

# SCIENTIFIC REPORTS

OPEN

## Antioxidant Lignans and Neolignans from *Acorus tatarinowii*

Yuanyuan Lu<sup>1,2</sup>, Yongbo Xue<sup>1</sup>, Shenjie Chen<sup>1</sup>, Hucheng Zhu<sup>1</sup>, Jinwen Zhang<sup>2</sup>, Xiao-Nian Li<sup>3</sup>, Jianping Wang<sup>1</sup>, JunJun Liu<sup>1</sup>, Changxing Qi<sup>1</sup>, Guang Du<sup>2</sup> & Yonghui Zhang<sup>1</sup>

Received: 30 November 2015

Accepted: 24 February 2016

Published: 10 March 2016

Eleven new lignans and neolignans, named acortatarinowins G–N (1–8), including three pairs of enantiomers (1a/1b–3a/3b) and five optically pure lignans and neolignans (4–8), along with five known analogs (9–14), were isolated from the rhizomes of *Acorus tatarinowii* Schott. Compounds 1–3 were successfully separated by chiral HPLC to afford 1a/1b–3a/3b. The planar structures of 1–8 were elucidated by extensive spectroscopic analyses including HRESIMS and NMR. Their absolute configurations were determined by analyses of single-crystal X-ray diffraction and a modified Mosher's method, assisted by experimental and calculated electronic circular dichroism (ECD) data. Compounds 1a and 1b were rare 7,8'-epoxy-8,7'-oxyneolignane. Compounds 1–8 were evaluated for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing antioxidant power assay. Compound 6, exhibiting strong DPPH radical scavenging capacity with IC<sub>50</sub> value of 16.4 ± 0.22 µg/mL, could interpret the herbal traditional usage.

Reactive oxygen free radical, produced in normal or pathological cell metabolism, is oxygenated to provide energy to fuel biological processes for many living organisms<sup>1</sup>. However, many diseases, such as cirrhosis and arteriosclerosis, rheumatoid arthritis, cancer as well as neurodegenerative diseases associated with aging, are due to the accumulation of oxygen derived free radicals<sup>1–3</sup>.

*Acorus tatarinowii* Schott (family Araceae) is a well-known traditional Chinese medical plant, whose rhizomes are historically used to treat neurodegenerative diseases, such as apoplexy and dementia, amnesia, epilepsy, improvement of learning and memory, especially Alzheimer's disease (AD)<sup>4,5</sup>. AD is a devastating neurodegenerative disease, and no effective treatment is available, affecting more than 35 million people worldwide<sup>6</sup>. Therefore, it is increasingly urgent to search for bioactive compounds on the cure of AD from traditional Chinese medicine.

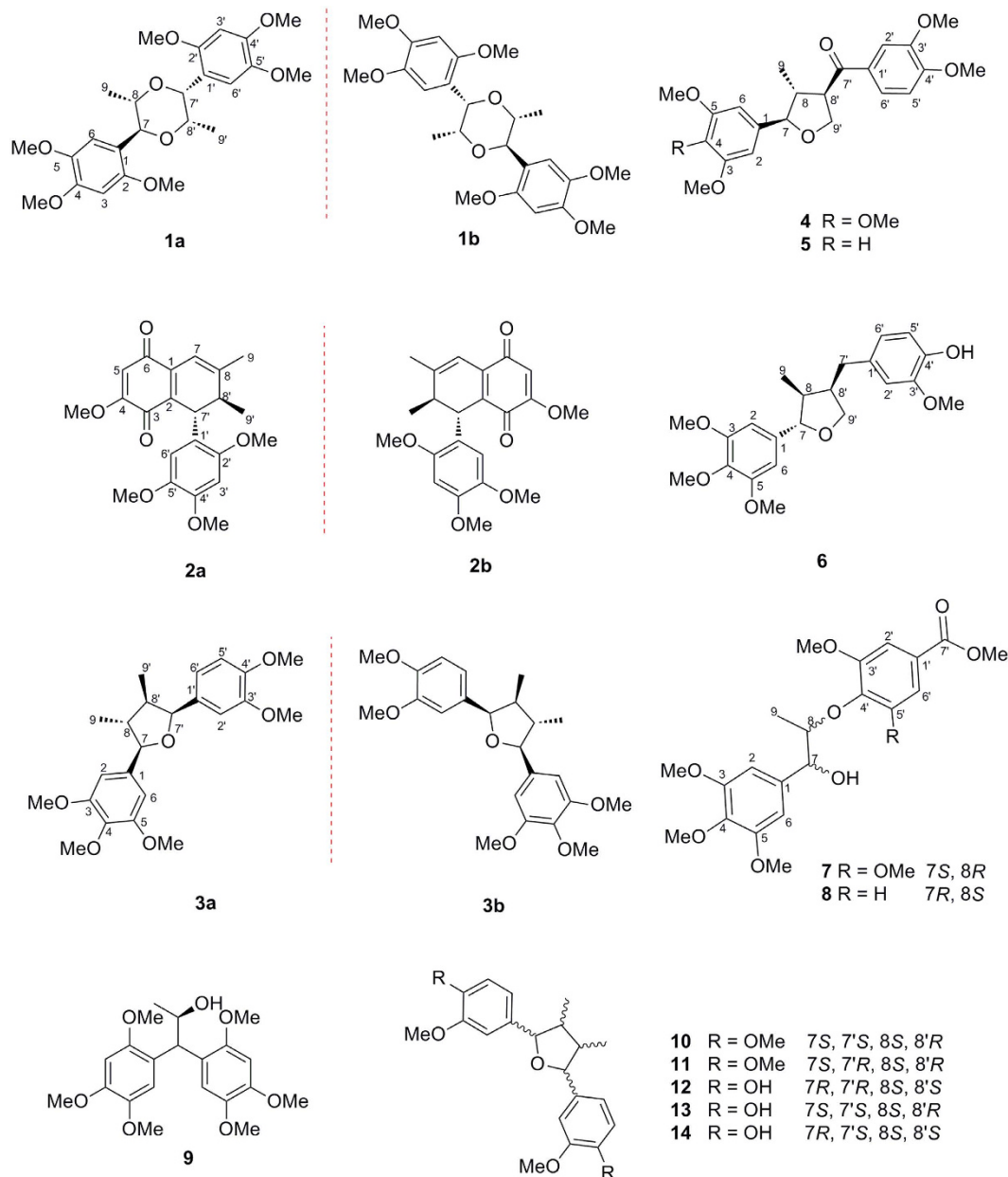
As part of our continuing efforts to discover bioactive natural products in the medical plant *A. tatarinowii*, a series of enantiomeric lignans and norlignans were previously reported<sup>7</sup>. In our current study, further investigation on chemical constituents of the rhizomes of *A. tatarinowii* in different regions has led to the isolation of eleven new lignans and neolignans (1a/1b–3a/3b and 4–8), and six known analogs (9–14). All of these new isolates were evaluated for their antioxidant activities to interpret the herbal traditional usage and to discover new potential agent for AD. Herein, we report the isolation, structural elucidation, and the absolute configuration determination of all new compounds.

### Results and Discussion

**Structure Elucidation.** The EtOAc extract of the air-dried rhizomes of *A. tatarinowii* was subjected to a series of chromatographic separations, to yield eleven new (1a/1b–3a/3b and 4–8) and six known (9–14) neolignans and lignans (Fig. 1). The structures of the known compounds (9–14) were identified as tatarinoid C (9)<sup>8</sup>, (+)-veraguensin (10)<sup>9</sup>, (–)-galgravin (11)<sup>10</sup>, and (+)-saucermetindiol (12)<sup>11</sup>, (–)-machilin-I (13)<sup>12</sup> and (+)-verrucosin (14)<sup>11</sup>, respectively, by comparing their NMR data with those reported in literatures.

(±)-Acortatarinowin G (1a/1b), obtained as block crystals in MeOH (mp 186–187 °C), had a molecular formula of C<sub>24</sub>H<sub>32</sub>O<sub>8</sub> based on <sup>13</sup>C NMR data and a sodiated molecular ion in positive HRESIMS at *m/z* 471.1977 [M + Na]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>32</sub>NaO<sub>8</sub>, 471.1995). The <sup>1</sup>H NMR (Table 1) data of 1 showed signals for

<sup>1</sup>Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, People's Republic of China. <sup>2</sup>Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, People's Republic of China. <sup>3</sup>State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China. Correspondence and requests for materials should be addressed to G.D. (email: tjyxb@sina.com) or Y.Z. (email: zhangyh@mails.tjmu.edu.cn)



**Figure 1.** Structures of isolated compounds.

two 1,2,4,5-tetrasubstituted phenyl groups ( $\delta_{\text{H}}$  7.08, 1H, s; 7.07, 1H, s; 6.51, 1H, s; and 6.49, 1H, s), four oxygenated methines ( $\delta_{\text{H}}$  5.25, 1H, d,  $J = 3.2$  Hz;  $\delta_{\text{H}}$  5.02, 1H, d,  $J = 9.3$  Hz;  $\delta_{\text{H}}$  4.34, 1H, m; and  $\delta_{\text{H}}$  3.76, 1H, d, m), six methoxy groups ( $\delta_{\text{H}}$  3.90, 2\*3.89, 3.88, 3.83, and 3.81), and two methyl signals ( $\delta_{\text{H}}$  1.12, 3H,  $J = 6.8$  Hz and  $\delta_{\text{H}}$  1.08, 3H,  $J = 6.3$  Hz). The  $^{13}\text{C}$  NMR and DEPT (Table 2) of **1** revealed 24 carbons, which were resolved into 12 aromatic carbons, four oxygenated methine carbons, six methoxy groups, and two methyl carbons. The spectroscopic data suggested that **1** was a 7,8-dioxane neolignan which was very similar to verimol H except that two 1,4-dimethoxyphenyl moieties in verimol H were replaced by two 1,2,4,5-tetramethoxyphenyl groups in **1**<sup>13</sup>. This conclusion was supported by the HMBC correlations from H-7 ( $\delta_{\text{H}}$  5.02) to C-1 ( $\delta_{\text{C}}$  119.8), C-2 ( $\delta_{\text{C}}$  151.8), C-6 ( $\delta_{\text{C}}$  111.7), C-8 ( $\delta_{\text{C}}$  78.8), C-9 ( $\delta_{\text{C}}$  17.1), and C-8' ( $\delta_{\text{C}}$  70.8), and from H-7' ( $\delta_{\text{H}}$  5.25) to C-1' ( $\delta_{\text{C}}$  119.5), C-2' ( $\delta_{\text{C}}$  150.0), C-6' ( $\delta_{\text{C}}$  111.2), C-8' ( $\delta_{\text{C}}$  70.8), C-9' ( $\delta_{\text{C}}$  12.3), and C-8 ( $\delta_{\text{C}}$  78.8). In addition, the HMBC correlations of OMe-2/C-2, OMe-4/C-4, OMe-5/C-5, OMe-2'/C-2', OMe-4'/C-4' and OMe-5'/C-5', verified that compound **1** was 2,4,5, 2',4',5'-hexamethoxy-7,8'-epoxy-8,7'-oxyneolignan.

The relative configuration of compound **1** was determined through a NOESY experiment and the coupling constants, and further confirmed by X-ray crystallographic analyses. The large coupling constant of H-7/H-8 ( $J_{7,8} = 9.3$  Hz) suggested a *trans*-relationship between H-7 and H-8, and the small coupling constant of H-7'/H-8' ( $J_{7',8'} = 3.2$  Hz) indicated H-7' and H-8' were in a *cis* position. The key cross-peak of H-7'/H-8 in the NOESY spectrum indicated the relative configuration of acortatarinowin G was (7R\*, 8R\*, 7'S\*, 8'R\*). Fortunately,

No.	1 <sup>b</sup>	2 <sup>b</sup>	3 <sup>a</sup>	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>
2			6.82 s	6.62 s	7.04 s	6.63 s	6.76 s	6.77 s
3	6.51 s							
4					6.93 s			
5		5.88 s						
6	7.07 s		6.82 s	6.62 s	6.93 s	6.63 s	6.76 s	6.77 s
7	5.02 d (9.3)	6.45 s	4.41 d (9.3)	4.38 d (9.5)	4.41 d (9.0)	4.52 d (7.4)	4.71 d (5.9)	4.73 d (4.9)
8	3.76 m		1.77 m	2.62 m	2.45 m	2.24 m	4.42 m	4.65 m
9	1.09 d (6.3)	1.86 s	1.08 d (6.6)	1.07 d (6.6)	1.03 d (6.8)	1.12 d (7.0)	1.13 d (6.3)	1.31 d (6.1)
2'			6.956 d (2.0)	7.56 d (2.0)	7.58 d (2.0)	6.78 d (1.8)	7.33 s	7.53 d (2.0)
3'	6.50 s	6.53 s						
5'			6.97 d (8.2)	6.92 d (8.8)	7.08 d (8.5)	6.72 d (8.0)		6.97 d (8.5)
6'	7.08 s	6.45 s	6.97 dd (8.2, 2.0)	7.55 dd (8.8, 2.0)	7.71 dd (8.5, 2.0)	6.64 dd (8.0, 1.8)	7.33 s	7.57 dd (8.5, 2.0)
7'	5.25 d (3.2)	4.44 s	5.15 d (8.8)			2.83 dd (13.4, 5.0), 2.45 dd (13.4, 11.1)		
8'	4.34 m	2.31 q (7.3)	2.28 m	3.83 m	4.03 m	2.61 m		
9'a	1.12 d (6.8)	1.11 d (7.2)	0.66 d (7.0)	4.30 dd (9.3, 8.8)	4.28 dd (9.0, 8.5)	4.02 dd (8.4, 6.3)		
9'b				4.15 dd (9.3, 7.5)	4.11 dd (9.0, 6.8)	3.73 dd (8.4, 5.1)		
OMe-2	3.82 s							
OMe-3			3.87 s	3.87 s	3.86 s	3.83 s	3.83 s	3.82 s
OMe-4	3.89 s	3.79 s	3.78 s	3.83 s		3.75 s	3.74 s	3.70 s
OMe-5	3.88 s		3.87 s	3.87 s	3.86 s	3.83 s	3.83 s	3.82 s
OMe-2'	3.83 s	3.90 s						
OMe-3'			3.83 s	3.94 s	3.90		3.89 s	3.85 s
OMe-4'	3.89 s	3.84 s	3.83 s	3.95 s	3.93	3.83 s		
OMe-5'	3.90 s	3.64 s					3.89 s	
OMe-7'							3.90 s	3.86 s

**Table 1.** <sup>1</sup>H NMR Data for Compounds **1**–**8** (400 MHz, *J* in Hz). <sup>a</sup>in methanol-*d*<sub>4</sub>. <sup>b</sup>in CDCl<sub>3</sub>.

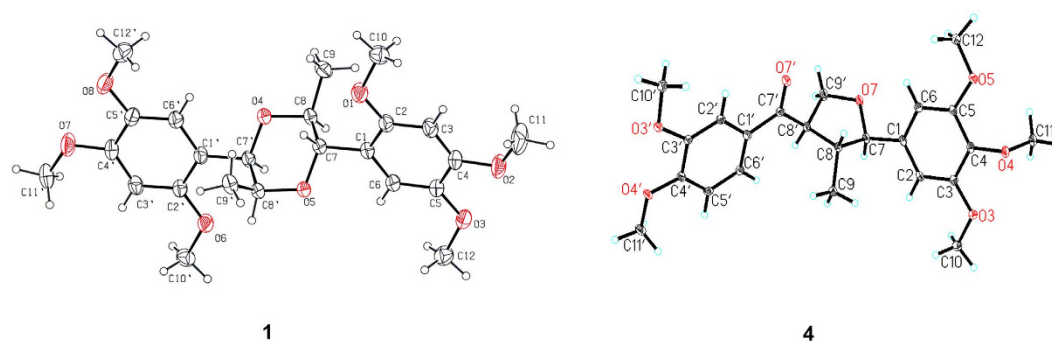
crystals of **1** were obtained from MeOH, and a single X-ray diffraction experiment was performed using Cu K $\alpha$  radiation (Fig. 2), which supported the above conclusion. Surprisingly, the crystal of **1** had the centrosymmetric space group *P*-2<sub>1</sub>*c*, indicating its racemic nature<sup>13,14</sup>, which was supported by the absence of optical rotation and any Cotton effects in the ECD spectrum. Subsequent chiral resolution led to the isolation of **1a** and **1b** (Fig. 3, **1a:1b** = 1:1) with opposite specific rotation and mirror imaged Cotton effects in their ECD spectrum (Fig. 4). An 8*S* configuration for **1a** was established by a positive Cotton effect around 230 nm in the ECD spectrum (Fig. 4)<sup>15–17</sup>. Furthermore, the experiment ECD spectrum of **1a/1b** matched well with calculated ECD spectrum of (7*S*, 8*S*, 7'*R*, 8'*S*)-**1** and (7*R*, 8*R*, 7'*S*, 8'*R*)-**1** (Fig. 4), respectively. Thus, the absolute configurations of **1a** and **1b** were established as shown (Fig. 1).

(±)-Acortatarinowin H (**2a/2b**), obtained as brown-red amorphous powder, had a molecular formula of C<sub>22</sub>H<sub>24</sub>O<sub>6</sub> as revealed by HRESIMS spectrum (*m/z* 407.1493 [M + Na]<sup>+</sup>) and <sup>13</sup>C NMR data. The <sup>1</sup>H NMR data showed signals (Table 1) attributable to two methyl groups, two methine protons, four methoxy protons, two olefinic protons, and two aromatic protons. The <sup>13</sup>C-NMR spectrum showed the presence of 22 carbon signals, corresponding to two methyl carbons, two methine carbons, four methoxy groups, 12 olefinic carbons and two conjugated carbonyl carbons, which resembled those of 7'-(2',4',5'-trimethoxyphenyl)-4-methoxy-8,8'-dimethyl-2,5 quinone<sup>18</sup>. A side-by-side comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data of **1** with those of 7'-(2',4',5'-trimethoxyphenyl)-4-methoxy-8,8'-dimethyl-2,5-quinone revealed that the small differences were the absence of two olefinic carbons and the appearance of two methine carbons in **2**. Based on the aforementioned evidence, **2** was determined to be 4,2',4',5'-tetramethoxy-7-ene-3,6-dione-2,7'-cycloignane, which was further supported by the <sup>1</sup>H–<sup>1</sup>H COSY correlations of H-7'/H-8' and H-8'/H-9', and the key HMBC correlations from H-7' ( $\delta_{\text{H}}$  4.44) to C-1' ( $\delta_{\text{C}}$  121.4), C-2' ( $\delta_{\text{C}}$  151.1), C-6' ( $\delta_{\text{C}}$  112.2), C-8' ( $\delta_{\text{C}}$  41.5), C-9' ( $\delta_{\text{C}}$  19.5), C-1 ( $\delta_{\text{C}}$  137.3), C-3 ( $\delta_{\text{C}}$  181.5), C-2 ( $\delta_{\text{C}}$  132.5), and C-8 ( $\delta_{\text{C}}$  153.3).

The relative configurations at C-7' and C-8' of the core skeleton of **2** were established by analyses of coupling constants aided by molecular models generated by Chem3D. According to those models, H-7' and H-8' in a *trans* position matched well with the coupling constant ( $J_{7',8'} = 0$  Hz), which was supported by a single peak at  $\delta_{\text{H}}$  4.44 (H-7') and a quartet peak at  $\delta_{\text{H}}$  2.31 (H-8'). The absence of any Cotton effects in the ECD spectrum and optical rotation revealed compound **2** was a pair of racemic mixture as well. These findings were supported by chiral HPLC analyses of **2** (Fig. 3). Compounds **2a** and **2b** were successfully obtained using a Daicel IC column, showing opposite specific rotations (**2a**:  $[\alpha]_{20}^{\text{D}}$  +14; **2b**:  $[\alpha]_{20}^{\text{D}}$  –14) and opposite Cotton effects (Fig. 4) [**2a**: 213 ( $\Delta\epsilon$  +26.05), 243 ( $\Delta\epsilon$  –12.82), 315 ( $\Delta\epsilon$  –9.33); **2b**: 213 ( $\Delta\epsilon$  –27.01), 243 ( $\Delta\epsilon$  +13.02), 315 ( $\Delta\epsilon$  +10.31)]. A negative Cotton effect around 315 nm indicated an 8'*S* for **2a**<sup>19,20</sup>. Therefore, the absolute configurations of **2a** and **2b** were determined as 7'*R*, 8'*S* and 7'*S*, 8'*R*, respectively.

No.	1 <sup>b</sup>	2 <sup>b</sup>	3 <sup>a</sup>	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>
1	119.8	137.3	138.7	136.0	134.3	140.0	138.4	138.7
2	151.8	132.5	104.9	103.7	111.4	104.2	105.5	105.6
3	97.7	181.5	154.6	153.4	150.7	154.5	154.2	154.1
4	149.4	159.6	138.1	137.8	112.7	138.4	138.4	138.3
5	143.3	106.2	154.6	153.4	150.7	154.5	154.2	154.1
6	111.7	186.6	104.9	103.7	120.8	104.2	105.5	105.6
7	67.9	113.3	88.9	89.2	90.2	88.6	78.5	77.1
8	78.8	153.3	49.8	45.9	47.7	46.6	84.9	80.0
9	17.1	23.4	15.1	15.4	15.1	12.8	17.1	15.6
1'	119.5	121.4	135.1	130.3	131.4	133.5	126.4	123.9
2'	150.0	151.1	112.4	110.2	111.8	113.5	107.9	114.0
3'	97.0	99.1	149.9	149.4	150.5	145.8	154.4	150.1
4'	148.8	148.9	150.2	153.9	155.6	149.0	142.2	153.1
5'	143.6	143.0	112.7	110.5	111.9	116.2	154.4	115.4
6'	111.2	112.2	120.9	123.2	124.9	122.2	107.9	124.6
7'	75.1	35.0	84.6	197.6	200.2	34.3	168.1	168.4
8'	70.8	41.5	47.1	54.4	55.1	45.4		
9'	12.3	19.5	15.3	70.8	71.5	73.4		
OMe-2	56.8							
OMe-3			56.7	56.3	56.5	56.6	56.7	56.5
OMe-4	56.3	56.3	61.1	60.9		61.1	61.1	61.1
OMe-5	56.3		56.7	56.3	56.5	56.6	56.7	56.5
OMe-2'	56.8	57.2						
OMe-3'			56.5	56.1	56.5		56.6	56.5
OMe-4'	56.3	56.3	56.5	56.2	56.6	56.4		
OMe-5'	56.7	57.2					56.6	
OMe-7'							52.8	52.5

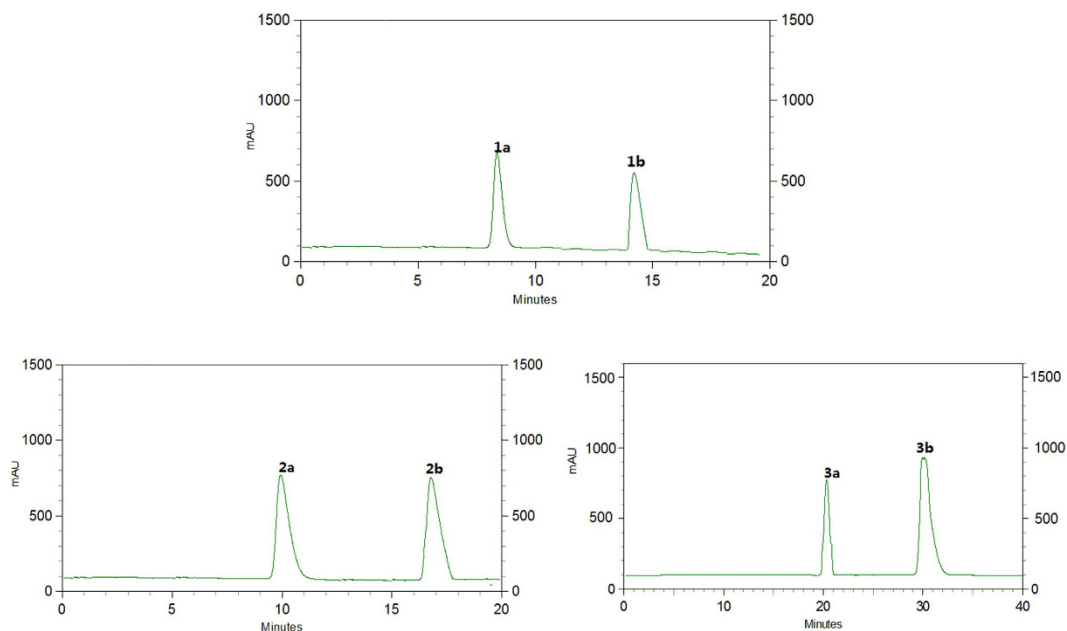
**Table 2.** <sup>13</sup>C NMR Data for Compounds 1–8 (100 MHz). <sup>a</sup>in methanol-*d*<sub>4</sub>. <sup>b</sup>in CDCl<sub>3</sub>.



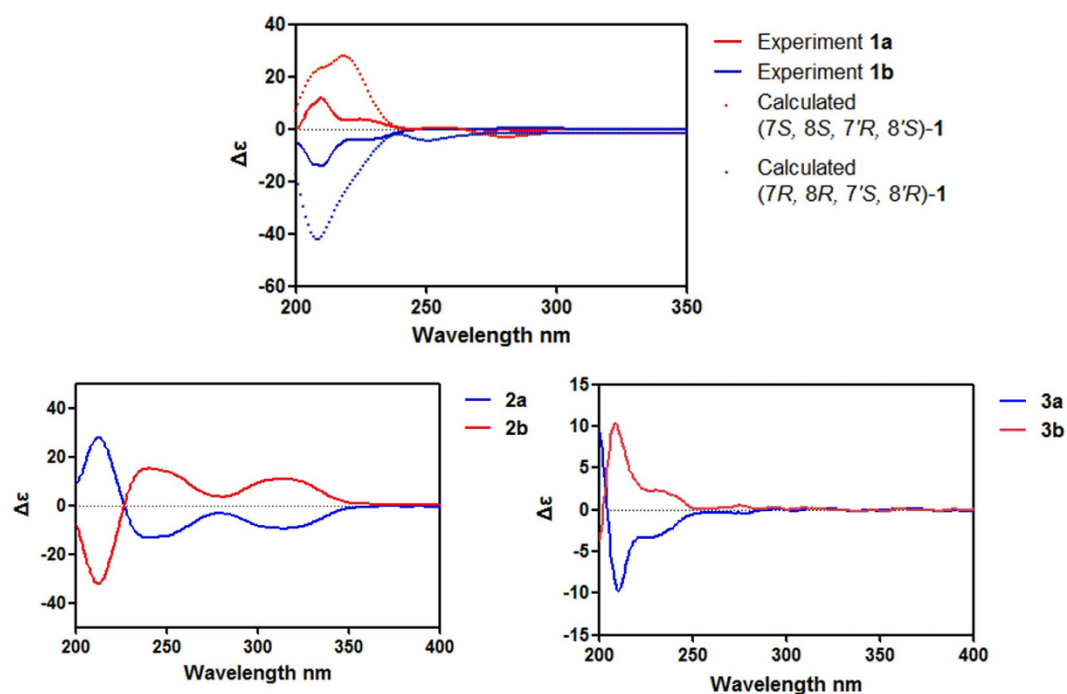
**Figure 2.** ORTEP drawing of compounds 1 and 4.

(±)-Acortatarinowin I (**3a/3b**), obtained as a light yellow gum, its NMR data and positive optical rotation were the same as those of 3,4,5,3',4'-pentamethoxy-7,7'-epoxylignan reported in the literature with absolute configuration not determined<sup>21</sup>. Based on our previous research<sup>7</sup>, compound **3** was subjected to a Daicel IC column to check its partial racemic nature, and (–)-**3a** and (+)-**3b** (Fig. 3, **3a:3b** ≈ 1:3) were successfully obtained, showing typical antipodal ECD curves (Fig. 4) and specific rotations of opposite sign. The absolute configuration for **3b** was determined as 7*S*,8*S*,7'*R*,8'*S* by comparing the ECD curve of **3b** with that of closely related compound hydroxyvera<sup>22</sup>. Hence, that of **3a** was determined as 7*R*,8*R*,7'*S*,8'*R* (Fig. 1).

Acortatarinowin J (**4**), was isolated as colourless crystals (MeOH) with mp 158–159 °C. The molecular formula of **4** was determined as C<sub>23</sub>H<sub>28</sub>O<sub>7</sub> according to <sup>13</sup>C NMR data and HREIMS data *m/z* 439.1718 [M + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>28</sub>NaO<sub>7</sub>, 439.1733). The IR spectrum indicated the presence of conjugated carbonyl group (1674 cm<sup>-1</sup>) and aromatic rings (1594 and 1515 cm<sup>-1</sup>). The NMR data (Tables 1 and 2) showed signals attributable to a 1,3,4,5-tetrasubstituted and a 1,3,4-trisubstituted aromatic moiety, five methoxy groups, three methines including an oxygenated methine, an oxygenated methylene unit, a methyl group and a conjugated carbonyl group. These findings indicated that compound **4** is a 7,9'-epoxylignan similar to magnone B<sup>23</sup>. Comparison of the NMR data indicated that the only small difference between **4** and magnone B was that the hydroxymethyl group (C-8) in



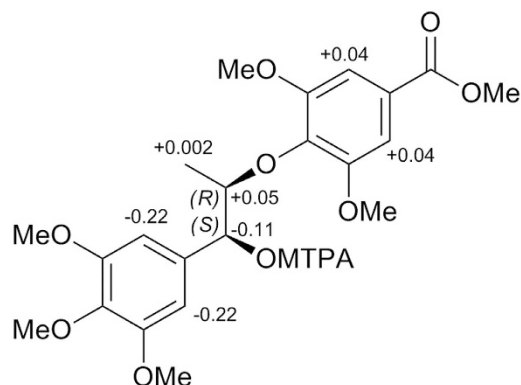
**Figure 3.** Chiral HPLC separation profiles of compounds 1a/1b–3a/3b.



**Figure 4.** The experimental ECD spectra of 1a/1b–3a/3b, and the calculated ECD spectra for 1a/1b.

magnone B was reduced to a methyl group. This deduction was supported by the HMBC correlations from H-7 ( $\delta_{\text{H}}$  4.38) to C-1 ( $\delta_{\text{C}}$  136.0), C-2,6 ( $\delta_{\text{C}}$  103.7), C-8 ( $\delta_{\text{C}}$  45.9), C-9 ( $\delta_{\text{C}}$  15.4), C-8' ( $\delta_{\text{C}}$  54.4), and C-9' ( $\delta_{\text{C}}$  70.8), from H-8 ( $\delta_{\text{H}}$  2.62) to C-1 ( $\delta_{\text{C}}$  136.0), C-7 ( $\delta_{\text{C}}$  89.2), C-8' ( $\delta_{\text{C}}$  54.4), and C-7' ( $\delta_{\text{C}}$  197.6), and from H-2'/6' ( $\delta_{\text{H}}$  7.55/7.56) to C-7' ( $\delta_{\text{C}}$  197.6). In addition, the HMBC correlations for OMe-3/C-3, OMe-4/C-4, OMe-5/C-5, OMe-3'/C-3', and OMe-4'/C-4' verified the location of these substituents. Thus, the structure of **4** was determined as 3,3',4,4',5-pentamethoxy-7,9'-epoxylignan-7'-one.

The relative configuration of **4** was established through a NOESY experiment and coupling constant. The big coupling constant ( $J = 9.5$  Hz) between H-7 and H-8 indicated the *trans*-orientation, and the strong NOESY correlation of H-7 and H-8' verified they were in a *cis*-orientation. Therefore, the 2D structure of **4** was determined as (7*R*<sup>\*</sup>, 8*R*<sup>\*</sup>, 8'*R*<sup>\*</sup>)-3,3',4,4',5-pentamethoxy-7,9'-epoxylignan-7'-one. Fortunately, the crystals of **4** were obtained



**Figure 5.**  $\Delta\delta_{H(S-R)}$  values (in ppm) for the MTPA esters of **7**.

from MeOH, and the absolute configuration of acortatarinowin J was finally elucidated as *7R,8R,8'R* on the basis of single crystal X-ray diffraction (Fig. 2).

Acortatarinowin K (**5**), obtained as white amorphous powder, possesses the molecular formula  $C_{22}H_{26}O_6$ , as determined from a sodiated molecular ion in the positive HRESIMS at  $m/z$  387.1797  $[M + H]^+$  (calcd for  $C_{22}H_{27}O_6$ , 387.1808) and its NMR data. A side-by-side comparison of the  $^{13}C$  NMR data of **5** with those of **4** revealed that **5** was closely related to **4** except the absence of a methoxy group. Correspondingly, the signals attributable to a symmetrical 1,3,4,5-tetrasubstituted aromatic moiety in **4** was replaced by those of a 1,3,5-trisubstituted aromatic moiety in the  $^1H$  NMR spectrum. Therefore, compound **5** was surmised to be 3,3',4',5-tetramethoxy-7,9'-epoxylignan-7'-one. This deduction was confirmed by 2D NMR data analyses. The big coupling constant ( $J_{7,8} = 9.5$  Hz) and key NOESY correlation from H-7 to H-8' indicated the relative configuration of **5** was the same as **4**. Furthermore, the experiment ECD spectrum of **5** matched well with that of **4** (Supporting Information, SI, Figs S37 and S47). Thus, the absolute configuration of **5** was determined as *7R,8R,8'R*.

Acortatarinowin L (**6**), had the molecular formula  $C_{22}H_{28}O_6$ , determined on the basis of a sodiated molecular ion in the positive HRESIMS at  $m/z$  411.1774  $[M + Na]^+$  (calcd for  $C_{22}H_{28}NaO_6$ , 411.1784) and its NMR data. A comparison of the NMR data (Tables 1 and 2) between **6** and **4** indicated that they differed in the presence of a methylene unit and the absence of a conjugated carbonyl and a methoxy group in **6**. Thus, compound **6** was determined as 4'-hydroxy-3,3',4,5-tetramethoxy-7,9'-epoxylignan. The NOESY correlations from H-9 to H-7' and H-7 revealed H-7 was oriented opposite to H-8 and H-8'. Thus, the relative configuration of **6** was determined as *7R\**, *8R\**, *8'S\**. Compound **6** displayed a coupled Cotton effect, negative at 235 nm and positive at 215 nm (SI, Fig. S57), indicating exciton coupling in the  $\pi \rightarrow \pi^*$  transition of the phenyl chromophores<sup>24</sup>. The positive chirality ( $[\alpha]_{20}^D + 18$ ) revealed the *7R*, *8R*, *8'S* configuration for **6** on the basis of the ECD exciton chirality rule<sup>17,24,25</sup>. Therefore, the absolute configuration of **6** was assigned as *7R*, *8R*, *8'S*.

Acortatarinowin M (**7**) was obtained as a light yellow gum. The HRESIMS ion at  $m/z$  459.1619  $[M + Na]^+$  (calcd for  $C_{22}H_{28}NaO_9$ , 459.1631) and the NMR data established its molecular formula as  $C_{22}H_{28}O_9$ . The NMR data (Tables 1 and 2) displayed signals for two symmetric tetrasubstituted aromatic moieties, six methoxy groups, two oxygenated methines, a methyl, and a conjugated carbonyl. These findings indicated that compound **7** was an 8-*O*-4' type neolignan and was similar to acortatarinowin C with the difference in the presence of an methoxy group in **7**. The key HMBC correlation from H-2'/H-6' to C-7' revealed compound **7** was 5'-methoxy derivative of acortatarinowin C. The absolute configuration at C-7 was determined as *7S* by modified Mosher's method (Fig. 5), and C-8 was *R* due to a negative Cotton effect around 237 nm<sup>15-17</sup>.

Acortatarinowin N (**8**) was obtained as a light yellow gum. The NMR data and an HRESIMS  $[M + Na]^+$  ion at  $m/z$  429.1464 (calcd for  $C_{21}H_{26}NaO_8$ , 429.1525) allowed the assignment of the molecular formula of  $C_{21}H_{26}O_8$  the same as acortatarinowin C<sup>7</sup>. A side-by-side comparison of the  $^1H$  and  $^{13}C$  NMR data of **8** with those of acortatarinowin C indicated there were only small changes in chemical shifts from H-7/C-7 to H-9/C-9 [ $\delta_H$  4.73 (1H, d,  $J = 4.9$  Hz, H-7),  $\delta_C$  77.1 (C-7); 4.65 (1H, dq,  $J = 6.1, 4.9$  Hz, H-8), 80.0 (C-8); and 1.31 (3H, d,  $J = 6.1$  Hz, H-9, 15.6 (C-9 in **8**);  $\delta_H$  4.75 (1H, d,  $J = 5.4$  Hz, H-7),  $\delta_C$  77.1 (C-7); 4.69 (1H, dq,  $J = 6.2, 5.4$  Hz, H-8), 80.0 (C-8); 1.17 (3H, d,  $J = 6.2$  Hz, H-9, 16.1 (C-9 in acortatarinowin C)]. On the basis of these observations, along with 2D NMR analyses, compound **8** was deduced to be the stereoisomer of acortatarinowin C with an *erythro* configuration (*7R\**, *8S\**) due to its small coupling constant ( $J_{7,8} = 4.9$  Hz). The positive ECD Cotton effect near 238 nm of **8** agreed well with that of (–)-acortatarinowin B<sup>7</sup> and its structurally related neolignan compound (*7R*, *8S*)-balanophonin<sup>25,26</sup>. Thus, the absolute configuration of acortatarinowin N was determined as *7R,8S*.

**Antioxidant Activity Evaluation.** The abilities of the compounds **1–8** to scavenge DPPH radical were evaluated from 6.25 to 100  $\mu\text{g/mL}$ . Trolox (vitamin E) was used as positive control for antioxidant activity (Table 3). Among the compounds tested, compound **6** exhibited the most potent antioxidant activity with  $IC_{50}$  value of  $16.437 \pm 0.22$   $\mu\text{g/mL}$ , whereas the other compounds did not exhibit any activity with the maximum concentration 100  $\mu\text{g/mL}$ . By comparing the structures of all the compounds tested, it was concluded that the presence of phenolic hydroxyl functionality was required for the antioxidant activity of these lignans and neolignans.

Compounds	Concentrations ( $\mu\text{g/mL}$ )	Scavenging ratio (%)	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
1a	100	$-2.292 \pm 0.263$	
1b	100	$-2.893 \pm 0.278$	
2a	100	$-2.477 \pm 0.351$	
2b	100	$-4.892 \pm 0.088$	
3a	100	$-1.487 \pm 0.351$	
3b	100	$-1.363 \pm 0$	
4	100	$-1.301 \pm 0.263$	
5	100	$-1.796 \pm 0.088$	
6	100	$84.784 \pm 0.756$	$16.437 \pm 0.22$
	50	$71.794 \pm 0.611$	
	25	$59.629 \pm 0.328$	
	12.5	$43.712 \pm 0.435$	
	6.25	$29.609 \pm 0.993$	
7	100	$-1.796 \pm 0.088$	
8	100	$-2.145 \pm 0.078$	
Trolox	50	$95.629 \pm 0.372$	$3.895 \pm 0.38$
	25	$94.805 \pm 0.611$	
	12.5	$94.433 \pm 0.682$	
	6.25	$69.238 \pm 3.335$	
	3.125	$41.114 \pm 4.279$	

**Table 3.** DPPH Radical Scavenging Activity of New Compounds *in vitro*. The data are expressed as the means  $\pm$  SEM. Three independent experiments were performed. Trolox (vitamin E) was used as the positive control.

## Conclusion

In this study, eleven new lignans and neolignans, including three pairs of enantiomers **1a/1b–3a/3b**, were isolated from the rhizomes of *A. tatarinowii*. Compounds **1a** and **1b** were rare 7,8'-epoxy-8,7'-oxyneolignanes. The absolute configurations of the two 7,8'-epoxy-8,7'-oxyneolignanes (**1a/1b**) were determined for the first time by comparison of their experimental and calculated ECD spectrum. Biologically, the antioxidant activities of the new isolates were evaluated. Compound **6** possessing a phenolic hydroxyl moiety exhibited the most potent antioxidant activity, whereas the other compounds, containing no phenolic hydroxyl groups in their structures, did not show activity in DPPH assay. Currently, pathogenetic and biochemical studies have consistently suggested oxidative stress have played an important role in AD pathogenesis<sup>27</sup>, and many studies have focused on searching therapeutic agents for AD through inhibiting reactive oxygen species production<sup>28</sup>. Thus, the results of the study could elucidate the bioactivity of *A. tatarinowii* in some extent. Further investigations on *in vivo* animal experiments and structure-function relationship for developing more excellent agent for AD are performing.

## Methods

**General experimental procedures.** Melting points were measured using a Beijing Tech X-5 micro-melting point apparatus and were uncorrected. UV, FT-IR, and ECD spectra were obtained from a Varian Cary 50, a Bruker Vertex 70, and a JASCO-810 spectrometer, respectively. Optical rotations were measured in MeOH/CH<sub>2</sub>Cl<sub>2</sub> on a Perkin-Elmer 341 polarimeter equipped with a 1 dm 75 microcell and a sodium lamp (589 nm). 1D and 2D NMR spectra were acquired on a Bruker AM-400 spectrometer and the <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts were referenced to the solvent or solvent impurity peaks for methanol-*d*<sub>4</sub> ( $\delta_{\text{H}}$  3.31 and  $\delta_{\text{C}}$  49.0) and CDCl<sub>3</sub> ( $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.0). High-resolution electrospray ionization mass spectra (HRESIMS) were acquired in the positive ion mode with a Thermo Fisher LC-LTQ-Orbitrap XL spectrometer. Column chromatography was performed using silica gel (100–200 and 200–300 mesh; Qingdao Marine Chemical Inc., China), octadecylsilyl (ODS, 50  $\mu\text{m}$ , YMC Co. Ltd., Japan), and Sephadex LH-20 (40–70  $\mu\text{m}$ , Amersham Pharmacia Biotech AB, Uppsala, Sweden, Sweden). The crystallographic data were acquired on a Bruker SMART APEX-II CCD diffractometer equipped with graphite-monochromatised Cu K $\alpha$  radiation ( $\lambda = 1.54178 \text{ \AA}$ ). Semi-preparative HPLC was carried out on an Agilent 1200 quaternary system with a UV detector or on a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 autosampler injector, and an Ultimate 3000 diode array detector (DAD) using a reversed-phased C<sub>18</sub> column (5  $\mu\text{m}$ , 10  $\times$  250 mm) at a flow rate of 2.5 mL/min. Thin-layer chromatography (TLC) was performed with silica gel 60 F<sub>254</sub> (Yantai Chemical Industry Research Institute) and RP-C<sub>18</sub> F<sub>254</sub> plates (Merck, Germany).

**Plant material.** The rhizomes of *A. tatarinowii* were collected at Jiujiang, Jiangxi Province, P. R. China in September 2013, and were identified by one of the authors, Prof. J. P. Wang. A voucher specimen (No. 2013–0916A) was deposited with the Herbarium of Materia Medica, Faculty of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, China.

**Extraction and isolation.** The air-dried rhizomes of *A. tatarinowii* (40 kg) were extracted with 95% EtOH three times at room temperature, and the extract was partitioned with petroleum ether, EtOAc, and *n*-BuOH

against water. The EtOAc fraction (400.0 g) was separated by chromatography to obtain six fractions (Fr.1–6) using silica gel CC eluting with a gradient of petroleum ether–acetone (20:1–1:1). Fr. 2 was further purified by column chromatography (silica gel CC, 10 × 100 cm), eluting with a gradient of petroleum ether–EtOAc (15:1–1:1), to yield five fractions (Fr.2.1–2.5). Fr. 2.3 were subjected to an ODS column (MeOH–H<sub>2</sub>O, 30:70–70:30) and a Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 1:1) column to yield three mixtures (A–C). Mixture A was then purified by semi-preparative HPLC (CH<sub>3</sub>CN–H<sub>2</sub>O, 45:55) to obtain compound **1** (*t*<sub>R</sub> 56.0 min, 10.0 mg). Compounds **9** (19.5 mg) and **10** (12.6 mg) were got from mixture B using an RP C<sub>18</sub> column eluting with MeOH–H<sub>2</sub>O (65:35), and compound **11** (5 mg) was obtained from Mixture C. Fr.2.4 was separated by repeated silica gel CC eluted with a petroleum ether–EtOAc gradient and Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 1:1) to yield two mixtures (D and F). Compounds **2** (*t*<sub>R</sub> 60.0 min, 8.2 mg, CH<sub>3</sub>CN–H<sub>2</sub>O, 50:50) and **3** (*t*<sub>R</sub> 90.0 min, 6.0 mg, CH<sub>3</sub>CN–H<sub>2</sub>O, 45:55) were purified from mixtures D and F by semi-preparative HPLC, respectively. Compound **1** (**1a/1b**) was further purified by chiral HPLC eluting with EtOH to give **1a** (*t*<sub>R</sub> 7.0 min, 2.0 mg) and **1b** (*t*<sub>R</sub> 11.5 min, 2.0 mg). Compounds **2a** (*t*<sub>R</sub> 10.0 min, 2.8 mg), **2b** (*t*<sub>R</sub> 17.0 min, 2.8 mg), **3a** (*t*<sub>R</sub> 20.0 min, 1.5 mg), and **3b** (*t*<sub>R</sub> 30.0 min, 4.0 mg) were successfully separated using the same IC column as **1** eluting with EtOH–*n*-hexane 15:85 and 25:75, respectively. Fr. 4 was subjected to a silica gel again eluting with petroleum ether–EtOAc (10:1–3:1) to yield four subfractions, named Fr.4.1–4.4. Fr.4.2 and Fr.4.3 were further partitioned with ODS column (MeOH–H<sub>2</sub>O, 30:70–70:30) and Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 1:1) to obtain four subfractions, named Fr.4.2.1–4.2.2 and Fr.4.3.1–4.3.2, respectively. Compounds **7** (*t*<sub>R</sub> 49.0 min, 4.0 mg) and a mixture of **4** and **5** (*t*<sub>R</sub> 44.0 min, 27.0 mg) were separated by semi-preparative HPLC (MeOH–H<sub>2</sub>O, 55:45) equipped with an RP-C<sub>18</sub> column from Fr.4.2.1, and compounds **4** and **5** were finally purified by semi-preparative HPLC eluting with CH<sub>3</sub>CN–H<sub>2</sub>O (45:55). Compound **8** (*t*<sub>R</sub> 63.5 min, 6.0 mg) was obtained from Fr.4.2.2 by semi-preparative HPLC (MeOH–H<sub>2</sub>O, 50:50). Fr.4.3.1 was subjected to an RP-C<sub>18</sub> column to obtain compound **6** (*t*<sub>R</sub> 87.4 min, 4.0 mg) and a mixture of **12–14** (*t*<sub>R</sub> 74.0 min, 15.0 mg) eluting with CH<sub>3</sub>CN–H<sub>2</sub>O (40:60). Compounds **12–14** were purified by repeated semi-preparative HPLC equipped with an RP C<sub>18</sub> column and a SiO<sub>2</sub> column, respectively.

(±)-*Acortatarinowin G* (**1**). Colorless block crystals (MeOH), mp 186–187 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0 (*c* 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (4.91), 230 (4.41), 291 (4.13) nm; IR (KBr)  $\nu_{\max}$  1612, 1511, 1465, 1399, 1317, 1206, 1130, 1086, 1034, 862, 781 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data, see Table 2; (+)-HREIMS *m/z* 471.1977 [M + Na]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>32</sub>NaO<sub>8</sub>, 471.1995).

(+)-*Acortatarinowin G* (**1a**). White amporous powder; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +28 (*c* 0.1, MeOH); ECD (MeOH) 210 ( $\Delta\epsilon$  +13.03), 230 ( $\Delta\epsilon$  +3.33), 260 ( $\Delta\epsilon$  +0.52).

(-)-*Acortatarinowin G* (**1b**). White amporous powder; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -28 (*c* 0.1, MeOH); ECD (MeOH) 210 ( $\Delta\epsilon$  -13.51), 230 ( $\Delta\epsilon$  -3.53), 260 ( $\Delta\epsilon$  -0.54).

(±)-*Acortatarinowin H* (**2**). Brown-red amorphous powder, [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0 (*c* = 0.2, CH<sub>2</sub>Cl<sub>2</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) = 208 (4.21), 243 (3.83), 290 (3.43), and 315 (3.42) nm; IR  $\nu_{\max}$  = 1650, 1573, 1509, 1456, 1299, 1243, 1218, 1036, 843, 810 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data, see Table 2; HRESIMS *m/z* 407.1493 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>24</sub>NaO<sub>6</sub>, 407.1471).

(+)-*Acortatarinowin H* (**2a**). Brown-red amorphous powder; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +14 (*c* 0.1, CH<sub>2</sub>Cl<sub>2</sub>); ECD (MeOH) 213 ( $\Delta\epsilon$  +26.05), 243 ( $\Delta\epsilon$  -12.82), 315 ( $\Delta\epsilon$  -9.33).

(-)-*Acortatarinowin H* (**2b**). Brown-red amorphous powder; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -14 (*c* 0.1, CH<sub>2</sub>Cl<sub>2</sub>); ECD (MeOH) 213 ( $\Delta\epsilon$  -27.01), 243 ( $\Delta\epsilon$  +13.02), 315 ( $\Delta\epsilon$  +10.31).

(±)-*Acortatarinowin I* (**3**). Light yellow gum, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +13 (*c* = 0.4, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) = 207 (4.72), 232 (4.51), and 278 (4.04) nm; IR  $\nu_{\max}$  = 927, 1590, 1509, 1454, 1417, 1381, 1328, 1233, 1125, 1027, 812, 766, and 713 cm<sup>-1</sup>; <sup>1</sup>H NMR (methanol-*d*<sub>4</sub>, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (methanol-*d*<sub>4</sub>, 100 MHz) data, see Table 2; HRESIMS *m/z* 425.1958 [M + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>30</sub>NaO<sub>6</sub>, 425.1940).

(-)-*Acortatarinowin I* (**3a**). Light yellow gum; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -32 (*c* 0.1, MeOH); ECD (MeOH) 210 ( $\Delta\epsilon$  -9.71), 233 ( $\Delta\epsilon$  -3.10);

(+)-*Acortatarinowin I* (**3b**). light yellow gum; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +32 (*c* 0.3, MeOH); ECD (MeOH) 210 ( $\Delta\epsilon$  +10.32), 230 ( $\Delta\epsilon$  +2.55).

*Acortatarinowin J* (**4**). Colorless block crystals, mp 158–159 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -1.4 (*c* = 0.4, CH<sub>2</sub>Cl<sub>2</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) = 207 (5.19), 231 (4.60), 278 (4.11), and 303 (3.95) nm; ECD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 210 (-3.51), 237 (+1.32), 275 (-0.80), 305 (-0.92) nm; IR  $\nu_{\max}$  = 2934, 2030, 1674, 1594, 1514, 1486, 1419, 1266, 1127, 1023, 829, and 766 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data, see Table 2; HRESIMS *m/z* 439.1718 [M + Na]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>28</sub>NaO<sub>7</sub>, 439.1733).

*Acortatarinowin K* (**5**). White amorphous powder, [ $\alpha$ ]<sub>D</sub><sup>20</sup> -1.2 (*c* = 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) = 205 (4.45), 231 (4.24), 278 (3.93), and 308 (3.77) nm; ECD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 210 (-1.05), 233 (+0.71), 266 (-0.20), 303 (-0.48) nm; IR  $\nu_{\max}$  = 2934, 2031, 1673, 1633, 1518, 1446, 1419, 1299, 1166, 1022, 813, and 765 cm<sup>-1</sup>; <sup>1</sup>H



NMR (methanol- $d_4$ , 400 MHz) data, see Table 1;  $^{13}\text{C}$  NMR (methanol- $d_4$ , 100 MHz) data, see Table 2; HRESIMS  $m/z$  387.1797  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{22}\text{H}_{27}\text{O}_6$ , 387.1808).

**Acortatarinowin L (6).** Light yellow gum,  $[\alpha]_{\text{D}}^{20} + 18$  ( $c = 0.1$ , MeOH); UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 205 (4.81), 230 (4.15), and 280 (3.53) nm; ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 210 (+3.92), 228 (+0.81), 275 (+0.44) nm; IR  $\nu_{\text{max}} = 3428$ , 2934, 1590, 1513, 1461, 1418, 1370, 1327, 1271, 1234, 1125, 1032, 1005, 799, and  $707\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 400 MHz) data, see Table 1;  $^{13}\text{C}$  NMR (methanol- $d_4$ , 100 MHz) data, see Table 2; HRESIMS  $m/z$  411.1774  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{22}\text{H}_{28}\text{NaO}_6$ , 411.1784).

**Acortatarinowin M (7).** Light yellow gum,  $[\alpha]_{\text{D}}^{20} + 12$  ( $c = 0.1$ , MeOH); UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 208 (4.73) and 272 (4.01) nm; ECD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 210 (+0.02), 221 (−1.41), 241 (−0.93), and 296 (−0.41) nm; IR  $\nu_{\text{max}} = 3479$ , 2937, 1717, 1590, 1500, 1461, 1416, 1335, 1228, 1126, 1033, 1000, 831, and  $763\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 400 MHz) data, see Table 1;  $^{13}\text{C}$  NMR (methanol- $d_4$ , 100 MHz) data, see Table 2; HRESIMS  $m/z$  459.1619  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{22}\text{H}_{28}\text{NaO}_9$ , 459.1631).

**Acortatarinowin N (8).** Light yellow gum,  $[\alpha]_{\text{D}}^{20} - 23$  ( $c = 0.1$ , MeOH); UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 207 (4.77), 263 (4.22), and 293 (3.93) nm; ECD (MeOH) 215 ( $\Delta\epsilon - 0.70$ ), 224 ( $\Delta\epsilon - 0.78$ ), 238 ( $\Delta\epsilon + 2.13$ ), 296 (−1.68) nm; IR (KBr)  $\nu_{\text{max}} 3456$ , 2939, 2714, 1714, 1593, 1508, 1463, 1416, 1328, 1270, 1219, 1126, 1057, 1004,  $762\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 400 MHz) data, see Table 1;  $^{13}\text{C}$  NMR (methanol- $d_4$ , 100 MHz) data, see Table 2; (+)-HRESIMS  $m/z$  429.1464  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{21}\text{H}_{26}\text{NaO}_8$ , 429.1525).

**(S)-MTPA Derivative of 7.**  $^1\text{H}$  NMR (methanol- $d_4$ , 400 MHz)  $\delta_{\text{H}}$  7.50–7.15 (5H, overlap, aromatic protons), 6.59 (2H, s, H-2,6), 6.10 (1H, d,  $J = 7.2$  Hz, H-7), 4.85 (m, H-8), 1.016 (1H, d,  $J = 6.4$  Hz, H-9), 7.35 (2H, s, H-2',6'), HRESIMS  $m/z$  675.2020  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{32}\text{H}_{35}\text{F}_3\text{NaO}_{11}$ , 675.2029).

**(R)-MTPA Derivative of 7.**  $^1\text{H}$  NMR (methanol- $d_4$ , 400 MHz)  $\delta_{\text{H}}$  7.50–7.15 (5H, overlap, aromatic protons), 6.81 (2H, s, H-2,6), 6.21 (1H, d,  $J = 7.2$  Hz, H-7), 4.80 (m, H-8), 1.014 (1H, d,  $J = 6.4$  Hz, H-9), 7.31 (2H, s, H-2',6'), HRESIMS  $m/z$  675.2019  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{32}\text{H}_{35}\text{F}_3\text{NaO}_{11}$ , 675.2029).

#### Single Crystal X-ray Diffraction Analyses and Crystallographic Data of Compounds ( $\pm$ )-1 and 4.

The crystallographic data of ( $\pm$ )-1 (CCDC 1434906) and 4 (CCDC 1028833) have been deposited in the Cambridge Crystallographic Data Centre.

**Crystallographic Data of Compound ( $\pm$ )-1.**  $\text{C}_{24}\text{H}_{32}\text{O}_8$ ,  $M = 448.5$ , monoclinic,  $a = 14.8444(2)$  Å,  $b = 10.0477(2)$  Å,  $c = 8.05590(10)$  Å,  $\alpha = 90.00^\circ$ ,  $\beta = 91.0230(10)^\circ$ ,  $\gamma = 90.00^\circ$ ,  $V = 1201.36(3)$  Å<sup>3</sup>,  $T = 296(2)$  K, space group  $P-2_1c$ ,  $Z = 2$ ,  $\mu(\text{CuK}\alpha) = 1.54178\text{ mm}^{-1}$ , 9895 reflections measured. The final  $R_1$  values were 0.0226 ( $I > 2\sigma(I)$ ). The final  $wR(F^2)$  values were 0.0911 ( $I > 2\sigma(I)$ ). The final  $R_1$  values were 0.0338 (all data). The final  $wR(F^2)$  values were 0.0906 (all data). The goodness of fit on  $F^2$  was 1.048. Flack parameter = 0.19 (12).

**Crystallographic Data of Compound 4.**  $\text{C}_{23}\text{H}_{28}\text{O}_7$ ,  $M = 416.45$ , monoclinic,  $a = 9.8071(4)$  Å,  $b = 7.9422(3)$  Å,  $c = 13.6711(5)$  Å,  $\alpha = 90.00^\circ$ ,  $\beta = 99.0390(10)^\circ$ ,  $\gamma = 90.00^\circ$ ,  $V = 1051.62(7)$  Å<sup>3</sup>,  $T = 100(2)$  K, space group  $P2_1$ ,  $Z = 2$ ,  $\mu(\text{CuK}\alpha) = 0.801\text{ mm}^{-1}$ , 8314 reflections measured, 3314 independent reflections ( $R_{\text{int}} = 0.0370$ ). The final  $R_1$  values were 0.0338 ( $I > 2\sigma(I)$ ). The final  $wR(F^2)$  values were 0.0983 ( $I > 2\sigma(I)$ ). The final  $R_1$  values were 0.0339 (all data). The final  $wR(F^2)$  values were 0.0983 (all data). The goodness of fit on  $F^2$  was 1.094. Flack parameter = 0.17(15). The Hooft parameter is 0.09(5) for 1354 Bijvoet pairs.

**Preparation of the (R) and (S)-MTPA Ester of 7<sup>29</sup>.** Compound 7 (0.6 mg) was dissolved in 2.0 mL anhydrous  $\text{CH}_2\text{Cl}_2$ . Dimethylaminopyridine (30.0 mg), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (20  $\mu\text{L}$ ), and (R)-MTPA chloride (25.0  $\mu\text{L}$ ) were added in sequentially. The reaction mixture was stirred for 3 h at room temperature under  $\text{N}_2$ . The solution was evaporated under reduced pressure. The residue was passed to a small silica gel CC eluting with petroleum ether–acetone (5:1) to afford the (S)-MTPA ester of 7. The (R)-MTPA ester of 7 was prepared with (S)-MTPA chloride in the same manner.

**DPPH Radical Scavenging Activity.** The free radical scavenging activity of each isolated new neolignans and lignans was tested using a DPPH assay previously described<sup>30</sup>. Briefly, the tested compounds (6.25, 12.5, 25, 50, 75, and 100  $\mu\text{g}/\text{mL}$ ) were reacted with DPPH (100  $\mu\text{g}/\text{mL}$ ) in EtOH (with 5% DMSO). The reaction was placed in the wells of 96-well plates at room temperature in the dark. After 30 min of incubation, the optical density of the reaction mixture at 515 nm was read using a microplate reader. All the tests were performed three times, and the results were averaged. Vitamin E was used as the positive control. An EtOH solution (with 5% DMSO) was used as a control.

#### References

- Mau, J.-L., Chao, G.-R. & Wu, K.-T. Antioxidant Properties of Methanolic Extracts from Several Ear Mushrooms. *J Agr Food Chem* **49**, 5461–5467 (2001).
- Saleh, M. A., Clark, S., Woodard, B. & Deolu-Sobogun, S. A. Antioxidant and free radical scavenging activities of essential oils. *Ethn Dis* **20**, S1–78–82 (2010).
- Hazra, B., Biswas, S. & Mandal, N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complem Altern M* **8**, 63 (2008).
- Liu, P., Kong, M., Yuan, S., Liu, J. & Wang, P. History and Experience: A Survey of Traditional Chinese Medicine Treatment for Alzheimer's Disease. *Evid-Based Compl Alt* **2014**, 5 (2014)
- Tang, C., Ye, Y., Feng, Y. & Quinn, R. J. TCM, brain function and drug space. *Nat. Prod. Rep.* **33**, 6–25 (2016)

6. Prince, M., Prina, M. & Guerchet, M. *World Alzheimer Report*. Alzheimer's Disease International: London, UK; pp 1–92 (2013).
7. Lu, Y. *et al.* (±)-Acortatarinowins A–F, Norlignan, Neolignan, and Lignan Enantiomers from *Acorus tatarinowii*. *J Nat Prod* **78**, 2205–2214 (2015).
8. Tong, X.-G. *et al.* Compounds from *Acorus tatarinowii*: Determination of Absolute Configuration by Quantum Computations and cAMP Regulation Activity. *J. Nat. Prod.* **73**, 1160–1163 (2010).
9. Prasad, A. K. *et al.* Neolignans and a lignan from *Piper clarkii*. *Phytochemistry* **39**, 655–658 (1995).
10. Li, X.-N. *et al.* Lignans with Anti-HIV Activity from *Schisandra propinqua* var. *sinensis*. *J. Nat. Prod.* **72**, 1133–1141 (2009).
11. Nishiwaki, H., Nakayama, K., Shuto, Y. & Yamauchi, S. Synthesis of All Stereoisomers of 3,3'-Dimethoxy-7,7'-epoxylignane-4,4'-diol and Their Plant Growth Inhibitory Activity. *J. Ag. Food Chem.* **62**, 651–659 (2014).
12. Shimomura, H., Sashida, Y. & Oohara, M. Lignans from *Machilus thunbergii*. *Phytochemistry* **27**, 634–636 (1988).
13. Sy, L.-K. & Brown, G. D. Novel Phenylpropanoids and Lignans from *Illicium verum*. *J. Nat. Prod.* **61**, 987–992 (1998).
14. Zhu, H. *et al.* A pair of unprecedented cyclohexylethanoid enantiomers containing unusual trioxabicyclo[4.2.1]nonane ring from *Clerodendrum bungei*. *Tetrahedron Lett.* **55**, 2277–2279 (2014).
15. Harada, N. & Nakanishi, K. A Method for Determining the Chirality of Two Aromatic Chromophores and the Absolute Configurations of Chromomycin A3 and Related Antibiotics. *J. Am. Chem. Soc.* **91**, 5896 (1969).
16. Huo, C., Liang, H., Zhao, Y., Wang, B. & Zhang, Q. Neolignan glycosides from *Symplocos caudata*. *Phytochemistry* **69**, 788–795 (2008).
17. Li, Y. *et al.* Bioactive Neolignans and Lignans from the Bark of *Machilus robusta*. *J. Nat. Prod.* **74**, 1444–1452 (2011).
18. Lee, S. Y., Moon, E., Kim, S. Y., Choi, S. U. & Lee, K. R. Quinone Derivatives from the Rhizomes of *Acorus gramineus* and Their Biological Activities. *Biosci., Biotech., and Bioch.* **77**, 276–280 (2013).
19. da Silva, T. & Lopes, L. M. X. Aryltetralone lignans and 7,8-seco-lignans from *Holostylis reniformis*. *Phytochemistry* **65**, 751–759 (2004).
20. da Silva, T. & Lopes, L. M. X. Aryltetralol and aryltetralone lignans from *Holostylis reniformis*. *Phytochemistry* **67**, 929–937 (2006).
21. Lopes, N. P., de Almeida Blumenthal, E. E., Cavalheiro, A. J., Kato, M. J. & Yoshida, M. Lignans,  $\gamma$ -lactones and propiophenones of *Virola surinamensis*. *Phytochemistry* **43**, 1089–1092 (1996).
22. Sawasdee, K. *et al.* New neolignans and a lignan from *Milusa fragrans*, and their anti-herpetic and cytotoxic activities. *Tetrahedron Lett.* **54**, 4259–4263 (2013).
23. Jung, K. Y. *et al.* Magnone A and B, Novel Anti-PAF Tetrahydrofuran Lignans from the Flower Buds of *Magnolia fargesii*. *J. Nat. Prod.* **61**, 808–811 (1998).
24. Harada, N. & Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books, Mill Valley, CA, and Oxford University Press: Oxford (1983).
25. León, A., Cogordán, J. A., Sterner, O. & Delgado, G. Enantiomeric Derivatives of Tokinolide B: Absolute Configuration and Biological Properties. *J. Nat. Prod.* **75**, 859–864 (2012).
26. Solyomváry, A. *et al.* Identification and isolation of new neolignan and sesqueneolignan species: Their acid-catalyzed ring closure and specific accumulation in the fruit wall of *Cirsium eriophorum* (L.) Scop. *Process Biochem.* **50**, 853–858 (2015).
27. Pratico, D. & Sung, S. Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease. *J. Alzheimers Dis.* **6**, 171–175 (2004).
28. Pontikia, E., Kontogiorgis, C., Xu, Y., Hadjipavlou-Litina, D. & Luo, Y. New Lipoygenase Inhibitors of Reactive Oxygen Species Production in Cellular Models of Amyloid (A2) Toxicities. *J. Alzheimers Dis.* **34**, 215–230 (2013).
29. Ohtani, I., Kusumi, T., Kashman, Y. & Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. *J. Am. Chem. Soc.* **113**, 4092–4096 (1991).
30. Krishnaiah, D., Sarbatly, R. & Nithyanandam, R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Process* **89**, 217–233 (2011).

## Acknowledgements

The authors would like to thank the Analytical and Testing Center at Huazhong University of Science and Technology for assistance in conducting ECD and IR analyses. This work was financially supported by the Program for New Century Excellent Talents in University, State Education Ministry of China (NCET-2008-0224), the National Natural Science Foundation of China (Nos. 31370372, 81573316, 31570361, and 31200258).

## Author Contributions

G.D. and Y.Z. designed the experiments and commented the manuscript. Y.L. conducted the main experiments with the help of C.Q. analyzed the data, and wrote the manuscript; X.L. conducted the single crystal X-ray diffraction analyses; J.L. did the ECD calculations; S.C. performed the Mosher's experiment; Y.X. and J.W. authenticated the plant material; H.Z., J.Z. and Y.X. polished this manuscript; All authors reviewed the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at <http://www.nature.com/srep>

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Lu, Y. *et al.* Antioxidant Lignans and Neolignans from *Acorus tatarinowii*. *Sci. Rep.* **6**, 22909; doi: 10.1038/srep22909 (2016).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>