂	1.90, Overlapped ^a	45.4, CH	11''	1.82, m	36.9, CH ₂
12''	1.71, Overlapped ^a	38.8, CH ₂	1.68, Overlapped ^a	25.8, CH ₂	12''	1.71, Overlapped ^a	
13''	1.15, Overlapped ^a		1.68, Overlapped ^a	38.9, CH ₂	13''		72.3, C
14''		40.2, C		40.1, C	14''	1.10, s	28.7, CH ₃
15''		83.0, C		82.9, C	15''	1.12, s	28.5, CH ₃
	1.36, s	23.0, CH ₃	1.37, s	23.0, CH ₃		0.95, s	16.0, CH ₃
	1.20, s	31.0, CH ₃	1.21, s	30.9, CH ₃			168.1, C
		174.6, C		174.3, C			
	1.08, s	23.6, CH ₃	1.08, s	23.8, CH ₃			

^a Overlapped signals without designating multiplicity and assigned from HMBC and HSQC spectra.

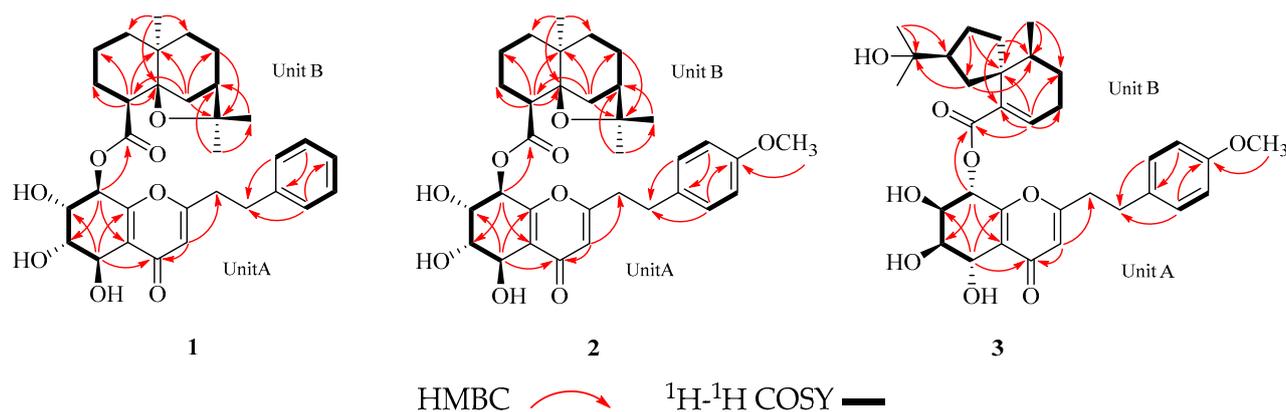


Figure 2. Key HMBC and ^1H - ^1H COSY correlations of compounds 1–3.

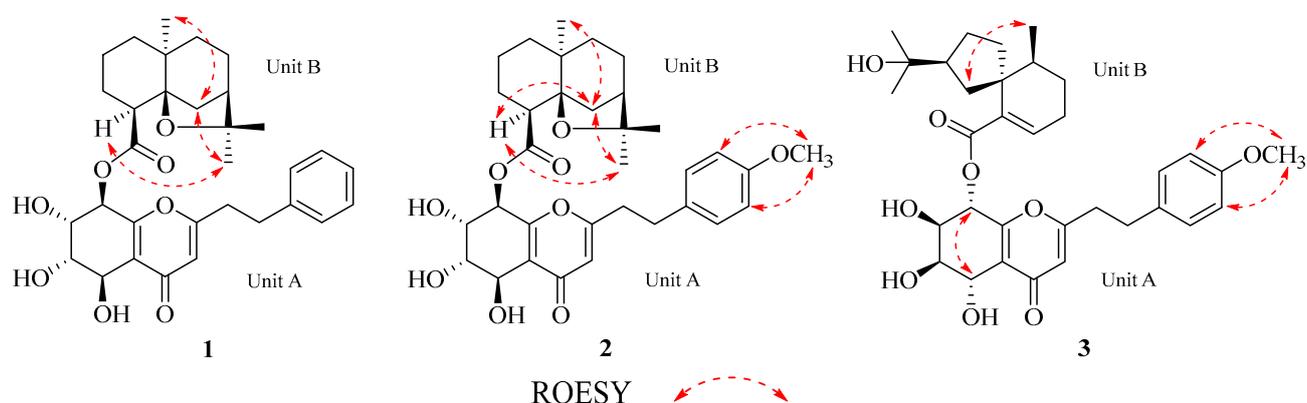


Figure 3. Key ROESY correlations of compounds 1–3.

The coupling constants, with H-7/H-8 showing a large value ($^3J = 8.1$ Hz) and H-6/H-7 showing a small one ($^3J = 2.2$ Hz), suggest that H-7 and H-8 adopt an axial half-chair conformation, whereas H-6 is equatorial. The ROESY spectrum observed no NOE effect between H-5 and H-7, confirming that H-5 is equatorial. The relative configuration of unit A was similar to the 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone unit of aquifilarone A [23], and the relative configuration of compound 1 was established through an analysis of 3J coupling constants and the ROESY spectrum (Figure 3). The relative configuration of unit B was determined to be identical to that of baimuxifuranic acid [12]. This conclusion was supported by NOE correlations observed for H-4''/H-13'', H-6''/H-13'', and H-6''/H-15'', which were found to be syn-oriented based on the analysis of ROESY data (Figure 3). As illustrated in Figure 4, the cotton effect of the experimentally observed ECD spectrum demonstrates consistency with the theoretically calculated spectrum. As a result, the structure of compound 1 was determined and given the name aquisinenin G.

Compound 2 was obtained as a yellow oil. It has the molecular formula $\text{C}_{33}\text{H}_{42}\text{O}_9$ (m/z 605.2719 $[\text{M} + \text{Na}]^+$, calcd. for $\text{C}_{33}\text{H}_{42}\text{NaO}_9$, 605.2721), established by HRESIMS (Figure S8), indicating the addition of a methoxy group to aquisinenin G. The ^1H and ^{13}C NMR spectra closely resembled those of aquisinenin G, with the addition of an extra methoxy group (Table 1). The ^1H NMR spectra of compound 2 indicated a para-disubstituted benzene ring with signals at δ_{H} 6.84 (2H, d, $J = 8.4$ Hz, H-3'/5') and δ_{H} 7.11 (2H, d, $J = 8.3$ Hz, H-2'/6'), implying a methoxy group at C-4' (δ_{C} 159.8). This deduction was validated by the HMBC correlation between 4'-OCH₃ (δ_{H} 3.76) and C-4' (δ_{C} 159.8), as well as the NOE correlation from 4'-OCH₃ to H-3' and H-5' (Figure 2). The relative configuration of unit B was indicated by NOE correlations observed for H-4''/H-6''/H-13'', and H-6''/H-15''. The absolute configuration was determined through electronic circular dichroism (ECD)

calculations, with the calculated ECD spectrum closely matching the experimental ECD spectrum in Figure 5. The structure of compound 2 was determined as depicted in Figure 1 and designated as aquisitionin H.

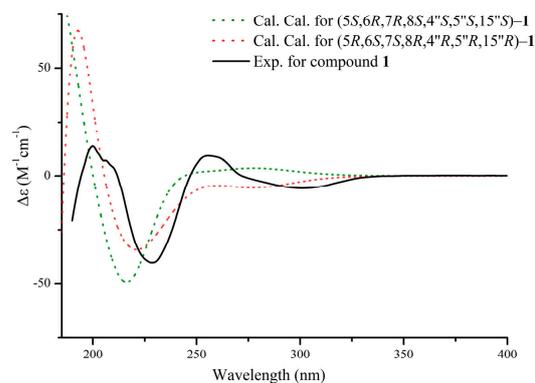


Figure 4. The experimental and calculated ECD spectra of compound 1.

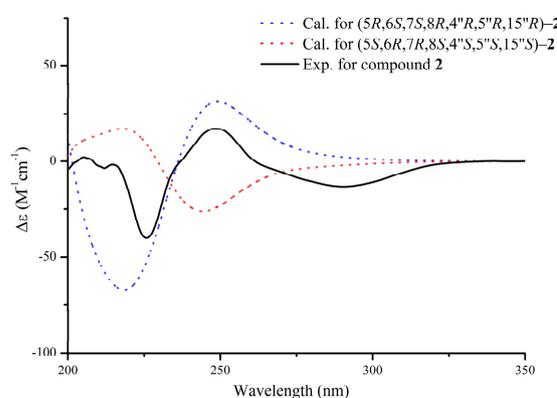


Figure 5. The experimental and calculated ECD spectra of compound 2.

Compound 3 was obtained as a yellow oil. Its molecular formula $C_{33}H_{42}O_9$ was deduced from HRESIMS data (Figure S15) (m/z 605.2718 $[M + Na]^+$, calcd. for $C_{33}H_{42}NaO_9$, 605.2721), suggesting 13 degrees of unsaturation. 1H NMR data (Table 1) indicated a para-substituted benzene ring [δ_H 7.05 (2H, d, $J = 8.5$ Hz, H-2', 6'), 6.79 (2H, d, $J = 8.6$ Hz, CH-3', 5'), 2.88 (2H, m, H-7'), and 2.85 (2H, m, H-8')], four sequential hydroxylated methines [δ_H 4.90 (1H, m, H-5), 4.06 (1H, d, $J = 2.6$ Hz, H-6), 4.05 (1H, d, $J = 2.4$ Hz, H-7), and 6.03 (1H, d, $J = 5.5$ Hz, H-8)], three methines [δ_H 6.12 (1H, s, H-3), 6.86 (1H, t, $J = 3.8$ Hz, H-2''), 1.70 (1H, m, H-5''), and 2.45 (1H, m, H-8'')], and three methyl groups [δ_H 1.12 (3H, s, H-12''), 1.14 (3H, s, H-13''), and 0.96 (3H, s, H-14'')]. The ^{13}C NMR data of 3 (Table 1) showed 33 carbon resonances comprising ten quaternary (two carbonyl at δ_C 181.7 and 168.1, four oxygenated at δ_C 171.2, 160.5, 72.3, and 159.8), twelve methine (four oxygenated at δ_C 66.2, 70.2, 72.4, and 71.4), seven methylene, and four methyl (one *O*-methyl) carbons. The 1H - 1H COSY spectrum of compound 3 displayed spin-coupling systems for H-5/H-6/H-7/H-8 and H-2'/H-3'/H-5'/H-6' (Figure 2). Analysis and comparison with the known 2-(2-phenylethyl)chromone [2], along with the HMBC correlations (Figure 2), indicated that compound 3 contains a 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone (unit A). This is supported by correlations from H-2', 6' (δ_H 7.05), to C-7' (δ_C 32.8); 4'-OCH₃ (δ_H 3.74) to C-4' (δ_C 159.8); H-3 (δ_H 6.12) to C-8' (δ_C 36.5), C-4 (δ_C 181.7); H-5 (δ_H 4.90) to C-9 (δ_C 160.5), C-4 (δ_C 181.7); and H-8 (δ_H 6.03) to C-10 (δ_C 123.6). The 1H - 1H COSY spectrum of compound 3 identified spin-coupling systems for H-2'' to H-5'' and H-7'' to H-10'' (Figure 2). Analysis of the HMBC correlations (Figure 2), except for the unit A, from H-2''

(δ_{H} 6.86) to C-4'' (δ_{C} 40.4), C-6'' (δ_{C} 48.1) and C-15'' (δ_{C} 168.1); H-9'' (δ_{H} 2.18) to C-6'' (δ_{C} 48.1), C-7'' (δ_{C} 27.5); and H-14'' (δ_{H} 0.95) to C-4'' (δ_{C} 40.4), C-6'' (δ_{C} 48.1), indicated that unit B of compound **3** included both a six- and a five-membered ring structure skeleton, a structural feature consistent with previously reported analogues. Unit B of compound **3** was an agarospirane-type sesquiterpenoid (Figure 1). The ester linkage (C-8/O/C-15'') connecting units A and B was determined by the HMBC correlation from H-8 (δ_{H} 6.03) to C-15'' (δ_{C} 168.1) (Figure 2).

The relative configuration of unit A was determined based on ROESY data and $^3J_{\text{H-H}}$ coupling constants ($^3J_{6,7} = 2.6$ Hz, $^3J_8 = 5.5$ Hz), revealing H-6 and H-7 in equatorial cis-adjacent positions, while H-5 and H-8 adopt syn-facial orientations with the presence of the NOE correlation between H-5 and H-8. The NOE correlations of H-7''/H-14'' indicate that these protons are cofacial and β -oriented, establishing the relative configuration of unit B (Figure 3) as identical to that of baimuxifuranic acid [24]. The relative configuration was determined by comparing the experimental and calculated ECD spectra (Figure 6). The structure of compound **3** was identified as depicted in Figure 1 and designated as aquisinenin I.

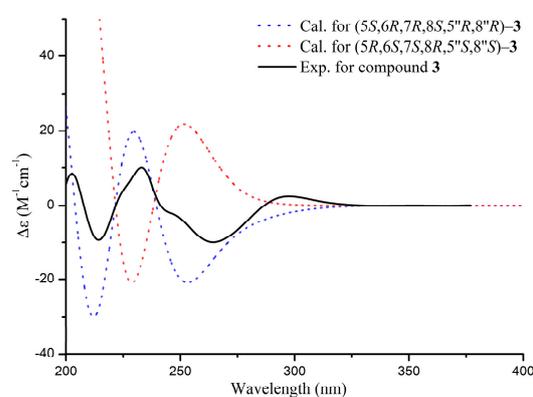


Figure 6. The experimental and calculated ECD spectra of compound **3**.

Compounds **1–3** are unique, consisting of a 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone linked to a sesquiterpene at C-8 of the chromone unit by an ester bond. The agarofuran- and agarospirane-type sesquiterpene units in the 2-(2-phenylethyl)chromone-sesquiterpene hybrids have not been previously reported in studies on these compounds.

2.2. Spectroscopic Data of Compounds

2.2.1. Aquisinenin G (**1**)

Yellow oil; $[\alpha]_{\text{D}}^{25} -10$ (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) at 193 (+9.59), 231 (−18.02), 281 (+11.38) nm; UV (MeOH) λ_{max} ($\log \epsilon$): at 254 (1.68) nm; ^1H and ^{13}C NMR data are provided in Table 1; HRESIMS m/z 575.2613 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{32}\text{H}_{40}\text{NaO}_8$, 575.2615).

2.2.2. Aquisinenin H (**2**)

Yellow oil; $[\alpha]_{\text{D}}^{25} -92$ (*c* 0.10, MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) at 200 (+14.08), 228 (−40.51), 255 (+9.38), 301 (−5.89) nm; UV (MeOH) λ_{max} ($\log \epsilon$): at 262 (1.96) nm; ^1H and ^{13}C NMR data are provided in Table 1; HRESIMS m/z 605.2719 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{42}\text{NaO}_9$, 605.2721).

2.2.3. Aquisinenin I (3)

Yellow oil; $[\alpha]_D^{25} +51$ (*c* 0.10, MeOH); ECD (MeOH) λ_{\max} ($\Delta\epsilon$) at 203 (+8.45), 214 (−9.40), 233 (+10.10), 264 (−9.97), 297 (+2.51) nm; UV (MeOH) λ_{\max} ($\log \epsilon$): at 267 (2.17) nm; ^1H and ^{13}C NMR data are provided in Table 1; HRESIMS m/z 605.2718 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{42}\text{NaO}_9$, 605.2721).

2.3. Biological Activity

2.3.1. Anti-Inflammatory Assay

The bioactivity assessments demonstrated that compound 1 exhibited potent inhibitory activity against LPS-induced NO production in RAW264.7 cells with IC_{50} values of $22.31 \pm 0.42 \mu\text{M}$, approximating the efficacy of the positive controls Indomethacin (IC_{50} , $33.25 \pm 4.47 \mu\text{M}$) and quercetin (IC_{50} , $16.10 \pm 1.07 \mu\text{M}$). In contrast, compound 3 exhibited no significant inhibitory activity, which is potentially attributable to its significant cytotoxicity. Compared with compounds 2 and 3, the results underscored the significant anti-inflammatory efficacy of compound 1 through its modulation of NO-mediated inflammatory pathways.

2.3.2. Neuroprotective Assay

The results of the neuroprotective assay demonstrated that compounds 1 and 3 enhanced cell viability at concentrations of 12.5, 25, 50, and 100 μM , achieving improvements of $62.54 \pm 6.39\%$, $66.84 \pm 8.59\%$, $67.42 \pm 5.38\%$, $75.04 \pm 8.59\%$, and $60.50 \pm 8.54\%$, $64.69 \pm 7.45\%$, $73.59 \pm 8.24\%$, and $70.86 \pm 7.13\%$ (Figures 7 and 8), respectively, as compared to the control group ($59.45 \pm 3.15\%$). Notably, compounds 1 and 3 exhibited concentration-dependent improvements in cell viability, with compound 3 showing maximal enhancement ($73.59 \pm 8.24\%$) at 50 μM and compound 1 achieving optimal efficacy ($75.04 \pm 8.59\%$) at 100 μM , both significantly surpassing the baseline viability of $59.45 \pm 3.15\%$ in untreated controls.

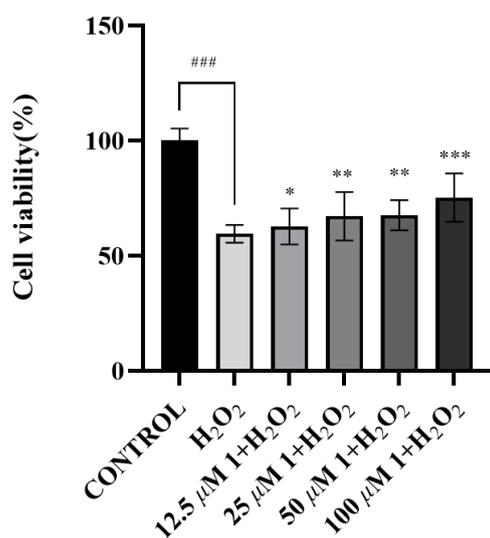


Figure 7. The survival rates of SH-SY5Y cells treated with compound 1. (All data of Figure 7 are expressed as mean \pm (SEM), derived from three independent replicates. Statistical analyses were conducted using a one-way analysis of variance (ANOVA), with a post hoc Welch's *t*-test. Significance thresholds were defined as follows: ### $p < 0.001$, relative to blank control group; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.1$, versus H₂O₂-induced oxidative stress model group.

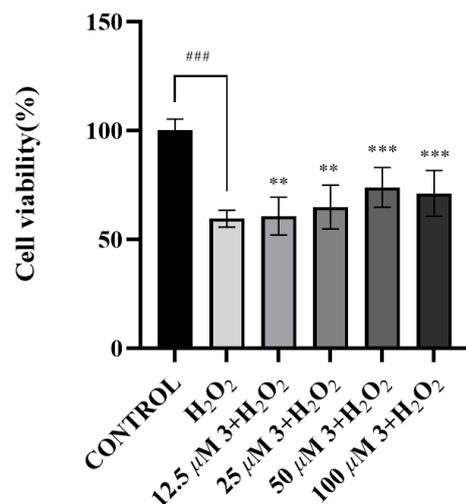


Figure 8. The survival rates of SH-SY5Y cells treated with compound 3. (All data of Figure 8 are expressed as mean \pm (SEM), derived from three independent replicates. Statistical analyses were conducted using a one-way analysis of variance (ANOVA), with a post hoc Welch's *t*-test. Significance thresholds were defined as follows: ### $p < 0.001$, relative to blank control group; *** $p < 0.001$, ** $p < 0.01$, versus H₂O₂-induced oxidative stress model group.

2.3.3. Cytotoxicity Assay

Compounds 1–3 were evaluated for cytotoxic effects on K562, BEL-7402, SGC-7901, A549, and Hela tumor cell lines *in vitro*. Compound 1 demonstrated cytotoxicity with IC₅₀ values of 72.37 ± 0.20 μ M against K562 and 61.47 ± 0.22 μ M against BEL-7402, while compounds 2 and 3 showed cytotoxicity across all five tested human cancer cell lines (Table 2). These findings highlight the differential cytotoxic profiles of the tested compounds, with compound 1 displaying selective activity and compounds 2 and 3 demonstrating broad-spectrum anticancer potential.

Table 2. Cytotoxic activities of compounds 1–3. (IC₅₀, μ M).

Compound	K-562	BEL-7402	SGC-7901	A-549	Hela
1	72.37 ± 0.20	61.47 ± 0.22	—	—	—
2	27.58 ± 0.07	24.55 ± 0.17	31.68 ± 0.26	19.86 ± 0.26	23.18 ± 0.19
3	30.68 ± 0.12	41.24 ± 0.26	36.21 ± 0.73	61.16 ± 1.01	53.23 ± 0.07
Cisplatin ^a	3.08 ± 0.05	4.02 ± 0.06	4.11 ± 0.02	1.93 ± 0.02	11.29 ± 0.15

K-562: lymphoblast cells isolated from the bone marrow of a chronic myelogenous leukemia patient. BEL-7402: a cell line derived from surgical specimens of human liver cancer patients. SGC-7901: a human gastric adenocarcinoma cell line established from a surgical specimen of a gastric cancer patient. A549: a cell line isolated from the lung tissue of a male with lung cancer. Hella: a cell line derived from cervical cancer cells of a woman. — No activity at a concentration of 20 μ g/mL. ^a Positive control.

3. Materials and Methods

3.1. General Experimental Procedures

High-resolution electrospray ionization mass spectrometry (HRESIMS) was conducted using an API QSTAR Pulsar mass spectrometer (Bruker, Karlsruhe, Germany). ¹H, ¹³C, and ²D NMR spectra were recorded using a Bruker AV III spectrometer (Karlsruhe, Germany) and a Quantum-IPlus 600 spectrometer (Quantum Design China, Beijing, China). Optical rotations were determined using an Anton Paar Modular Circular Polarimeter 500 (Graz, Austria). ECD and UV spectra were obtained using a MOS-500 spectrometer from Biologic, Clermont-Ferrand, France. Analytic HPLC was conducted using an Agilent Technologies 1260 Infinity II system with a DAD G1315D detector (Agilent, Santa Clara, CA, USA). The separation process utilized COSMOSIL-packed C18 and π NAP columns, both 5 μ m,

250 mm × 4.6 mm. Semipreparative HPLC utilized reversed-phase columns (COSMOSIL C18, Japan, 5 μm, 250 mm × 10 mm). The separation process utilized ODS gel (20–45 μm, Fuji Silysia Chemical Co., Ltd., Greenville, NC, USA), silica gel (60–80, 200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and Sephadex LH-20 (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on precoated silica gel G plates from Qingdao Marine Chemical Co., Ltd., China. The detection of spots was achieved by spraying with 5% sulfuric acid in ethanol and subsequent heating. GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses.

3.2. Plant Material

The plant material (Hainan agarwood) was procured from Hainan Province, China, in August 2018 and was authenticated as originating from *A. sinensis* by Prof. Dr. Haofu Dai. A voucher specimen (No. 201808) was deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

3.3. Extraction and Isolation

The dried Hainan agarwood (1.0 kg) was crushed and subjected to reflux extraction with 95% EtOH (3.0 L × 3, 3.0 h each). The combined extract was concentrated, dissolved in water, and sequentially partitioned with ethyl acetate (3.0 L × 3) and *n*-butanol (3.0 L × 3). The EtOAc-soluble fraction (232.6 g) was fractionated by silica gel vacuum liquid chromatography (VLC) with a PET-EtOAc gradient (1:0 → 0:1, stepwise), resulting in 18 fractions (Fr.1–Fr.18).

Fr.17 (30.9 g) was further separated via an ODS gel column eluted with a gradient of MeOH/H₂O (3:7 → 1:0, *v/v*), generating 60 subfractions (Fr.17.1–Fr.17.60). Fr.17.34 (564.0 mg) was subjected to chromatography using a Sephadex LH-20 column with methanol as the eluent, yielding four subfractions (Fr.17.34.1–Fr.17.34.4). Fr.17.34.2 (331.4 mg) was purified via semi-preparative HPLC (C₁₈ column; MeOH/H₂O, 70:30, *v/v*; 4.0 mL/min; UV 210/254 nm), resulting in three fractions: Fr.17.34.2.1–Fr.17.34.2.3. Further purification of Fr.17.34.2.2 (62.3 mg) under identical HPLC conditions but with MeCN/H₂O (55:45, *v/v*) yielded compound **1** (8.1 mg, *t_R* = 19.0 min). Similarly, Fr.17.34.2.3 (37.7 mg) was processed to yield compound **2** (23.9 mg, *t_R* = 17.0 min).

Fr.17.32 (371.0 mg) was separated using a Sephadex LH-20 column (MeOH eluent), resulting in three subfractions (Fr.17.32.1–Fr.17.32.3). Fr.17.32.2 (170.6 mg) was purified by semi-preparative HPLC (C₁₈ column; MeOH/H₂O, 60:40, *v/v*; 4.0 mL/min; UV 210/254 nm) to produce three fractions: Fr.17.32.2.1–Fr.17.32.2.3. Fr.17.34.1 (27.5 mg) was further separated using semi-preparative HPLC (C₁₈ column; MeCN/H₂O, 45:55, *v/v*; 4.0 mL/min; UV 210/254 nm), generating two fractions: Fr.17.34.2.11 and Fr.17.34.2.12. Fr.17.34.2 (55.9 mg) was subjected to the same protocol, yielding two fractions (Fr.17.34.2.21 and Fr.17.34.2.22). A final enrichment of Fr.17.34.2.1B and Fr.17.34.2.22 yielded compound **3** (13.9 mg, *t_R* = 26.0 min).

3.4. Anti-Inflammatory Assay

The inhibitory effects of compounds **1–3** on nitric oxide (NO) production were evaluated *in vitro* using the Griess assay on lipopolysaccharide (LPS)-stimulated RAW264.7 cells [25,26]. Quercetin and Indomethacin served as positive controls, while the medium with DMSO was used as the negative control. RAW264.7 mouse mononuclear macrophages were obtained from the Stem Cell Bank of the Chinese Academy of Sciences. Compounds were dissolved in DMSO at concentrations of 100, 50, 25, 12.5, and 6.25 μM using the double dilution method. RAW264.7 cells were plated in 96-well microtiter plates at 5 × 10⁴ cells/mL (100 μL per well) and incubated for 24 h in a humidified environment with 5% CO₂ and 90% air at 37 °C. Transfected cells were pretreated with the test solutions

for 1 h, followed by stimulation with 500 ng/mL LPS (Sigma, St. Louis, MO, USA) for 24 h. Subsequently, 100 μ L of supernatant from each well was transferred to new 96-well microtiter plates, and 100 μ L of Griess reagent (40 mg/mL, Sigma, USA) was added. Finally, the absorbance of each well was measured at 540 nm to calculate the IC₅₀ values of the tested compounds.

3.5. Neuroprotective Assay

The MTT assay was used to assess the protective effects of compounds 1–3 on SH-SY5Y human neuroblastoma cells against H₂O₂-induced oxidative stress [27]. The SH-SY5Y cells (1.2×10^4 cells/mL) were cultured at 37 °C in a 5% CO₂ and 95% air atmosphere in 96-well plates for 48 h. Subsequently, the cells were treated with 2-fold serial dilutions of compounds (100, 50, 25, 12.5, and 0 μ M) for 3 h, followed by the addition of 1000 μ M H₂O₂. After six hours, 20 μ M MTT (5 mg/mL in PBS) was introduced to each well and incubated for an additional four hours. Subsequently, the medium was discarded, and DMSO was employed to dissolve the formazan. Cell viability was quantified as a percentage of the control group (100%) by measuring absorbance at 490 nm with a Tecan microplate reader. Statistical analysis and group comparisons were conducted using GraphPad Prism software.

3.6. Cytotoxicity Assay

The cytotoxic effects of the compounds were assessed on five human cancer cell lines: myeloid leukemia (K562), hepatocellular carcinoma (BEL-7402), gastric adenocarcinoma (SGC-7901), non-small cell lung cancer (A549), and cervical carcinoma (HeLa) using the MTT assay [28,29]. Cells in the logarithmic growth phase were cultured at 37 °C with 5% CO₂ in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μ g/mL streptomycin. Cells were seeded into 96-well plates at 5×10^4 cells/mL and incubated for 24 h. Test compounds, dissolved in DMSO with a solvent concentration $\leq 0.1\%$, or cisplatin as a positive control, were then added and incubated for 72 h. MTT solution (20 μ L, 5 mg/mL in PBS) was subsequently added to each well and incubated for 4 h and absorbance was measured at 490 nm using a microplate reader. Dose–response curves were plotted, and IC₅₀ values were calculated by nonlinear regression analysis.

3.7. ECD Calculations

The absolute structures of compounds 1–3 were confirmed by optimizing potential configurations with Chem3D and XTB 6.6.0 software using the MMFF94 and gfn0 methods, respectively, followed by screening with the XTB (CREST) software package [30–34]. The ground state of the possible conformations was calculated by the Gaussian 16 program package, and the method # opt freq b3lyp/tzvp was selected (solvent method iefpcm, solvent = methanol). Then, the TD = (nstates = 20) wB97xd/TZVP (IEFPPCM, solvent = methanol) was selected to calculate the excited states. Theoretical ECD spectrograms were generated using Multiwfn 3.8 software based on the Boltzmann distribution [35]. Origin 8.5 software was used to compare the calculated curves with the experimental CD spectra.

4. Conclusions

In summary, an investigation into the constituents of Hainan agarwood (*Aquilaria sinensis*) led to the isolation of three novel 2-(2-phenylethyl)chromone-sesquiterpene hybrids (1–3). These compounds consisted of a 5,6,7,8-tetrahydro-2-(2-phenylethyl)chromone unit bound to an agarofuran-type sesquiterpene unit (compounds 1, 2) or an agarospirane-type sesquiterpene unit (compound 3) via an ester linkage.

The results of three biological activity tests indicate that compounds **1** and **3** might be promising lead candidates for the treatment of neurodegenerative diseases, and collectively position compound **1** as a dual-function candidate with anti-inflammatory and neuroprotective potential. In contrast, compounds **2** and **3** warrant further exploration as antitumor agents. A differential bioactivity assessment underscored the structure-dependent pharmacological effects of these compounds, exhibiting their viability as candidates for therapeutic development.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules30091984/s1>: Figure S1: The HRESIMS spectrum of compound **1**; Figures S2–S7: The ^1D and ^2D NMR spectra of compound **1** in $\text{MeOH-}d_4$; Figure S8: The HRESIMS spectrum of compound **2**; Figures S9–S14: The ^1D and ^2D NMR spectra of compound **2** in $\text{MeOH-}d_4$; Figure S15: The HRESIMS spectrum of compound **3**; Figures S16–S21: The ^1D and ^2D NMR spectra of compound **3** in $\text{MeOH-}d_4$; Figures S22–S27: ECD calculation images of compound **1–3**.

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References

1. Hashim, Y.Z.; Kerr, P.G.; Abbas, P.; Mohd, S.H. *Aquilaria* spp. (agarwood) as source of health beneficial compounds: A review of traditional use, phytochemistry and pharmacology. *J. Ethnopharmacol.* **2016**, *189*, 331–360. [[CrossRef](#)]
2. Li, W.; Chen, H.Q.; Wang, H.; Mei, W.L.; Dai, H.F. Natural products in agarwood and *Aquilaria* plants: Chemistry, biological activities and biosynthesis. *Nat. Prod. Rep.* **2021**, *38*, 528–565. [[CrossRef](#)]
3. Chen, D.; Xu, Z.R.; Chai, X.Y.; Zeng, K.W.; Jia, Y.X.; Bi, D.; Ma, Z.Z.; Tu, P.F. Nine 2-(2-phenylethyl) chromone derivatives from the resinous wood of *Aquilaria sinensis* and their inhibition of LPS-induced NO production in RAW 264.7 cells. *Eur. J. Org. Chem.* **2012**, *2012*, 5389–5397. [[CrossRef](#)]
4. Chen, L.L.; Liu, Y.; Li, Y.; Yin, W.; Cheng, Y. Anti-Cancer Effect of Sesquiterpene and Triterpenoids from Agarwood of *Aquilaria sinensis*. *Molecules* **2022**, *27*, 5350. [[CrossRef](#)]
5. Huang, X.L.; Zhou, Y.T.; Yan, Y.M.; Cheng, Y.X. Sesquiterpenoid-Chromone Heterohybrids from Agarwood of *Aquilaria sinensis* as Potent Specific Smad3 Phosphorylation Inhibitors. *J. Org. Chem.* **2022**, *87*, 7643–7648. [[CrossRef](#)]
6. Huang, X.L.; Cai, D.; Gao, P.; Wang, J.G.; Cheng, Y.X. Aquilariperoxide A, a Sesquiterpene Dimer from Agarwood of *Aquilaria sinensis* with Dual Antitumor and Antimalarial Effects. *J. Org. Chem.* **2023**, *88*, 8352–8359. [[CrossRef](#)]
7. Wei, S.Y.; Hu, D.B.; Xia, M.Y.; Luo, J.F.; Yan, H.; Yang, J.H.; Wang, Y.S.; Wang, Y.H. Sesquiterpenoids and 2-(2-Phenylethyl)chromone Derivatives from the Resinous Heartwood of *Aquilaria sinensis*. *Nat. Prod. Bioprospect* **2021**, *11*, 545–555. [[CrossRef](#)]
8. Zhang, H.; Ma, J.L.; Chang, C.; Ta, H.; Zhao, Y.F.; Shi, S.P.; Song, Y.L.; Tu, P.F.; Huo, H.X.; Zheng, J.; et al. Gastroprotective 2-(2-phenylethyl)chromone-sesquiterpene hybrids from the resinous wood of *Aquilaria sinensis* (Lour.) Gilg. *Bioorg. Chem.* **2023**, *133*, 106396. [[CrossRef](#)]

9. Yang, J.S.; Chen, Y.W. Studies on the constituents of *Aquilaria sinensis* (Lour.) Gilg. I. Isolation and structure elucidation of two new sesquiterpenes, baimuxinic acid and baimuxinal. *Acta Pharm. Sin. B* **1983**, *18*, 191–198.
10. Yang, J.S.; Chen, Y.W. Studies on the chemical constituents of *Aquilaria sinensis* (Lour.) Gilg. II. Isolation and structure of baimuxinol and dehydrobaimuxinol. *Acta Pharm. Sin. B* **1986**, *21*, 516–520.
11. Xu, J.F.; Zhu, L.F.; Lu, B.Y.; Liu, C.T. Study on the Chemical Constituents of Essential Oil of *Aquilaria sinensis* Lour Gilg. *J. Integr. Plant Biol.* **1988**, *30*, 635–638.
12. Yang, J.S.; Wang, Y.L.; Su, Y.L.; He, C.H.; Zheng, Q.T.; Yang, J. Studies on the chemical constituents of *Aquilaria sinensis* (Lour) Gilg. III. Elucidation of the structure of isobaimuxinol and isolation and identification of the constituents of lower boiling fraction of the volatile oil. *Acta Pharm. Sin. B* **1989**, *24*, 264–272.
13. Yang, L.; Qiao, L.; Xie, D.; Dai, J.; Guo, S. Sesquiterpenes and monoterpene from *Aquilaria sinensis*. *China J. Chin. Mater. Med.* **2012**, *37*, 1973–1976.
14. Yang, J.S.; Wang, Y.L.; Su, Y.L. Studies on the chemical constituents of *Aquilaria sinensis* (Lour.) Gilg. IV. Isolation and characterization of 2-(2-phenylethyl) chromone derivatives. *Acta Pharm. Sin. B* **1989**, *24*, 678–683.
15. Yang, J.S.; Wang, Y.L.; Su, Y.L. Studies on the chemical constituents of *Aquilaria sinensis* (Lour.) Gilg. V. Isolation and characterization of three 2-(2-phenylethyl) chromone derivatives. *Acta Pharm. Sin. B* **1990**, *25*, 186–190.
16. Liu, J.M.; Gao, Y.H.; Xu, H.H.; Chen, H.Y. Chemical constituents of *Aquilaria sinensis* (I). *Chin. Tradit. Herb. Drugs* **2006**, *37*, 325–327.
17. Liu, J.M.; Gao, Y.H.; Xu, H.H.; Xu, Z.Q. Chemical constituents of Lignum *Aquilariae* Resinatum (I). *Chin. Tradit. Herb. Drugs* **2007**, *38*, 1138–1140.
18. Yang, L.; Qiao, L.; Xie, D.; Yuan, Y.; Chen, N.; Dai, J.; Guo, S. 2-(2-phenylethyl)chromones from Chinese eaglewood. *Phytochemistry* **2012**, *76*, 92–97. [[CrossRef](#)]
19. Dai, H.F.; Liu, J.; Han, Z.; Zeng, Y.B.; Wang, H.; Mei, W.L. Two new 2-(2-phenylethyl)chromones from Chinese eaglewood. *J. Asian Nat. Prod. Res.* **2010**, *12*, 134–137. [[CrossRef](#)]
20. Mei, W.L.; Liu, J.; Li, X.N.; Dai, H.F. Study on the chemical constituents from Chinese eaglewood in Hainan. *J. Trop. Subtrop.* **2010**, *18*, 573–576.
21. Gao, Y.H.; Liu, J.M.; Lu, H.X.; Wei, Z.X. Two New 2-(2-Phenylethyl) chromen-4-ones from *Aquilaria sinensis* (Lour.) Gilg. *Helv. Chim. Acta* **2012**, *95*, 951–954. [[CrossRef](#)]
22. Huang, X.L.; Yan, Y.M.; Wang, D.W.; Cheng, Y.X. Spiroaquilarenes A–E: Unprecedented anti-inflammatory sesquiterpene polymers from agarwood of *Aquilaria sinensis*. *J. Org. Chem.* **2022**, *9*, 2070–2078. [[CrossRef](#)]
23. Yang, H.R.; Wang, P.; Liu, F.Z.; Yuan, J.Z.; Cai, C.H.; Wu, F.; Jiang, B.; Mei, W.L.; Dai, H.F. Dimeric 2-(2-phenethyl)chromones from agarwood of *Aquilaria filaria*. *Fitoterapia* **2023**, *165*, 105422. [[CrossRef](#)]
24. He, C.H.; Bao, G.H.; Xu, C.F.; Mu, S.T. Studies on the crystal structure of baimuxiangic acid. *Acta Pharm. Sin. B* **1982**, *17*, 597–602.
25. Li, M.M.; Su, X.Q.; Sun, J.; Gu, Y.F.; Huang, Z.; Zeng, K.W.; Zhang, Q.; Zhao, Y.F.; Ferreira, D.; Zjawiony, J.K.; et al. Anti-inflammatory ursane- and oleanane-type triterpenoids from *Vitex negundo* var. *cannabifolia*. *J. Nat. Prod.* **2014**, *77*, 2248–2254. [[CrossRef](#)]
26. Liu, F.Z.; Wang, H.; Li, W.; Yang, L.; Yang, J.L.; Yuan, J.Z.; Wei, Y.M.; Jiang, B.; Mei, W.L.; Dai, H.F. Filarones A and B, new anti-inflammatory dimeric 2-(2-phenethyl) chromones from agarwood of *Aquilaria filaria*. *Phytochem. Lett.* **2021**, *46*, 11–14. [[CrossRef](#)]
27. Cheng, Z.Y.; Lou, L.L.; Yang, P.Y.; Song, X.Y.; Wang, X.B.; Huang, X.X.; Song, S.J. Seven new neuroprotective sesquiterpenes isolated from the seeds of *Crataegus pinnatifida*. *Fitoterapia* **2019**, *133*, 225–230. [[CrossRef](#)]
28. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]
29. Yang, Y.; Mei, W.L.; Kong, F.D.; Chen, H.Q.; Li, W.; Chen, Z.B.; Dai, H.F. Four new bi-2-(2-phenylethyl)chromone derivatives of agarwood from *Aquilaria crassna*. *Fitoterapia* **2017**, *119*, 20–25. [[CrossRef](#)]
30. Pracht, P.; Bauer, C.A.; Grimme, S. Automated and efficient quantum chemical determination and energetic ranking of molecular protonation sites. *J. Comput. Chem.* **2017**, *38*, 2618–2631. [[CrossRef](#)]
31. Grimme, S. Exploration of Chemical Compound, Conformer, and Reaction Space with Meta-Dynamics Simulations Based on Tight-Binding Quantum Chemical Calculations. *J. Chem. Theory Comput.* **2019**, *15*, 2847–2862. [[CrossRef](#)]
32. Pracht, P.; Bohle, F.; Grimme, S. Automated exploration of the low-energy chemical space with fast quantum chemical methods. *Phys. Chem. Chem. Phys.* **2020**, *22*, 7169–7192. [[CrossRef](#)]
33. Pracht, P.; Grimme, S. Calculation of absolute molecular entropies and heat capacities made simple. *Chem. Sci.* **2021**, *12*, 6551–6568. [[CrossRef](#)]

34. Spicher, S.; Plett, C.; Pracht, P.; Hansen, A.; Grimme, S. Automated Molecular Cluster Growing for Explicit Solvation by Efficient Force Field and Tight Binding Methods. *J. Chem. Theory Comput.* **2022**, *18*, 3174–3189. [[CrossRef](#)]
35. Lu, T.; Chen, F. Multiwfn: A multifunctional wavefunction analyzer. *J. Comput. Chem.* **2012**, *33*, 580–592. [[CrossRef](#)] [[PubMed](#)]

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