JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Natural β-Dihydroagarofuran-Type Sesquiterpenoids as Cognition-Enhancing and Neuroprotective Agents from Medicinal Plants of the Genus *Celastrus*

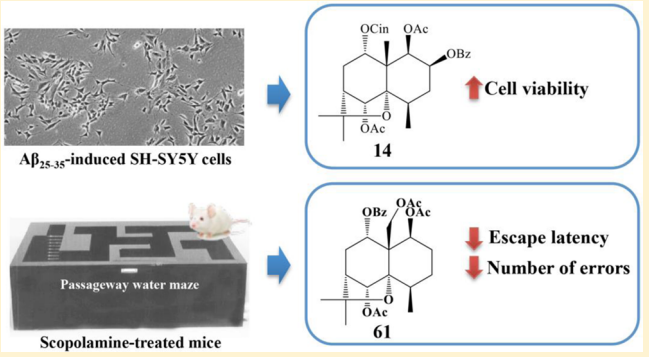
Ruonan Ning,[†,§] Yun Lei,[‡,§] Shuangzhu Liu,[†] Huan Wang,[‡] Rujun Zhang,[†] Wei Wang,[‡] Yingdong Zhu,[†] Haiyan Zhang,[*,‡] and Weimin Zhao[*,†]

[†]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

[‡]CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

Supporting Information

ABSTRACT: Alzheimer's disease (AD) is an irreversible, multifaceted, and progressive neurodegenerative disorder. Over the past 30 years, the search for anti-AD drugs has been primarily based on the cholinergic deficiency hypothesis and/or the β-amyloid (Aβ) cascade hypothesis. In this study, we report the identification of 16 new and 38 known β-dihydroagarofuran-type sesquiterpenoids from *Celastrus flagellaris* and *Celastrus angulatus*. The β-dihydroagarofuran-type sesquiterpenoids **58**, **59**, **61**, and **63** significantly attenuated scopolamine-induced prolonged escape latency and increased number of errors compared with the control group. At 10 μM, 21 of the 62 tested β-dihydroagarofuran-type sesquiterpenoids rescued $A\beta_{25-35}$-induced SH-SY5Y cells from viability reduction, which increased the cell viability from 64.6% for the model to more than 74.0%. The majority of the β-dihydroagarofuran-type sesquiterpenoids with ester groups exhibited stronger activity than those with free hydroxy groups or without substituents at the same positions. These results identified a new chemical skeleton as drug lead for the investigation of novel therapeutic agents against AD.



Alzheimer's disease (AD), characterized by the loss of cognitive functions, is the most common cause of dementia, which seriously affects the life quality of patients.[1] The cholinergic pathway plays an important role in the regulation of cognition, learning, and memory processes.[2] A large body of evidence emphasizes the abnormal accumulation of β-amyloid (Aβ) in the pathology of AD.[3] Therefore, the research on AD drugs over the past 30 years has been primarily based on the cholinergic deficiency and Aβ cascade hypotheses. However, the currently approved AD drugs do not prevent the progression of the disease and show only marginal benefits in moderately to severely affected patients,[4] which emphasizes the necessity of discovering new effective therapeutic agents.[2b,5]

Natural products have been shown to be reliable resources in drug discovery and development processes. In the 2012 Newman and Cragg's review, of the 1355 new drugs approved from 1980 to 2010, 26.8% are either natural products or therapeutic agents directly derived from natural products.[6] In the area of dementia research, natural products have played an important role in providing potentially valuable compounds for drug discovery, such as curcumin from *Curcuma longa*,[7] galanthamine from *Leucojum aestivum*,[8] and betulinic acid from *Bacopa monnieria*.[9]

The family Celastraceae is widespread in the tropical and subtropical regions of the world, including North Africa, South America, and East Asia, and is composed of approximately 88 genera, including trees, shrubs, and liana plants.[10] β-Dihydroagarofuran-type sesquiterpenoids are characteristic natural products of the family Celastraceae and are regarded as privileged structures due to their chemical diversity and attractive biological activities, including their ability to exert multidrug-resistant,[11] antitumor-promoting,[12] antiplasmodial,[13] antitubercular,[14] anti-inflammatory,[15] anti-HIV,[16] cytotoxic,[17] immunosuppressant,[18] and insecticidal effects.[19] The fruits of *Celastrus orbiculatus* Thunb. var. *cancatus* (Rehd. et Wils.) have been used in China for their activity against amnesia.[20] The seeds of *Celastrus paniculatus* Willd. have been used in China to refresh mental activity and have a long history of use in India to alleviate cognitive disturbances, and are revered as "Elixir of Life" in the latter country.[20] The methanol extract of the seeds

Received: March 17, 2015

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

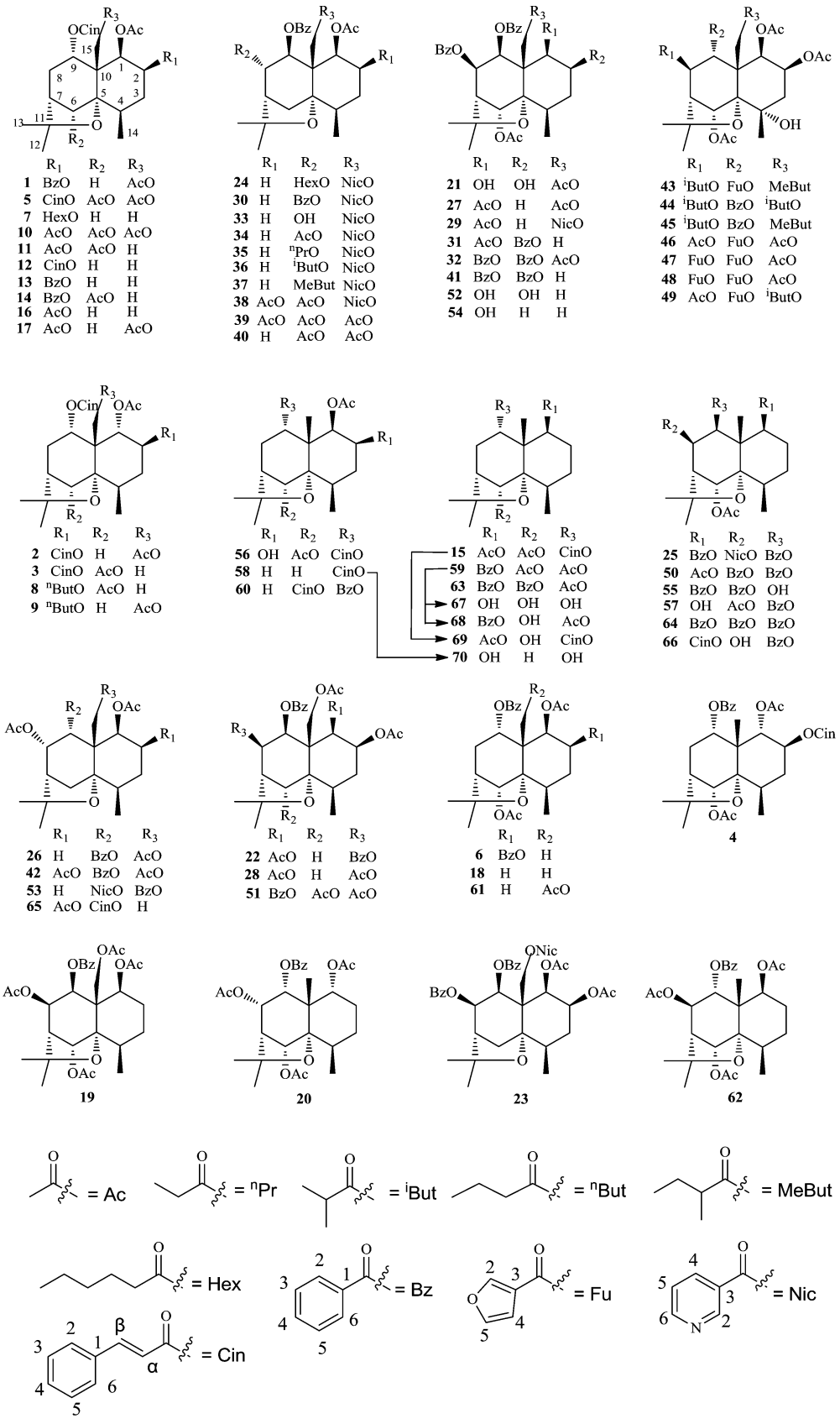


Figure 1. *β*-Dihydroagarofuran-type sesquiterpenoids isolated from *C. flagellaris*, *C. angulatus*, and *C. orbiculatus*.

of *C. paniculatus* was found to attenuate H_2O_2-induced injury in embryonic rat forebrain neuronal cells.[21] We recently reported the identification of dimeric trinorditerpenoids with neuroprotective activity from the root bark of *C. orbiculatus*.[22] Herein, we report the identification of new *β*-dihydroagarofuran-type sesquiterpenoids from *Celastrus* plants and their neuro-

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

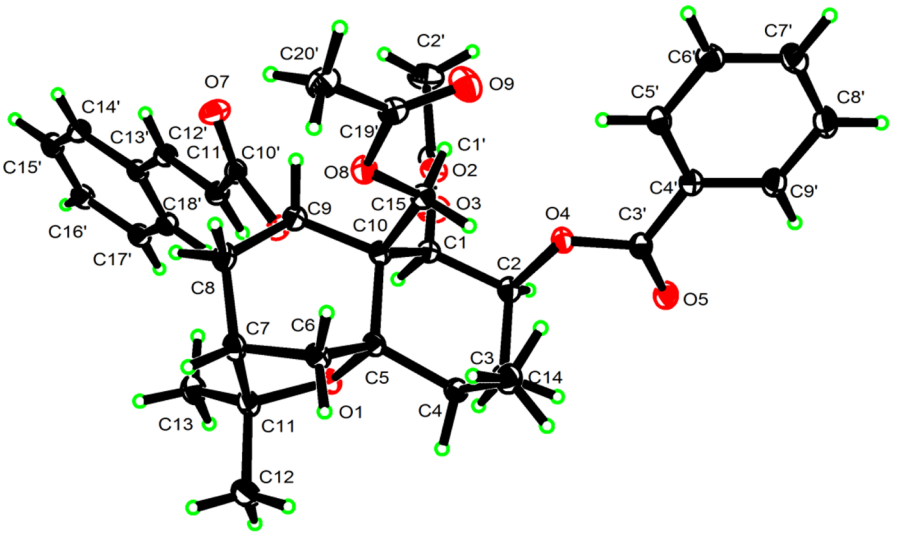


Figure 2. ORTEP drawing of compound **1**.

protective effects against Aβ_{25-35} toxicity, and their nootropic effects against scopolamine-induced memory impairment in mice, as measured using a water maze.

RESULTS AND DISCUSSION

Ten new (**1**–**10**) and 10 known (**11**–**20**) β-dihydroagarofuran-type sesquiterpenoids were isolated from the seeds of *Celastrus flagellaris* Rupr. Six new (**21**–**26**) and 28 known analogues (**27**–**54**) were obtained from the seeds and root bark of *Celastrus angulatus* Maxim. by using various chromatographic methods (Figure 1).

Compound **1** showed 35 signals in its ^{13}C NMR spectrum and an accurate $[M + Na]^+$ ion at *m*/*z* 627.2579 in the HRTOFMS, corresponding to the molecular formula $C_{35}H_{40}O_9Na$ (*m*/*z* 627.2570) and indicative of 16 indices of hydrogen deficiency. Its ^{1}H NMR spectrum indicated signals for 12 protons in the aromatic region for the benzoyl and cinnamoyl groups at δ_H 8.13 (d, 2H, *J* = 7.0 Hz), 7.69 (d, 1H, *J* = 16.0 Hz), 7.57 (m, 3H), 7.50 (t, 2H, *J* = 7.5 Hz), 7.39 (m, 3H), and 6.39 (d, 1H, *J* = 16.0 Hz), two acetyl groups at δ_H 1.77 and 2.17 (s, each 3H), three acylated oxymethine protons at δ_H 5.80 (m, 2H) and 5.23 (d, 1H, *J* = 6.9 Hz), and a pair of acylated oxymethylene protons at δ_H 5.12 and 4.48 (d, each 1H, *J* = 12.1 Hz). The ^{13}C NMR spectrum exhibited 35 carbon signals, including a benzoyl, a cinnamoyl, and two acetyl moieties, as well as 15 sp^3 carbon signals, comprising an oxygenated primary carbon (C-15), three oxygenated secondary carbons (C-1, C-2, C-9), two oxygenated tertiary carbons (C-5, C-11), three primary carbons (C-12, C-13, C-14), three secondary carbons (C-3, C-6, C-8), two tertiary carbons (C-4, C-7), and a quaternary (C-10) carbon, hence representing three indices of hydrogen deficiency and reflecting the tricyclic molecular backbone of **1**. These data suggested that compound **1** is a tetrasubstituted β-dihydroagarofuran-type sesquiterpenoid.

The locations of two of the four ester groups were defined based on the HMBC correlations between H-9 (δ_H 5.23) and the CinO-9 carbonyl carbon (δ_C 166.3) and between H$_2$-15 (δ_H 5.12, 4.48) and the AcO-15 carbonyl carbon (δ_C 170.9). However, the locations of the remaining ester groups could not be deduced via NMR analysis due to the overlapping of the two acylated oxymethine protons at δ_H 5.80 (m, 2H) in the ^{1}H NMR spectrum. Single-crystal X-ray diffraction analysis with Cu Kα radiation was used to establish the structure and absolute configuration of **1** [Flack parameter: 0.14 (14)] (Figure 2). Thus, the structure of **1** was unequivocally defined as (1*R*,2*S*,4*R*,5*S*,7*R*,9*S*,10*S*)-1,15-diacetoxy-2-benzoyloxy-9-cinnamoyloxy-β-dihydroagarofuran.

Compound **2** had the molecular formula $C_{37}H_{42}O_9$, as determined by HRTOFMS (*m*/*z* 653.2739 $[M + Na]^+$, calcd 653.2727) and its ^{13}C NMR data. A β-dihydroagarofuran skeleton was established from the ^{1}H–^{1}H COSY cross signals for the H-1/H-2/H-3/H-4/H$_3$-14 and H-6/H-7/H-8/H-9 spin systems and the HMBC correlations between H$_2$-15 and C-1, C-9, between H-9, H$_3$-14 and C-5, and between H-4, H-6 and C-10. The ^{1}H and ^{13}C NMR data revealed **2** to be a tetrasubstituted β-dihydroagarofuran-type sesquiterpenoid with two cinnamoyloxy and two acetoxy groups. In the HMBC spectrum, cross peaks between H-1 (δ_H 5.75) and the AcO-1 carbonyl carbon (δ_C 170.0), between H-2 (δ_H 5.66) and the CinO-2 carbonyl carbon (δ_C 166.2), between H-9 (δ_H 5.24) and the CinO-9 carbonyl carbon (δ_C 166.2), and between H$_2$-15 (δ_H 5.03, 4.49) and the AcO-15 carbonyl carbon (δ_C 170.8) indicated the locations of the four substituents of **2** (Figure 3). The relative configuration of **2** was established by the coupling constants of the key proton signals and the NOESY data. Signals at δ_H 5.75 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1), δ_H 5.66 (1H, dd, $J_{1,2 \text{ and } 2,3}$ = 3.4, 7.5 Hz, H-2), and δ_H 5.24 (1H, d, $J_{8,9}$ = 6.8 Hz,

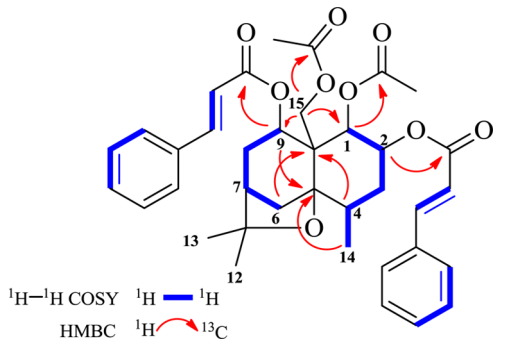


Figure 3. ^{1}H–^{1}H COSY (bold) and selected HMBC (arrows) correlations of compound **2**.

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 1. ^{1}H NMR Data of the Carbon Framework of Compounds 1−10 (δ ppm, J in Hz in Parentheses) in $CDCl_3$

no.	1	2	3	4	5	6	7[a]	8[a]	9	10
1	5.80 brs	5.75 d (3.4)	5.65 d (3.9)	5.65 d (3.2)	5.79 d (3.5)	5.70 d (3.3)	5.96 d (3.4)	5.73 d (3.6)	5.68 d (3.2)	5.73 d (3.6)
2	5.80 m	5.66 dd (7.5, 3.4)	5.70 dd (7.8, 3.9)	5.73 dd (7.5, 3.2)	5.68 dd (6.9, 3.5)	5.66 dd (7.5, 3.4)	6.00 dd (7.4, 3.4)	6.00 dd (7.5, 3.6)	5.56 dd (7.3, 3.2)	5.54 dd (7.5, 3.4)
3α	1.94 dd (15.2, 3.2)	1.88 dd (15.0, 3.5)	1.89 dd (15.0, 3.0)	1.90 dd (15.1, 3.3)	1.90 dd (15.2, 3.2)	1.97 dd (15.0, 3.5)	1.77 dd (15.0, 3.2)	1.78 dd (15.0, 3.5)	1.75 dd (15.0, 3.0)	1.78 dd (15.0, 3.0)
3β	2.56 ddd (15.2, 6.9, 3.2)	2.50 ddd (15.0, 7.5, 3.5)	2.44 m	2.45 m	2.49 ddd (15.2, 6.9, 3.2)	2.48 ddd (15.0, 7.5, 3.5)	2.49 ddd (15.0, 7.4, 3.2)	2.33 ddd (15.0, 7.5, 3.5)	2.46 ddd (15.0, 7.3, 3.0)	2.42 ddd (15.0, 7.5, 3.0)
4	1.98 t (6.9)	2.40 t (7.7)	2.37 t (7.5)	2.40 t (7.7)	2.38 t (7.0)	2.42 t (7.7)	1.90 m	2.45 m	1.92 t (6.9)	2.35 t (7.6)
6α	2.13 m	2.11 m					2.06 m		2.06 m	
6β	2.31 m	2.29 m	5.42 s	5.46 s	5.97 s	5.48 s	2.02 m	5.67 s	2.33 m	5.94 s
7	2.11 m	2.11 m	2.23 brs	2.26 t (7.0)	2.23 m	2.26 m	1.92 m	2.25 m	2.11 m	2.22 m
8α	2.28 m	2.27 m	2.23 m	2.20 m	2.50 m	2.53 m	2.21 m	2.50 m	2.24 m	2.46 m
8β	2.12 m	2.11 m	2.16 m	2.16 m	2.26 m	2.20 m	2.14 m	2.26 m	2.08 m	2.19 m
9	5.23 d (6.9)	5.24 d (6.8)	4.77 d (7.0)	4.99 d (6.9)	5.24 d (7.5)	4.99 d (6.9)	5.13 d (6.8)	5.11 d (6.9)	5.17 d (7.0)	5.18 d (7.1)
12	1.41 s	1.40 s	1.42 s	1.43 s	1.42 s	1.42 s	1.55 s	1.59 s	1.38 s	1.43 s
13	1.23 s	1.22 s	1.41 s	1.42 s	1.45 s	1.43 s	1.24 s	1.53 s	1.20 s	1.40 s
14	1.32 d (6.9)	1.30 d (7.7)	1.27 d (7.5)	1.27 d (7.7)	1.21 d (7.0)	1.39 d (7.7)	1.29 d (7.0)	1.28 d (7.0)	1.26 d (6.9)	1.18 d (7.6)
15	5.12 d (12.1)	5.03 d (12.6)	1.52 s	1.55 s	5.38 d (13.0)	1.60 s	1.46 s	1.55 s	4.84 d (12.7)	5.05 d (12.7)
	4.48 d (12.1)	4.49 d (12.6)			5.28 d (13.0)				4.40 d (12.7)	4.33 d (12.7)

[a]Recorded in pyridine-d_5.

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

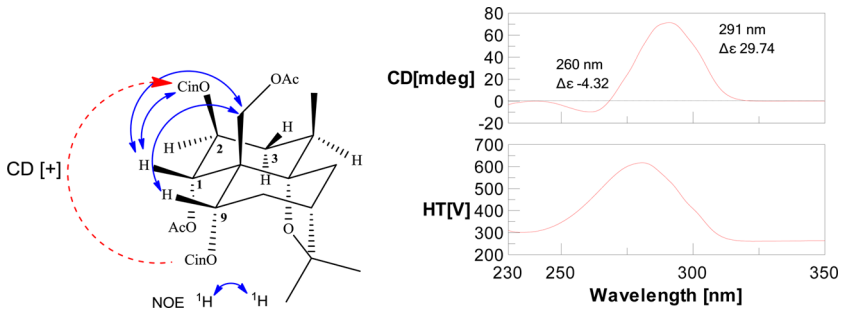


Figure 4. Key NOESY correlations, ECD exciton coupling, and ECD spectrum of compound **2**.

H-9) (Table 1) were compatible with equatorial orientations of H-1, H-2, and H-9, which are supported by the NOE associations between H-1/H-9 and H_3-15 and between H-1 and the CinO-2 olefinic protons. Its electronic circular dichroism (ECD) spectrum showed a Davidoff-type split curve with a positive first Cotton effect at 291 nm ($\Delta\varepsilon$ = 29.74) and a negative second Cotton effect at 260 nm ($\Delta\varepsilon$ = −4.32) (Figure 4),[23] due to the coupling of the two cinnamoyloxy chromophores at C-2β and C-9α. Therefore, the structure of **2** was characterized as (1*S*,2*S*,4*R*,5*S*,7*R*,9*S*,10*S*)-1,15-diacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran.

Compounds **3** and **4** were assigned the molecular formulas $C_{37}H_{42}O_9$ and $C_{35}H_{40}O_9$, respectively, according to their HRTOFMS (*m*/*z* 653.2742 $[M + Na]^+$, calcd 653.2727; *m*/*z* 627.2588 $[M + Na]^+$, calcd 627.2570) and ^{13}C NMR spectroscopic data. The NMR data showed that **3** and **4** are both tetrasubstituted β-dihydroagarofuran-type sesquiterpenoids. In the HMBC spectrum of **3**, cross peaks between H-1 (δ_H 5.65) and the AcO-1 carbonyl carbon (δ_C 170.2), between H-2 (δ_H 5.70) and the CinO-2 carbonyl carbon (δ_C 166.2), between H-6 (δ_H 5.42) and the AcO-6 carbonyl carbon (δ_C 170.3), and between H-9 (δ_H 4.77) and the CinO-9 carbonyl carbon (δ_C 166.3) suggested that an acetoxy group is located at C-6 in **3** instead of the acetoxy group at C-15 in **2**. The substitution of an acetoxy group at C-6 in **4** instead of the acetoxy group at C-15 in **2**, as well as a benzoyloxy group at C-9 in **4** instead of the cinnamoyloxy group in **2**, was verified by the HMBC correlations between H-6 (δ_H 5.46) and the AcO-6 carbonyl carbon (δ_C 170.3) and between H-9 (δ_H 4.99) and the BzO-9 carbonyl carbon (δ_C 165.7). Their relative configurations were deduced by analyses of key coupling constants ($J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 7.8 Hz, $J_{8,9}$ = 7.0 Hz in **3** and $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 7.5 Hz, $J_{8,9}$ = 6.9 Hz in **4**) and were confirmed by the NOESY experiment, in which NOE correlations were observed between H-1 and H-9/H_3-15; between H-6 and H_3-14 in **3**; and between H_3-15 and H-1/H-6/H-9 in **4**. The absolute configurations of **3** and **4** were established by analyses of their ECD spectra, which showed Davidoff-type split curves with a positive first Cotton effect at 292 nm ($\Delta\varepsilon$ = 38.52) and a negative second Cotton effect at 260 nm ($\Delta\varepsilon$ = −9.36) due to the coupling of the two cinnamoyloxy chromophores at C-2β and C-9α in **3** (Figure S29, Supporting Information), and with a positive first Cotton effect at 277 nm ($\Delta\varepsilon$ = 9.87) and a negative second Cotton effect at 238 nm ($\Delta\varepsilon$ = −1.65) due to the coupling of the cinnamoyloxy and benzoyloxy chromophores at C-2β and C-9α in **4** (Figure S39, Supporting Information).[23] Thus, the structures of **3** and **4** were assigned as (1*S*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,6-diacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran and (1*S*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,6-diacetoxy-2-cinnamoyloxy-9-benzoyloxy-β-dihydroagarofuran, respectively.

The structure of **5** was similar to that of **3**, except for an additional acetoxy group at C-15, whereas the structure of **6** was similar to that of **4**, except for replacement of a cinnamoyloxy by a benzoyloxy group at C-2. The relative configurations of **5** and **6** were deduced to be different from those of **3** and **4** at C-1 based on the NOE correlations between H-4 and H-1/H-2 in **5** and between H-1 and H-3α in **6**. Furthermore, the Davidoff-type split curve of **5** with a positive first Cotton effect at 292 nm ($\Delta\varepsilon$ = 27.36) and a negative second Cotton effect at 262 nm ($\Delta\varepsilon$ = −6.75) (Figure S49, Supporting Information), and the Davidoff-type split curve of **6** with a positive first Cotton effect at 235 nm ($\Delta\varepsilon$ = 22.88) and a negative second Cotton effect at 213 nm ($\Delta\varepsilon$ = 3.07) in the ECD spectrum[23] (Figure S59, Supporting Information) indicated their absolute configurations. Thus, the structures of **5** and **6** were defined as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,6,15-triacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran and (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,6-diacetoxy-2,9-dibenzoyloxy-β-dihydroagarofuran, respectively.

The ^1H and ^{13}C NMR data indicated that **7** is a trisubstituted β-dihydroagarofuran-type sesquiterpenoid with a cinnamoyloxy, an acetoxy, and a hexanoyloxy moiety, and **8** is a tetrasubstituted sesquiterpenoid with a cinnamoyloxy, a butanoyloxy, and two acetoxy groups. Analyses of the $^1H-^1H$ COSY, HSQC, and HMBC spectra of **7** and **8** enabled the establishment of their 2D structures. The relative configurations of **7** and **8** were determined from the coupling constants ($J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 7.4 Hz, $J_{8,9}$ = 6.8 Hz in **7**, and $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 7.5 Hz, $J_{8,9}$ = 6.9 Hz in **8**) of key adjacent protons and from the NOE correlations between H-1 and H-3α, between H-9 and H_3-15 in **7**, and between H_3-15 and H-1/H-6/H-9 in **8**. Thus, the structures of **7** and **8** were defined as 1β-acetoxy-2β-hexanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran and 1α,6α-diacetoxy-2β-butanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran, respectively.

Compound **9** possessed an identical molecular formula and similar 1D NMR data to those of **8**, except for the presence of a pair of acylated oxymethylene proton signals and the absence of an acylated oxymethine singlet proton signal. The NMR data of **10** were similar to those of **5**, except for the replacement of a cinnamoyloxy group by an acetoxy group at C-2 in **10**. Analyses of $^1H-^1H$ COSY, HSQC, and HMBC spectra enabled the deduction of the 2D structures of **9** and **10**. Their relative configurations were established using the methods described above and were defined as 1α,15-diacetoxy-2β-butanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran and 1β,2β,6α,15-tetraacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran, respectively.

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. ^{1}H NMR Data of the Carbon Framework of Compounds 21−26 (δ ppm, J in Hz in Parentheses) in $CDCl_3$

no.	21	22	23[a]	24	25	26
1	4.28 d (3.4)	5.57 d (3.2)	6.17 d (2.7)	5.44 dd (11.9, 4.3)	5.57 dd (10.4, 5.8)	5.50 dd (12.2, 4.2)
2α	4.10 brs	5.42 dd (6.4, 3.2)	5.82 dd (5.7, 2.7)	1.82 m	1.82 m	1.87 m
2β				1.58 m	1.79 m	1.48 m
3α	1.86 m	1.76 m	1.79 m	1.48 m	1.52 m	1.47 m
3β	2.25 m	2.47 m	2.49 m	2.27 m	2.24 m	2.28 m
4	2.29 m	1.94 m	2.00 m	1.88 m	2.31 m	1.92 m
6α		2.04 dd (13.0, 4.0)	2.17 dd (13.4, 3.2)	2.13 dd (12.8, 4.8)		2.20 dd (12.6, 4.8)
6β	6.49 s	2.92 d (13.0)	2.98 d (13.4)	2.65 d (12.8)	6.15 s	2.30 d (12.6)
7	2.66 d (4.1)	2.55 brt (3.6)	2.53 brt (3.7)	2.39 brt (3.9)	2.63 d (4.2)	2.26 m
8	5.81 dd (5.8, 4.1)	5.71 dd (5.6, 3.2)	6.13 dd (5.4, 3.4)	5.58 dd (9.8, 3.3)	5.82 dd (5.2, 4.2)	5.57 brs
9	5.91 d (5.8)	5.76 d (5.6)	6.20 d (5.4)	6.08 d (9.8)	5.78 d (5.2)	5.57 brs
12	1.61 s	1.59 s	1.50 s	1.59 s	1.65 s	1.52 s
13	1.45 s	1.26 s	1.24 s	1.21 s	1.47 s	1.22 s
14	1.25 d (7.6)	1.32 d (8.0)	1.41 d (8.0)	1.16 d (7.8)	1.21 d (7.4)	1.10 d (7.8)
15	5.24 d (12.5)	5.27 d (12.6)	5.97 d (12.0)	5.10 d (12.0)	1.66 s	4.56 d (12.1)
	4.89 d (12.5)	4.87 d (12.6)	5.47 d (12.0)	4.90 d (12.0)		4.42 d (12.1)

[a]Recorded in pyridine-d_5.

Analyses of the NMR and IR spectra of **21** revealed it to be a β-dihydroagarofuran-type sesquiterpenoid carrying two benzoyloxy, two acetoxy, and two secondary hydroxy groups. The HMBC correlations between H-8 (δ_H 5.81) and the BzO-8 carbonyl carbon (δ_C 166.3), between H-9 (δ_H 5.91) and the BzO-9 carbonyl carbon (δ_C 166.0), between H-6 (δ_H 6.49) and the AcO-6 carbonyl carbon (δ_C 170.0), and between H_2-15 (δ_H 4.89, 5.24) and the AcO-15 carbonyl carbon (δ_C 170.3) established the locations of the four substituents. The remaining two hydroxy groups were assigned to C-1 and C-2 according to the chemical shifts of H-1/C-1 at δ_H 4.28/δ_C 78.5, and H-2/C-2 at δ_H 4.10/δ_C 71.5, confirmed by the HSQC and HMBC data. Comparison of the NMR spectroscopic data of **22** with those of **21** indicated that the two hydroxy groups at C-1 and C-2 are acetylated, and the acetoxy group at C-6 in **21** is absent in **22**. Their relative configurations were established using the methods described above. In their ECD spectra, the observation of a negative first Cotton effect at 240 nm ($\Delta\varepsilon$ = −19.22) and a positive second Cotton effect at 222 nm ($\Delta\varepsilon$ = 15.82) in **21** (Figure S104, Supporting Information), as well as a negative first Cotton effect at 238 nm ($\Delta\varepsilon$ = −51.14) and a positive second Cotton effect at 219 nm ($\Delta\varepsilon$ = 4.17) in **22** (Figure S113, Supporting Information),[23] allowed the determination of their absolute configurations. Thus, the structures of **21** and **22** were defined as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,15-diacetoxy-8,9-dibenzoyloxy-1,2-dihydroxy-β-dihydroagarofuran and (1*R*,2*S*,4*R*,5*S*,7*R*,8*R*,9*S*,10*S*)-1,2,15-triacetoxy-8,9-dibenzoyloxy-β-dihydroagarofuran, respectively.

Compound **23** gave the molecular formula $C_{39}H_{41}NO_{11}$, as deduced from the HRESIMS (m/z 722.2588 $[M + Na]^+$, calcd 722.2577) and ^{13}C NMR data. The presence of a nicotinoyl function was revealed by the resonances at δ_H 9.14 (brs), 8.60 (brd, J = 4.8 Hz), 8.13 (brd, J = 8.0 Hz), and 7.07 (dd, J = 8.0, 4.8 Hz) in the ^{1}H NMR spectrum, and at δ_C 123.0 (d), 126.0 (s), 137.0 (d), 151.2 (d), 153.6 (d), 165.6 (s) in its ^{13}C NMR spectrum. The presence of two acetoxy and two benzoyloxy groups could also be deduced from the ^{1}H and ^{13}C NMR data (Tables 2 and 3). A three-proton multiplet centered at δ_H 5.75−5.80 in the ^{1}H NMR spectrum ($CDCl_3$) of **23** was resolved into two doublets at δ_H 6.17 (1H, J = 2.7 Hz) and 6.20 (1H, J = 5.4 Hz), and a doublet of doublets at δ_H 6.13 (1H, J = 5.4, 3.4 Hz) when the spectrum was recorded in pyridine-d_5. The locations of the five ester functions were established using ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. The relative configuration of **23** was determined as 1β,2β,8β,9β by analyses of the coupling constants ($J_{1,2}$ = 2.7 Hz, $J_{2,3}$ = 5.7 Hz, $J_{8,9}$ = 5.4 Hz) of key adjacent protons and NOE correlations between H-1 and H-9, and between H-2 and H-4. Thus, the structure of **23** was defined as 1β,2β-diacetoxy-8β,9β-dibenzoyloxy-15-nicotinoyloxy-β-dihydroagarofuran.

The 2D structures of **24** and **25** were established by analyses of their ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. They are also nicotinates of β-dihydroagarofuran-type sesquiterpenoids. The large coupling constant of H-8 with H-9 ($J_{8,9}$ = 9.8 Hz) in **24**, and the smaller coupling constant between H-8 and H-9 ($J_{8,9}$ = 5.2 Hz) in **25** revealed their *trans* and *cis* orientations, respectively. In their NOESY spectra, NOE cross peaks were observed between H-1 and H-4/H-9 in **24**, and between H-1 and H-6/H-9, between H-4 and H-6 in **25**. Thus, the structures of **24** and **25** were defined as 1β-acetoxy-9β-benzoyloxy-8α-hexanoyloxy-15-nicotinoyloxy-β-dihydroagarofuran and 6α-acetoxy-1β,9β-dibenzoyloxy-8β-nicotinoyloxy-β-dihydroagarofuran, respectively.

The spectroscopic data of **26** were similar to those of **42**, a β-dihydroagarofuran-type sesquiterpenoid previously identified from *C. paniculatus*.[24] Compound **26** lacks the C-2 acetoxy group of **42**, as is evidenced by the signals at δ_H 1.48 and 1.87 (H-2) and δ_C 22.4 (C-2) (Tables 2 and 3). The correlations between H-1 and H-4 and between H-8 and H-9/H-15a in the NOESY spectrum permitted the establishment of its relative configuration. Thus, the structure of **26** was defined as 1β,8α,15-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran.

The structures of the known compounds **11**,[25] **12**,[26] **13**,[27] **14**,[28] **15**,[29] **16**,[27] **17**,[30] **18**,[27] **19**,[25] **20**,[31] **32**,[32] **33**,[33] **34**,[33] **35**,[34] **36**,[34] **37**,[35] **38**,[36] **39**,[37] **40**,[37] **41**,[38] **42**,[24] **43**,[39] **44**,[39] **45**,[39] **46**,[40] **47**,[41] **48**,[19] **49**,[19] **50**,[38] **51**,[32] **52**,[38] **53**,[33] and **54**[38] were characterized by comparison of their observed and reported NMR data.

The fruits and seeds of several *Celastrus* plants have been used in China and India to enhance memory. According to our experimental results, the seeds of *C. flagellaris*, *C. angulatus*, and *C. orbiculatus* mainly contain fatty acids and β-dihydroagarofuran-type sesquiterpenoids. Linolenic acid, one of the fatty acids from the seeds of *Celastrus* plants, has been well-known to

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{13}C NMR Data of the Carbon Framework of Compounds 1–10 and 21–26 in $CDCl_3$

no.	1	2	3	4	5	6	7[a]	8[a]	9	10	21	22	23[a]	24	25	26
1	71.3 d	71.4 d	71.1 d	71.4 d	71.3 d	71.3 d	72.2 d	72.1 d	71.4 d	71.3 d	78.5 d	77.4 d	77.6 d	78.5 d	79.2 d	73.3 d
2	71.3 d	70.8 d	70.4 d	70.3 d	70.0 d	70.1 d	70.9 d	70.2 d	70.1 d	69.8 d	71.5 d	70.1 d	70.8 d	23.0 t	22.5 t	22.4 t
3	31.1 t	31.1 t	31.2 t	31.7 t	31.0 t	31.1 t	31.7 t	31.9 t	31.2 t	31.0 t	33.2 t	31.7 t	31.9 t	26.7 t	26.7 t	26.8 t
4	39.4 d	39.4 d	33.9 d	33.9 d	33.0 d	33.6 d	40.3 d	34.5 d	39.5 d	33.2 d	33.4 d	39.5 d	39.5 d	40.0 d	34.1 d	39.9 d
5	86.7 s	86.7 s	89.6 s	89.7 s	89.3 s	89.5 s	88.1 s	90.5 s	86.7 s	89.3 s	90.8 s	88.0 s	87.9 s	88.3 s	91.3 s	86.7 s
6	36.4 t	36.4 t	79.3 d	79.4 d	78.2 d	79.2 d	36.5 t	79.4 d	36.4 t	78.2 d	75.1 d	32.3 t	32.3 t	36.8 t	75.2 d	32.3 t
7	43.8 d	43.7 d	48.9 d	49.0 d	48.9 d	48.9 d	44.5 d	49.6 d	43.7 d	48.9 d	53.6 d	48.4 d	48.2 d	47.4 d	52.9 d	48.4 d
8	34.1 t	34.1 t	31.6 t	31.2 t	34.9 t	31.6 t	31.8 t	32.2 t	34.2 t	34.9 t	71.7 d	71.5 d	71.6 d	75.4 d	72.7 d	69.1 d
9	69.6 d	69.6 d	72.9 d	73.2 d	69.3 d	73.0 d	74.3 d	73.5 d	69.6 d	69.3 d	74.2 d	74.3 d	74.2 d	76.2 d	74.6 d	71.9 d
10	50.5 s	50.6 s	49.9 s	50.0 s	53.2 s	49.8 s	47.9 s	50.5 s	50.6 s	53.3 s	51.2 s	49.4 s	49.6 s	50.3 s	49.4 s	51.5 s
11	82.4 s	82.3 s	83.0 s	83.1 s	82.8 s	83.0 s	83.0 s	83.7 s	82.2 s	82.1 s	81.2 s	81.0 s	81.4 s	82.0 s	82.0 s	82.4 s
12	24.3 q	24.3 q	26.1 q	26.2 q	26.0 q	26.1 q	24.9 q	26.6 q	24.3 q	26.0 q	24.9 q	23.3 q	23.3 q	24.7 q	24.4 q	25.2 q
13	30.3 q	30.3 q	30.8 q	30.8 q	30.5 q	30.7 q	30.9 q	31.4 q	30.3 q	30.5 q	30.6 q	30.1 q	30.0 q	30.9 q	30.8 q	31.3 q
14	19.2 q	19.1 q	18.9 q	18.9 q	18.1 q	18.9 q	19.8 q	19.1 q	18.9 q	17.9 q	18.2 q	18.8 q	19.9 q	17.3 q	17.0 q	18.8 q
15	66.2 t	65.9 t	20.6 q	20.7 q	65.9 t	20.8 q	20.6 q	20.9 q	65.8 t	65.6 t	63.7 t	63.0 t	64.8 t	63.2 t	12.8 q	64.2 t

[a]Recorded in pyridine-d_5.

improve memory.[42] However, the biological activities relating to the cognition-enhancing and neuroprotective effects of β-dihydroagarofuran-type sesquiterpenoids of the genus *Celastrus* have not been reported. Scopolamine, a nonselective muscarinic antagonist, can block the cholinergic pathway.[43] On the basis of the blockade effects, the use of scopolamine on animals provides a simple and rapid method for testing the cognition-enhancing properties of new drugs.[44] Thus, the cognition-enhancing effects of compounds **58**, **59**, and **61**–**63**, which are available in sufficient quantities for animal testing, were evaluated on scopolamine-treated mice using a passageway water maze. As shown in Figure 5, mice injected with

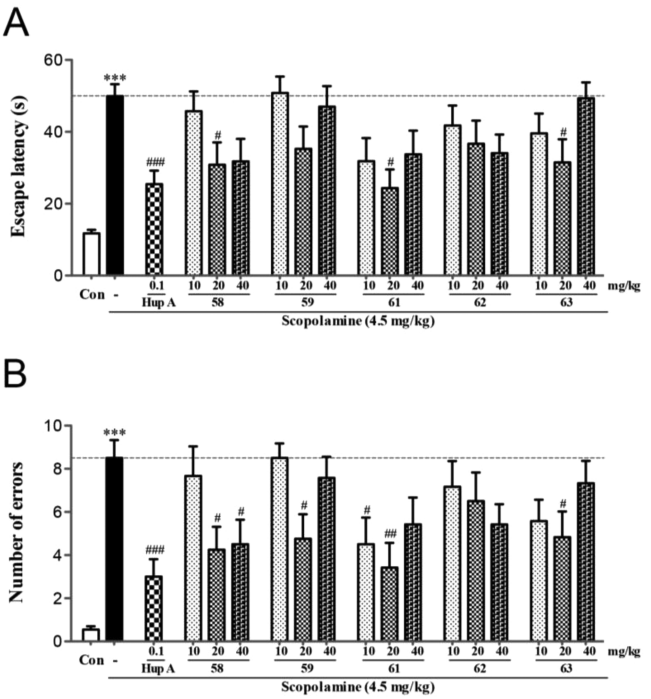


Figure 5. Effects of compounds **58**, **59**, and **61**–**63** on scopolamine-induced cognitive deficits of mice. (A) The escape latency of mice in passageway water maze. (B) The number of errors of mice in passageway water maze. Data are shown as mean ± SEM $n = 12$. $^{***}p < 0.001$ vs control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs scopolamine group. Control and huperzine A are, respectively, abbreviated as Con and Hup A.

scopolamine (4.5 mg/kg) exhibited longer escape latency and increased number of errors compared with the saline-injected control group. Compounds **58**, **59**, **61**, and **63**, as well as huperzine A (positive control), rescued the escape latency and significantly reduced the number of errors. Compound **58** showed cognition-enhancing effects in a dose-dependent manner, whereas compounds **59**, **61**, and **63** showed better cognition-enhancing effects at 20 mg/kg. Compound **62** showed no statistical difference compared with the scopolamine-treated group.

Aβ has generally been adopted as an inducer of neuronal injury to analyze the protective effects and mechanism of action of new pharmacotherapies for AD.[45] $A\beta_{25-35}$, a synthetic peptide that possesses the same β-sheet structure and exhibits large fibrils, possesses most of the physical and biological properties of full length-Aβ[46] and is often used to study the neuroprotective effects of various compounds predicted to modulate Aβ toxicity in vitro.[47] The 3-(4,5-dimethylthiazol-2-

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the neuroprotective effects of 62 β-dihydroagarofuran-type sesquiterpenoids, including the 47 sesquiterpenoids described above, 11 sesquiterpenoids isolated previously from *C. orbiculatus*,[28] and four synthetic sesquiterpenoid derivatives, against Aβ_{25-35}-induced SH-SY5Y cell injury. As shown in Table 4, 21 tested compounds increased the cell viability from

Table 4. Neuroprotective Effects of Selected Compounds Isolated from *Celastrus* Plants against Aβ_{25-35} Induced Neurotoxicity in SH-SY5Y Cells

	cell viability[a] (%)			cell viability[a] (%)	
compd	1 μM	10 μM	compd	1 μM	10 μM
1	–[b]	86.9	**39**	–	–
2	–	82.3	**40**	–	–
3	–	84.5	**41**	–	–
4	–	87.3	**42**	–	–
5	–	–	**43**	–	–
6	–	–	**44**	–	–
7	–	85.0	**45**	–	–
9	–	85.0	**46**	–	–
10	–	–	**47**	–	–
11	–	77.7	**48**	–	–
12	76.3	87.4	**49**	–	–
13	–	73.9	**50**	–	74.3
14	79.6	86.2	**51**	–	76.2
15	–	–	**52**	–	82.3
16	–	76.7	**53**	–	–
17	–	–	**54**	–	–
18	–	74.4	**55**	–	–
19	–	–	**56**	–	–
20	–	–	**57**	–	–
21	–	–	**58**	–	–
23	–	77.8	**59**	–	–
28	–	–	**60**	–	77.7
29	–	79.0	**61**	–	–
30	–	81.7	**62**	–	–
32	–	78.7	**64**	–	–
33	–	–	**65**	–	–
34	–	–	**66**	–	79.7
35	–	–	**67**	–	–
36	–	–	**68**	–	–
37	–	–	**69**	–	–
38	–	–	**70**	–	–
			EGCG	N.T.[c]	80.8

[a]The neuroprotective effect of these compounds on Aβ_{25-35}-induced neurotoxicity in SH-SY5Y cells. The cell viability in control was taken as 100%, and the average value of cell viability under Aβ_{25-35} exposure was 64.6 ± 1.4%. The positive control is epigallocatechin gallate (EGCG). [b]The – means not active. [c]N.T. means not tested.

64.6% for the model to more than 74.0%, significantly improving the cell viability at 10 μM. Among them, compounds **12** and **14** also showed neuroprotective effects at 1 μM. Comparison of the activities of **7**, **12**, **13**, **16**, **58** and **11**, **14**, **15** revealed that compounds containing a C-2 ester group are more potent than those without a substituent at the same position. Two derivatives obtained by hydrolysis, **67** and **70**, displayed weak activities, suggesting that β-dihydroagarofuran-type sesquiterpenoids with only two or three hydroxy groups provide no neuroprotective effect. Sesquiterpenoids with different ester groups at C-2 exhibited variations in the neuroprotective effect, i.e., **1** (BzO-2) > **17** (AcO-2); **11** (BzO-2) > **14** (AcO-2); **7** (HexO-2)/**12** (CinO-2) > **13** (BzO-2); and **7** (HexO-2)/**12** (CinO-2) > **16** (AcO-2). Therefore, the bulk of the substituents at C-2 may also play an important role in the neuroprotective effects of this kind of sesquiterpenoids. No unambiguous SAR could be deduced concerning the substituents at other positions.

In summary, we report the identification of 16 new and 38 known β-dihydroagarofuran-type sesquiterpenoids from the seed of *C. flagellaris* and the seed and root bark of *C. angulatus*. Twenty-one of the 62 tested compounds were found to possess neuroprotective effects against Aβ_{25-35}-induced toxicity on SH-SY5Y cells. According to animal behavior test results, four compounds rescued scopolamine-induced memory impairment in mice. These results revealed that certain β-dihydroagarofuran-type sesquiterpenoids may contribute to the memory-improving effect of several *Celastrus* plants used in Chinese and Indian folk medicines, and identified a new chemical skeleton as drug lead for the research of novel therapeutic agents against AD. Further in vitro and in vivo investigations on the neuroprotective and memory-enhancing effects, as well as their pharmacological mechanism, are required.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using a Büchi 510 melting point apparatus (Büchi, Flawil, Switzerland). Optical rotations were measured on a PerkinElmer 341 polarimeter, and IR spectra were recorded using KBr disks on a PerkinElmer 577 spectrometer (PerkinElmer, Waltham, MA). ECD spectra were obtained on a JASCO J-810 spectrometer (JASCO Corporation, Tokyo, Japan). UV spectra were obtained using a Varian Cary 50-vis spectrophotometer (Varian, Melbourne, Australia). NMR experiments were performed on Bruker AM-400, Bruker Advance III 500, Bruker Advance III 600 (Bruker, Ettlingen, Germany), or Varian-MERCURY Plus-400 (Varian, Palo Alto, CA) using TMS as an internal standard. ESIMS analyses were performed on a Shimadzu LC-MS-2020 spectrometer with a Shimadzu SPD-M20 (Shimadzu, Kyoto, Japan) diode array detector using CNW C_{18} (2.1 × 50 mm, 3.5 μm) or CNW C_{18} (2.1 × 100 mm, 3.5 μm) columns (Anpel Scientific Instrument Co., Ltd., Shanghai, People's Republic of China). HRESIMS analyses were performed on a Waters-Micromass QTOF Ultima Global mass spectrometer (Waters, Milford, MA). Semi-preparative HPLC was performed on a Unimicro EasySep-1010 binary pump system with a Unimicro EasySep-1010 detector (Unimicro, Shanghai, People's Republic of China) using YMC-Pack ODS-A (250 × 20 mm, 5 μm) or YMC-Pack ODS-A (250 × 10 mm, 5 μm) columns (YMC Co., Ltd., Kyoto, Japan). Precoated silica gel GF254 plates, silica gel (300–400 mesh) (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China), C_{18} reversed-phase (RP-18) silica gel (150–200 mesh) (Merck, Whitehouse Station, NJ), and CHP20P MCI gel (75–150 μm) (Mitsubishi Chemical Industries, Ltd., Tokyo, Japan) were used for column chromatography (CC) and TLC detection.

Plant Material, Extraction, and Isolation. The seeds of *C. flagellaris* were collected in October 2013 in the suburb of Jilin, Jilin Province, People's Republic of China, and were identified by Prof. Zhixin Ju of Jilin Agricultural University. A voucher specimen was deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM20131016-CB). The seeds of *C. flagellaris* (98 g) were powdered and ultrasonically extracted with CH_2Cl_2 at room temperature (1 L × 3) to give a crude extract (36 g). The crude extract was fractionated on a silica gel (300–400 mesh) column eluted with a petroleum ether/EtOAc gradient (from 40:1 to 10:1, v/v) to afford six fractions (Fr.1–Fr.6). Fr.2–5 were further separated using column chromatography over silica gel (petroleum ether/acetone of increasing polarity) and semipreparative HPLC to give the new compounds **1** (25 mg), **2** (6 mg), **3** (15 mg), **4** (4 mg), **5** (8 mg), **6** (5 mg), **7** (5

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX–XXX

mg), **8** (2 mg), **9** (4 mg), and **10** (5 mg), as well as the known compounds **11**−**20**.

The seeds of *C. angulatus* were collected in October 2010 in Xinxiang, Henan Province, People's Republic of China, and were identified by Prof. Jingui Shen of Shanghai Institute of Materia Medica. A voucher specimen was deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM20111010). The seeds of *C. angulatus* (1.8 kg) were powdered and ultrasonically extracted with EtOH at room temperature (10 L × 3) to yield a crude extract (300 g). The extract was fractionated over a silica gel (300−400 mesh) column and eluted with a petroleum ether/acetone gradient (from 20:1 to 1:1, v/v) to afford six fractions (Fr.1−Fr.6). Crude crystals (200 mg) precipitated from Fr.2 were chromatographed over silica gel (petroleum ether/EtOAc of increasing polarity) to give **26** (8 mg) and **40**. A portion (24 g) of the filtrate from Fr.2 was subjected to chromatography over C_{18} reversed-phase silica gel and semipreparative HPLC to give **24** (16 mg), **37**, and **50**. Fr.3 (18 g) and Fr.4 (16 g) were subjected to chromatography over silica gel (petroleum ether/acetone of increasing polarity) and semipreparative HPLC to afford the new compounds **22** (10 mg) and **25** (2 mg), as well as the known compounds **27**, **28**, **31**−**36**, **39**, **41**, **42**, **51**, **52**, and **54**. New compounds **21** (23 mg) and **23** (6 mg) and known compounds **29**, **30**, **38**, and **53** were obtained from Fr.5 (15 g) using isolation methods similar to those mentioned above.

The root bark of *C. angulatus* was collected in May 2010 in Xinxiang, Henan Province, People's Republic of China, and was identified by Prof. Jingui Shen of Shanghai Institute of Materia Medica. A voucher specimen was deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM20111011). The root bark of *C. angulatus* (1.8 kg) was powdered and ultrasonically extracted with EtOH at room temperature (10 L × 3) to give a crude extract (240 g). The crude extract was fractionated using silica gel (300−400 mesh) eluted with a petroleum ether/acetone gradient (from 20:1 to 1:1, v/v) to afford five fractions (Fr.1 ∼ Fr.5). Fr.2 (26 g) and Fr.4 (30 g) were further chromatographed over silica gel (petroleum ether/acetone of increasing polarity) and subjected to semipreparative HPLC to yield the known compounds **43**−**49**.

*(1R,2S,4R,5S,7R,9S,10S)-1,15-Diacetoxy-2-benzoyloxy-9-cinnamoyloxy-β-dihydroagarofuran (**1**):* colorless orthorhombic crystals (acetone); mp 218−220 °C; $[\alpha]^{22}_{D}$ + 143 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (4.40), 223 (4.41), 279 (4.42) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 238 (0.84), 275 (4.17) nm; IR (KBr) ν_{max} 1742, 1713, 1636, 1452, 1367, 1274, 1227, 1165, 776, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.77 (3H, s, AcO-1), 2.17 (3H, s, AcO-15), BzO-2 and CinO-9 [8.13 (d, 2H, *J* = 7.0 Hz), 7.69 (d, 1H, *J* = 16.0 Hz), 7.57 (m, 3H), 7.50 (t, 2H, *J* = 7.5 Hz), 7.39 (m, 3H), 6.39 (d, 1H, *J* = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 170.0 (s)], AcO-15 [21.5 (q), 170.9 (s)], BzO-2 and CinO-9 [118.2 (d), 128.4 × 2 (d), 128.8 × 2 (d), 129.0 × 2 (d), 130.0 (s), 130.0 × 2 (d), 130.5 (d), 133.3 (d), 134.5 (s), 145.6 (d), 166.2 (s), 166.3 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 627.2579 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_9Na$, 627.2570).

*(1S,2S,4R,5S,7R,9S,10S)-1,15-Diacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran (**2**):* white amorphous powder; $[\alpha]^{22}_{D}$ + 150 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.02), 279 (4.13) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 260 (−4.32), 291 (29.74) nm; IR (KBr) ν_{max} 1742, 1712, 1639, 1450, 1367, 1261, 1226, 1162, 1013, 769, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80 (3H, s, AcO-1), 2.18 (3H, s, AcO-15), CinO-2 and CinO-9 [7.76 (d, 1H, *J* = 16.2 Hz), 7.69 (d, 1H, *J* = 16.0 Hz), 7.64 (m, 2H), 7.55 (m, 2H), 7.39 (m, 6H), 6.43 (d, 1H, *J* = 16.2 Hz), 6.39 (d, 1H, *J* = 16.2 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 170.0 (s)], AcO-15 [21.6 (q), 170.8 (s)], CinO-2 and CinO-9 [118.2 × 2 (d), 128.4 × 4 (d), 129.0 × 4 (d), 130.5 × 2 (d), 134.5 × 2 (s), 145.3 (d), 145.6 (d), 166.2 × 2 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 653.2739 $[M + Na]^+$ (calcd for $C_{37}H_{42}O_9Na$, 653.2727).

*(1S,2S,4R,5S,6R,7R,9S,10S)-1,6-Diacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran (**3**):* white amorphous powder; $[\alpha]^{23}_{D}$ + 210 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.91), 279 (4.03) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 260 (−9.36), 292 (38.52) nm; IR (KBr) ν_{max} 1745, 1711, 1637, 1450, 1367, 1234, 1165, 1016, 767, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.81 (3H, s, AcO-1), 2.13 (3H, s, AcO-6), CinO-2 and CinO-9 [7.67 (d, 1H, *J* = 16.5 Hz), 7.63 (d, 1H, *J* = 16.5 Hz), 7.53 (m, 4H), 7.38 (m, 6H), 6.38 (d, 1H, *J* = 16.5 Hz), 6.37 (d, 1H, *J* = 16.5 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 170.2 (s)], AcO-6 [21.6 (q), 170.3 (s)], CinO-2 and CinO-9 [118.1 (d), 118.4 (d), 128.2 × 2 (d), 128.4 × 2 (d), 129.0 × 2 (d), 129.1 × 2 (d), 130.5 × 2 (d), 134.4 (s), 134.5 (s), 145.1 (d), 145.7 (d), 166.2 (s), 166.3 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 653.2742 $[M + Na]^+$ (calcd for $C_{37}H_{42}O_9Na$, 653.2727).

*(1S,2S,4R,5S,6R,7R,9S,10S)-1,6-Diacetoxy-2-cinnamoyloxy-9-benzoyloxy-β-dihydroagarofuran (**4**):* white amorphous powder; $[\alpha]^{21}_{D}$ + 87 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.20), 223 (4.11), 276 (4.23) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 238 (−1.65), 277 (9.87) nm; IR (KBr) ν_{max} 1742, 1713, 1639, 1392, 1366, 1280, 1227, 1100, 1015, 768, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62 (3H, s, AcO-1), 2.13 (3H, s, AcO-6), CinO-2 and BzO-9 [8.06 (d, 2H, *J* = 7.4 Hz), 7.63 (d, 1H, *J* = 16.1 Hz), 7.56 (t, 1H, *J* = 7.0 Hz), 7.51 (m, 2H), 7.44 (t, 2H, *J* = 7.5 Hz), 7.39 (m, 3H), 6.37 (d, 1H, *J* = 16.1 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.6 (q), 169.8 (s)], AcO-6 [21.6 (q), 170.3 (s)], CinO-2 and BzO-9 [118.3 (d), 128.3 × 2 (d), 128.4 × 2 (d), 129.1 × 2 (d), 129.6 (s), 130.2 × 2 (d), 130.6 (d), 133.4 (d), 134.4 (s), 145.1 (d), 165.7 (s), 166.3 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 627.2588 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_9Na$, 627.2570).

*(1R,2S,4R,5S,6R,7R,9S,10S)-1,6,15-Triacetoxy-2,9-dicinnamoyloxy-β-dihydroagarofuran (**5**):* white amorphous powder; $[\alpha]^{21}_{D}$ + 211 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.33), 279 (4.43) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 262 (−6.75), 292 (27.36) nm; IR (KBr) ν_{max} 1743, 1711, 1639, 1388, 1370, 1280, 1225, 1159, 1093, 1016, 768, 712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.79 (3H, s, AcO-1), 2.10 (3H, s, AcO-6), 2.27 (3H, s, AcO-15), CinO-2 and CinO-9 [7.76 (d, 1H, *J* = 16.1 Hz), 7.69 (d, 1H, *J* = 16.0 Hz), 7.64 (m, 2H), 7.55 (m, 2H), 7.39 (m, 6H), 6.44 (d, 1H, *J* = 16.1 Hz), 6.34 (d, 1H, *J* = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 169.8 (s)], AcO-6 [21.4 (q), 170.2 (s)], AcO-15 [21.5 (q), 170.8 (s)], CinO-2 and CinO-9 [117.8 (d), 118.0 (d), 128.4 × 4 (d), 129.0 × 4 (d), 130.6 × 2 (d), 134.4 × 2 (s), 145.4 (d), 146.0 (d), 165.9 (s), 166.1 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 711.2783 $[M + Na]^+$ (calcd for $C_{39}H_{44}O_{11}Na$, 711.2781).

*(1R,2S,4R,5S,6R,7R,9S,10S)-1,6-Diacetoxy-2,9-dibenzoyloxy-β-dihydroagarofuran (**6**):* white amorphous powder; $[\alpha]^{21}_{D}$ + 92 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 231 (4.33) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (3.07), 235 (22.88) nm; IR (KBr) ν_{max} 1747, 1730, 1718, 1456, 1367, 1280, 1230, 1111, 1096, 1009, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.59 (3H, s, AcO-1), 2.13 (3H, s, AcO-6), BzO-2 and BzO-9 [8.06 (dd, 2H, *J* = 7.0, 1.3 Hz), 7.98 (dd, 2H, *J* = 7.0, 1.3 Hz), 7.57 (t, 2H, *J* = 7.5 Hz), 7.45 (t, 4H, *J* = 7.0 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.5 (q), 169.7 (s)], AcO-6 [21.4 (q), 170.2 (s)], BzO-2 and BzO-9 [128.3 × 2 (d), 128.5 × 2 (d), 129.4 (d), 129.5 × 2 (d), 130.1 × 2 (d), 130.3 (s), 132.9 (d), 133.3 (d), 165.5 (s), 166.1 (s)], for other signals, see Table 3; HRTOFMS *m*/*z* 601.2421 $[M + Na]^+$ (calcd for $C_{33}H_{38}O_9Na$, 601.2414).

*1β-Acetoxy-2β-hexanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran (**7**):* white amorphous powder; $[\alpha]^{21}_{D}$ + 72 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.13), 223 (4.13), 277 (4.22) nm; IR (KBr) ν_{max} 2925, 1747, 1718, 1641, 1456, 1362, 1233, 1133, 1013, 878, 770, 713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66 (d, 1H, *J* = 16.0 Hz), 7.53 (m, 2H), 7.37 (m, 3H), 6.38 (d, 1H, *J* = 16.0 Hz), 5.55 (m, 2H), 4.75 (d, 1H, *J* = 6.5 Hz), 2.42 (ddd, 1H, *J* = 15.0, 6.5, 3.3 Hz), 2.28 (t, 2H, *J* = 7.4 Hz), 2.17 (m, 1H), 2.00−2.10 (m, 4H), 1.90 (t, 1H, *J* = 7.8 Hz), 1.80 (s, 3H), 1.73 (d, 1H, *J* = 15.4 Hz), 1.37 (s, 3H), 1.34 (s, 3H), 1.25 (d, 3H, *J* = 7.0 Hz), 1.20 (s, 3H), 0.88 (t, 3H, *J* = 6.5 Hz); ^1H NMR (pyridine-d_5) δ 1.97 (3H, s, AcO-1), HexO-2 [2.36 (t, 2H, *J* = 7.2 Hz), 2.11 (m, 2H), 1.66 (m, 2H), 1.27 (m, 2H), 0.85 (t, 3H, *J* = 7.1 Hz)], CinO-9 [8.03 (d, 1H, *J* = 16.0 Hz), 7.56 (m, 2H), 7.32 (m, 3H), 6.78 (d, 1H, *J* = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR (pyridine-d_5) δ AcO-1 [21.3 (q), 170.7 (s)], HexO-2 [14.5 (q), 23.0 (t), 25.4 (t), 32.0 (t), 35.2 (t), 173.4 (s)], CinO-9 [119.7 (d), 129.1 × 2 (d), 129.7 × 2 (d), 131.1 (d), 136.3 (s), 145.6 (d), 166.6 (s)], for

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

other signals, see Table 3; HRTOFMS m/z 563.2996 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_7Na$, 563.2985).

*1α,6α-Diacetoxy-2β-butanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran (**8**):* white amorphous powder; $[\alpha]^{21}_D$ + 96 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.01), 218 (4.03), 277 (4.21) nm; IR (KBr) ν_{max} 2925, 1744, 1709, 1637, 1388, 1367, 1234, 1165, 1014, 768,712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66 (d, 1H, J = 16.0 Hz), 7.53 (m, 2H), 7.38 (m, 3H), 6.38 (d, 1H, J = 16.0 Hz), 5.59 (m, 2H), 5.38 (s, 1H), 4.74 (d, 1H, J = 6.5 Hz), 2.41 (ddd, 1H, J = 15.0, 6.3, 3.0 Hz), 2.32 (t, 1H, J = 7.0 Hz), 2.26 (t, 2H, J = 7.4 Hz), 2.22 (m, 1H), 2.12 (s, 3H), 1.79 (s, 3H), 1.76 (m, 1H), 1.44 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H), 1.20 (d, 3H, J = 7.0 Hz), 0.94 (t, 3H, J = 6.5 Hz); ^1H NMR (pyridine-d_5) δ 1.94 (3H, s, AcO-1), 2.18 (3H, s, AcO-6), ButO-2 [2.31 (m, 2H), 1.65 (m, 2H), 0.92 (t, 3H, J = 7.0 Hz)], CinO-9 [8.03 (d, 1H, J = 16.0 Hz), 7.61 (m, 2H), 7.34 (m, 3H), 6.76 (d, 1H, J = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR (pyridine-d_5) δ AcO-1 [21.3 (q), 170.6 (s)], AcO-6 [21.7 (q), 170.5 (s)], ButO-2 [14.2 (q), 19.2 (t), 37.1 (t), 173.2 (s)], CinO-9 [119.3 (d), 129.1 × 2 (d), 129.8 × 2 (d), 131.2 (d), 135.3 (s), 146.0 (d), 166.3 (s)], for other signals, see Table 3; HRTOFMS m/z 593.2748 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_9Na$, 593.2727).

*1α,15-Diacetoxy-2β-butanoyloxy-9α-cinnamoyloxy-β-dihydroagarofuran (**9**):* white amorphous powder; $[\alpha]^{22}_D$ + 100 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.40), 218 (4.40), 279 (4.64) nm; IR (KBr) ν_{max} 1746, 1719, 1643, 1452, 1365, 1223, 1162, 1134, 1012, 879, 767, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.78 (3H, s, AcO-1), 2.16 (3H, s, AcO-15), ButO-2 [2.33 (m, 2H), 1.69 (m, 2H), 0.96 (t, 3H, J = 7.0 Hz)], CinO-9 [7.67 (d, 1H, J = 16.0 Hz), 7.54 (m, 2H), 7.38 (m, 3H), 6.36 (d, 1H, J = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 169.8 (s)], AcO-15 [21.6 (q), 170.8 (s)], ButO-2 [13.8 (q), 18.5 (t), 36.8 (t), 172.9 (s)], CinO-9 [118.2 (d), 128.4 × 2 (d), 129.0 × 2 (d), 130.5 (d), 134.5 (s), 145.5 (d), 166.2 (s)], for other signals, see Table 3; HRTOFMS m/z 593.2734 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_9Na$, 593.2727).

*1β,2β,6α,15-Tetraacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**10**):* white amorphous powder; $[\alpha]^{22}_D$ + 77 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.03), 218 (4.05), 279 (4.23) nm; IR (KBr) ν_{max} 1745, 1710, 1637, 1368, 1237, 1158, 1091, 878, 768 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.78 (3H, s, AcO-1), 2.10 (3H, s, AcO-2), 2.09 (3H, s, AcO-6), 2.23 (3H, s, AcO-15), CinO-9 [7.69 (d, 1H, J = 16.0 Hz), 7.55 (dd, 2H, J = 6.8, 2.3 Hz), 7.39 (m, 3H), 6.36 (d, 1H, J = 16.0 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 169.8 (s)], AcO-2 [21.4 (q), 170.1 (s)], AcO-6 [21.5 (q), 170.2 (s)], AcO-15 [21.5 (q), 170.7 (s)], CinO-9 [117.7 (d), 128.4 × 2 (d), 129.1 × 2 (d), 130.7 (d), 134.4 (s), 146.0 (d), 165.9 (s)], for other signals, see Table 3; HRTOFMS m/z 623.2458 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{11}Na$, 623.2468).

*(1R,2S,4R,5S,6R,7R,8R,9S,10S)-6,15-Diacetoxy-8,9-dibenzoyloxy-1,2-dihydroxy-β-dihydroagarofuran (**21**):* white amorphous powder; $[\alpha]^{22}_D$ −39 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.34), 273 (3.22) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 222 (15.82), 240 (−19.22) nm; IR (KBr) ν_{max} 3514, 2929, 1726, 1603, 1452, 1371, 1284, 1263, 1234, 1101, 1041, 860, 712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.12 (3H, s, AcO-6), 1.89 (3H, s, AcO-15), BzO-8 and BzO-9 [8.04 (d, 2H, J = 7.2 Hz), 7.87 (d, 2H, J = 7.2 Hz), 7.56 (t, 1H, J = 7.2 Hz), 7.50 (t, 1H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.2 Hz), 7.32 (t, 2H, J = 7.2 Hz)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-6 [21.5 (q), 170.0 (s)], AcO-15 [21.5 (q), 170.3 (s)], BzO-8 and BzO-9 [128.6 × 2 (d), 128.7 × 2 (d), 129.9 × 2 (d), 130.1 × 2 (d), 130.1 × 2 (s), 133.5 × 2 (d), 166.0 (s), 166.3 (s)], for other signals, see Table 3; HRESIMS m/z 633.2322 $[M + Na]^+$ (calcd for $C_{33}H_{38}O_{11}Na$, 633.2312).

*(1R,2S,4R,5S,7R,8R,9S,10S)-1,2,15-Triacetoxy-8,9-dibenzoyloxy-β-dihydroagarofuran (**22**):* white amorphous powder; $[\alpha]^{22}_D$ −126 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.39), 274 (3.34) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 219 (4.17), 238 (−51.14) nm; IR (KBr) ν_{max} 1739, 1603, 1452, 1369, 1284, 1255, 1234, 1115, 1088, 1026, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53 (3H, s, AcO-1), 2.01 (3H, s, AcO-2), 1.89 (3H, s, AcO-15), BzO-8 and BzO-9 [8.00 (d, 2H, J = 7.6 Hz), 7.90 (d, 2H, J = 7.6 Hz), 7.58 (t, 1H, J = 7.6 Hz), 7.49 (t, 1H, J = 7.6 Hz), 7.45 (t, 2H, J = 7.6 Hz), 7.31 (t, 2H, J = 7.6 Hz)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.8 (q), 170.1 (s)], AcO-2 [21.5 (q), 170.3 (s)], AcO-15 [21.5 (q), 171.0 (s)], BzO-8 and BzO-9 [128.5 × 2 (d), 128.7 × 2 (d), 129.8 × 2 (d), 129.9 × 2 (d), 130.3 × 2 (s), 133.4 × 2 (d), 165.1 (s), 166.2 (s)], for other signals, see Table 3; HRESIMS m/z 659.2479 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_{11}Na$, 659.2468).

*1β,2β-Diacetoxy-8β,9β-dibenzoyloxy-15-nicotinoyloxy-β-dihydroagarofuran (**23**):* white amorphous powder; $[\alpha]^{22}_D$ −164 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.48), 264 (3.63) nm; IR (KBr) ν_{max} 1743, 1720, 1603, 1591, 1450, 1367, 1284, 1292, 1271, 1244, 1115, 1084, 1026, 854, 712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.14 (brs, 1H), 8.60 (brd, 1H, J = 4.8 Hz), 8.13 (brd, 1H, J = 8.0 Hz), 8.05 (d, 2H, J = 7.6 Hz), 7.65 (d, 2H, J = 7.6 Hz), 7.56 (t, 1H, J = 7.6 Hz), 7.43 (t, 2H, J = 7.6 Hz), 7.22 (t, 1H, J = 7.6 Hz), 7.07 (dd, 1H, J = 8.0, 4.8 Hz), 6.89 (t, 2H, J = 7.6 Hz), 5.80 (d, 1H, J = 5.8 Hz), 5.76 (d, 1H, J = 3.1 Hz), 5.75 (dd, 1H, J = 5.8, 3.3 Hz), 5.58 (d, 1H, J = 12.1 Hz), 5.47 (dd, 1H, J = 6.1, 3.1 Hz), 5.17 (d, 1H, J = 12.1 Hz), 2.77 (d, 1H, J = 13.2 Hz), 2.60 (brt, 1H, J = 3.7 Hz), 2.51 (m), 2.14 (dd, 1H, J = 13.2, 4.1 Hz), 2.03 (m), 1.82 (m),1.73 (3H, s, AcO-1), 1.72 (3H, s, AcO-2), 1.42 (d, 1H, J = 8.0 Hz), 1.61 (s, 1H), 1.29 (s, 1H); ^1H NMR (pyridine-d_5) δ 1.92 (AcO-1), 1.81 (AcO-2), BzO-8 and BzO-9 [8.34 (d, 2H, J = 7.6 Hz), 7.97 (d, 2H, J = 7.6 Hz), 7.56 (t, 1H, J = 7.6 Hz), 7.48 (t, 2H, J = 7.6 Hz), 7.23 (overlapped, 1H), 6.95 (t, 2H, J = 7.6 Hz)], NicO-15 [9.54 (brs, 1H), 8.77 (brd, 1H, J = 4.8 Hz), 8.39 (brd, 1H, J = 8.0 Hz), 7.23 (overlapped, 1H)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-1 [21.5 (q), 170.6 (s)], AcO-2 [21.2 (q), 170.2 (s)], BzO-8 and BzO-9 [128.2 × 2 (d), 128.7 × 2 (d), 129.7 × 2 (d), 129.8 × 2 (d), 129.2 (s), 129.7 (s), 133.2 (d), 133.5 (d), 165.0 (s), 166.0 (s)], NicO-15 [123.0 (d), 126.0 (s), 137.0 (d), 151.2 (d), 153.6 (d), 165.6 (s)]; ^{13}C NMR (pyridine-d_5) δ AcO-1 [21.5 (q), 171.2 (s)], AcO-2 [21.5 (q), 170.5 (s)], BzO-8 and BzO-9 [128.9 × 2 (d), 129.4 × 2 (d), 130.2 × 2 (d), 130.6 × 2 (d), 130.1 (s), 130.7 (s), 133.8 (d), 134.2 (d), 165.9 (s), 166.5 (s)], NicO-15 [123.5 (d), 126.8 (s), 137.5 (d), 151.9 (d), 154.4 (d), 166.2 (s)], for other signals, see Table 3 ; HRESIMS m/z 722.2588 $[M + Na]^+$ (calcd for $C_{39}H_{41}NO_{11}Na$, 722.2577)

*1β-Acetoxy-9β-benzoyloxy-8α-hexanoyloxy-15-nicotinoyloxy-β-dihydroagarofuran (**24**):* white amorphous powder; $[\alpha]^{22}_D$ + 36 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.78), 263 (3.12) nm; IR (KBr) ν_{max} 1726, 1589, 1452, 1369, 1282, 1269, 1228, 1109, 1025, 977, 714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (3H, s, AcO-1), HexO-8 [2.12 (t, 2H, J = 7.6 Hz), 1.36 (m, 2H), 1.03 (m, 2H), 1.07 (m, 2H), 0.69 (t, 3H, J = 7.0 Hz)], BzO-9 [7.85 (d, 2H, J = 7.6 Hz), 7.48 (t, 1H, J = 7.6 Hz), 7.32 (t, 2H, J = 7.6 Hz)], NicO-15 [9.45 (brs, 1H), 8.86 (brs, 1H), 8.51 (brd, 1H, J = 7.8 Hz), 7.49 (dd, 1H, J = 7.8, 4.8 Hz)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-1 [21.3 (q), 170.2 (s)], HexO-8 [13.9 (q), 22.3 (t), 31.3 (t), 21.7 (t), 34.7 (t), 173.4 (s)], BzO-9 [128.7 × 2 (d), 129.6 × 2 (d), 129.9 (s), 133.2 (d), 165.7 (s)], NicO-15 [123.8 (d), 124.0 (s), 137.4 (d), 151.4 (d), 154.2 (d), 165.7 (s)], for other signals, see Table 3; HRESIMS m/z 636.3177 $[M + H]^+$ (calcd for $C_{36}H_{46}NO_9$, 636.3173).

*6α-Acetoxy-1β,9β-dibenzoyloxy-8β-nicotinoyloxy-β-dihydroagarofuran (**25**):* white amorphous powder; $[\alpha]^{22}_D$ −67 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.34), 263 (3.51) nm; IR (KBr) ν_{max} 1730, 1591, 1452, 1337, 1286, 1228, 1113, 1093, 1026, 872, 706 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13 (3H, s, AcO-6), BzO-1 [7.58 (d, 2H, J = 7.6 Hz), 7.27 (t, 1H, J = 7.6 Hz), 7.01 (t, 2H, J = 7.6 Hz)], NicO-8 [9.24 (brs, 1H), 8.80 (brd, 1H, J = 4.8 Hz), 8.32 (brd, 1H, J = 7.8 Hz), 7.43 (dd, 1H, J = 7.8, 4.8 Hz)], BzO-9 [7.48 (d, 2H, J = 7.6 Hz), 7.15 (t, 1H, J = 7.6 Hz), 6.88 (t, 2H, J = 7.6 Hz)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-1 [21.5 (q), 170.3 (s)], BzO-1 [128.1 × 2 (d), 129.4 × 2 (d), 129.9 (s), 132.7 (d), 165.7 (s)], NicO-8 [123.7 (d), 124.9 (s), 137.4 (d), 151.2 (d), 153.8 (d), 164.4 (s)], BzO-9 [127.8 × 2 (d), 129.4 × 2 (d), 129.6 (s), 132.4 (d), 165.1 (s)], for other signals, see Table 3; HRESIMS m/z 642.2708 $[M + H]^+$ (calcd for $C_{37}H_{40}NO_9$, 642.2703).

*1β,8α,15-Triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**26**):* white amorphous powder; $[\alpha]^{22}_D$ −4 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.05), 274 (2.95) nm; IR (KBr) ν_{max} 1743, 1722, 1363, 1281, 1228, 1095, 1072, 1045, 887, 715 cm^{-1}; ^1H NMR

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

($CDCl_3$) δ 1.62 (3H, s, AcO-1), 1.89 (3H, s, AcO-8), 2.25 (3H, s, AcO-15), BzO-9 [8.09 (d, 2H, J = 7.2 Hz), 7.45 (t, 1H, J = 7.2 Hz), 7.57 (t, 2H, J = 7.2 Hz)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ AcO-1 [20.9 (q), 169.9 (s)], AcO-8 [21.1 (q), 170.2 (s)], AcO-15 [21.5 (q), 170.3 (s)], BzO-9 [128.5 × 2 (d), 130.4 × 2 (d), 129.6 (s), 133.5 (d), 166.0 (s)], for other signals, see Table 3; HRESIMS m/z 539.2244 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_9Na$, 539.2257).

Preparation of 67 and 68. An excess amount of NaH (10 mg) was added to a solution of compound **59** (30 mg, 0.066 mmol) in MeOH (25 mL), and the mixture was refluxed for 24 h. The reaction mixture was cooled to room temperature, and the solution was acidified to pH 7 with 3 N HCl. After evaporation of the solvent, the residue was extracted with EtOAc, and the organic layer was washed with H_2O and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by column chromatography over silica gel (petroleum ether/acetone, 5:1) to give **67** (5 mg, 0.019 mmol) and **68** (3 mg, 0.0072 mmol). **67**: white amorphous powder; ^{1}H NMR ($CDCl_3$) data are identical to reported data.[48] **68**: white amorphous powder; ^{1}H NMR ($CDCl_3$) δ 5.46 (dd, 1H, J = 12.0, 4.0 Hz, H-1), 4.41 (1H, s, H-6), 4.96 (d, 1H, J = 7.0 Hz, H-9), 1.51 (3H, s, H-12); 1.39 (3H, s, H-13); 1.30 (3H, s, H-15); 1.60 (3H, s, AcO-9), BzO-1 [8.08 (d, 2H, J = 7.2 Hz), 7.45 (t, 1H, J = 7.2 Hz), 7.57 (t, 2H, J = 7.2 Hz)].

Preparation of 69 and 70. Compounds **69** (3 mg, 0.0068 mmol) and **70** (5 mg, 0.020 mmol) were prepared from **15** (30 mg, 0.062 mmol) and **58** (30 mg, 0.070 mmol), respectively, using the same chemical transformation method described above. **68** and **69**: white amorphous powder; ^{1}H NMR ($CDCl_3$) data are identical to reported data.[49]

X-ray Crystallographic Analysis of 1. All data were collected using a Bruker APEX-II CCD diffractometer with graphite-monochromated Cu Kα radiation (1.54178 Å). The structure was solved by direct methods using SHELXL-97 and was refined using full-matrix least-squares calculation on F^2 using SHELXL-97. The hydrogen atom positions were geometrically idealized and were allowed to ride on their parent atoms. Non-hydrogen atoms were refined anisotropically. Crystallographic data for **1** has been deposited at the Cambridge Crystallographic Data Centre under the deposition number CCDC 1401060. Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [Tel: (+44) 1223−336−408. Fax: (+44) 1223-336-033. E-mail: deposit@ccdc.cam.ac.uk].

Crystal Data of **1**: $C_{35}H_{40}O_9$, M_r = 604.67, orthorhombic, $P212121$, a = 9.1983 (2) Å, b = 18.6272 (4) Å, c = 18.7098 (4) Å, V = 3205.71 (12) Å^3, Z = 4, D_{calc} = 1.253 mg/m^3. Crystal dimensions: 0.2 × 0.1 × 0.05 mm^3, μ = 0.74 mm^{-1}, $F(000)$ = 1288, T = 296 K. Independent reflections: 5599 (R_{int} = 0.0063). The final R_1 values were 0.035, wR_2 = 0.087 [$I > 2\sigma(I)$]. Flack parameter: 0.14 (14).

Biological Assay. *Animals.* Imprinting control region (ICR) mice (18−20 g) were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. The detailed operation is described in the Supporting Information.

Behavioral Test. Animal behavior was determined by a passageway water maze, as described above.[50] The detailed operation is described in the Supporting Information.

Cell Culture and Compound Treatment. Human neuroblastoma SH-SY5Y cells were purchased from ATCC and were maintained in MEM/F12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin in a humid atmosphere of 5% CO_2 at 37 °C. Cells were pretreated with each compound (1 and 10 μM) and epigallocatechin gallate (EGCG, 10 μM) for 2 h, followed by exposure to 10 μM of Aβ_{25-35} (Sigma) in the presence of compounds for another 24 h.

MTT Assay. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sangon Biotech) reduction assay, as previously described.[22b] The detailed operation is described in Supporting Information.

Statistical Analysis. The detailed operation is described in Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00234.

UV, IR, MS, HRMS, ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, NOESY, HSQC, and HMBC spectra of all new compounds described and the ECD spectra of compounds **1**−**6**, **21**, and **22**; ^{1}H NMR spectra of compounds **67**−**70**; detailed operation of portions of the biological assays, such as animals, behavioral test, MTT assay, and statistical analysis (PDF)

Crystallographic data for compound **1** (CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel/Fax: 86-21-50806052. E-mail: wmzhao@simm.ac.cn (W.Z.).

*Tel/Fax: 86-21-50806710. E-mail: hzhang@simm.ac.cn (H.Z.).

Author Contributions

§These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (81473113) and the State Key Laboratory of Drug Research (SIMM1403KF-04). We are grateful to Prof. Zhixin Ju of Jilin Agricultural University for collection and identification of the plant material of *C. flagellaris*.

■ REFERENCES

(1) Holtzman, D. M.; Morris, J. C.; Goate, A. M. *Sci. Transl. Med.* **2011**, *3*, 77sr1.

(2) (a) Anand, P.; Singh, B. *Arch. Pharmacal Res.* **2013**, *36*, 375−399. (b) Schneider, L. S.; Mangialasche, F.; Andreasen, N.; Feldman, H.; Giacobini, E.; Jones, R.; Mantua, V.; Mecocci, P.; Pani, L.; Winblad, B.; Kivipelto, M. *J. Intern. Med.* **2014**, *275*, 251−283.

(3) (a) Karran, E.; Mercken, M.; De Strooper, B. *Nat. Rev. Drug Discovery* **2011**, *10*, 698−712. (b) Palop, J. J.; Mucke, L. *Nat. Neurosci.* **2010**, *13*, 812−818.

(4) (a) Herrmann, N.; Chau, S. A.; Kircanski, I.; Lanctot, K. L. *Drugs* **2011**, *71*, 2031−2065. (b) Mayeux, R. *N. Engl. J. Med.* **2010**, *362*, 2194−2201.

(5) Mangialasche, F.; Solomon, A.; Winblad, B.; Mecocci, P.; Kivipelto, M. *Lancet Neurol.* **2010**, *9*, 702−716.

(6) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311−335.

(7) Park, S. Y.; Kim, D. S. H. L. *J. Nat. Prod.* **2002**, *65*, 1227−1231.

(8) Halpin, C. M.; Reilly, C.; Walsh, J. J. *J. Chem. Educ.* **2010**, *87*, 1242−1243.

(9) Planchard, M. S.; Same, M. A.; Kumar, A.; Rangachari, V. *ACS Chem. Neurosci.* **2012**, *3*, 900−908.

(10) (a) Spivey, A. C.; Weston, M.; Woodhead, S. *Chem. Soc. Rev.* **2002**, *31*, 43−59. (b) Gao, J. M.; Wu, W. J.; Zhang, J. W.; Konishi, Y. *Nat. Prod. Rep.* **2007**, *24*, 1153−1189.

(11) (a) Perez-Victoria, J. M.; Tincusi, B. M.; Jimenez, I. A.; Bazzocchi, I. L.; Gupta, M. P.; Castanys, S.; Gamarro, F.; Ravelo, A. G. *J. Med. Chem.* **1999**, *42*, 4388−4393. (b) Kennedy, M. L.; Cortes-Selva, F.; Perez-Victoria, J. M.; Jimenez, I. A.; Gonzalez, A. G.; Munoz, O. M.; Gamarro, F.; Castanys, S.; Ravelo, A. G. *J. Med. Chem.* **2001**, *44*,

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX

4668−4676. (c) Reyes, C. P.; Munoz-Martinez, F.; Torrecillas, I. R.; Mendoza, C. R.; Gamarro, F.; Bazzocchi, I. L.; Nunez, M. J.; Pardo, L.; Castanys, S.; Campillo, M.; Jimenez, I. A. *J. Med. Chem.* **2007**, *50*, 4808−4817.
(12) Takaishi, Y.; Ujita, K.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. *Cancer Lett.* **1992**, *67*, 215.
(13) Mba'ning, B. M.; Lenta, B. N.; Noungoue, D. T.; Antheaume, C.; Fongang, Y. F.; Ngouela, S. A.; Boyom, F. F.; Rosenthal, P. J.; Tsamo, E.; Sewald, N.; Laatsch, H. *Phytochemistry* **2013**, *96*, 347−352.
(14) Chen, J. J.; Yang, C. S.; Peng, C. F.; Chen, I. S.; Miaw, C. L.; Huang, Y. M.; Wang, Y. S.; Chen, S. H. *Planta Med.* **2008**, *74*, 1039.
(15) Jin, H. Z.; Hwang, B. Y.; Kim, H. S.; Lee, J. H.; Kim, Y. H.; Lee, J. J. *J. Nat. Prod.* **2002**, *65*, 89−91.
(16) Gutierrez-Nicolas, F.; Oberti, J. C.; Ravelo, A. G.; Estevez-Braun, A. *J. Nat. Prod.* **2014**, *77*, 1853−1863.
(17) Kuo, Y. H.; King, M. L.; Chen, C. F.; Chen, H. Y.; Chen, C. H.; Chen, K.; Lee, K. H. *J. Nat. Prod.* **1994**, *57*, 263−269.
(18) Luo, Y. G.; Pu, X.; Luo, G. Y.; Zhou, M.; Ye, Q.; Liu, Y.; Gu, J.; Qi, H. Y.; Li, G. Y.; Zhang, G. L. *J. Nat. Prod.* **2014**, *77*, 1650−1657.
(19) Ji, Z. Q.; Wu, W. J.; Yang, H.; Shi, B. J.; Wang, M. G. *Nat. Prod. Res.* **2007**, *21*, 334−342.
(20) Chinese Medicinal Material Corporation. *Chinese Medicine Resources Notes* (Zhongguo Zhongyao Ziyuan Zhiyao); Science Press: Beijing, 1994; pp 692−694.
(21) Godkar, P. B.; Gordon, R. K.; Ravindran, A.; Doctor, B. P. *Phytomedicine* **2006**, *13*, 29−36.
(22) (a) Wang, L. Y.; Wu, J.; Yang, Z.; Wang, X. J.; Fu, Y.; Liu, S. Z.; Wang, H. M.; Zhu, W. L.; Zhang, H. Y.; Zhao, W. M. *J. Nat. Prod.* **2013**, *76*, 745−749. (b) Wang, X. J.; Wang, L. Y.; Fu, Y.; Wu, J.; Tang, X. C.; Zhao, W. M.; Zhang, H. Y. *Phytomedicine* **2013**, *20*, 1064−1070.
(23) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
(24) Weng, J. R.; Yen, M. H. *Helv. Chim. Acta* **2010**, *93*, 1716−1724.
(25) Guo, Y. Q.; Li, X.; Xu, J.; Meng, D. L.; Li, Y. S.; Ohizumi, Y. *Chem. Lett.* **2005**, *34*, 764−765.
(26) Wang, M. A.; Chen, F. H. *J. Nat. Prod.* **1997**, *60*, 602−603.
(27) Takaishi, Y.; Ohshima, S.; Nakano, K.; Tomimatsu, T.; Tokuda, H.; Nishino, H.; Iwashima, A. *J. Nat. Prod.* **1993**, *56*, 815−824.
(28) Zhu, Y. D.; Miao, Z. H.; Ding, J.; Zhao, W. M. *J. Nat. Prod.* **2008**, *71*, 1005−1010.
(29) Takaishi, Y.; Aihara, F.; Tamai, S.; Nakano, K.; Tomimatsu, T. *Phytochemistry* **1992**, *31*, 3943−3947.
(30) Guo, Y. Q.; Li, X.; Ma, Z. J.; Cui, W. S.; Wang, J. H.; Ju, Z. X. *J. Asian Nat. Prod. Res.* **2005**, *7*, 157−160.
(31) Sang, H.; Wang, H. Q.; Tu, Y. Q.; Chen, Y. Z. *Magn. Reson. Chem.* **1991**, *29*, 650−655.
(32) Munoz-Martinez, F.; Mendoza, C. R.; Bazzocchi, I. L.; Castanys, S.; Jimenez, I. A.; Gamarro, F. *J. Med. Chem.* **2005**, *48*, 4266−4275.
(33) Wang, Y. H.; Yang, L.; Tu, Y. Q.; Zhang, K.; Chen, Y. Z.; Fan, A. S. *J. Nat. Prod.* **1998**, *61*, 942−944.
(34) Liu, J. K.; Cheng, C. Q.; Wu, D. G. *Phytochemistry* **1993**, *32*, 379−381.
(35) Tu, Y. Q.; Ma, Y. X. *Phytochemistry* **1993**, *32*, 1339−1342.
(36) Tu, Y. Q.; C, Y. X.; Ma, Y. X. *Chin. Chem. Lett.* **1993**, *4*, 219−220.
(37) Cheng, C. Q.; Wu, D. G.; Liu, J. K. *Phytochemistry* **1992**, *31*, 2777−2780.
(38) Takaishi, Y.; Noguchi, H.; Murakami, K.; Nakano, K.; Tomimatsu, T. *Phytochemistry* **1990**, *29*, 3869−3873.
(39) Wu, W.; Wang, M.; Zhu, J.; Zhou, W.; Hu, Z.; Ji, Z. *J. Nat. Prod.* **2001**, *64*, 364−367.
(40) Wu, W. J.; Zhao, T. Z.; Shang, Y. J.; Fu, J. G.; Zhang, H. Y. *Chin. Chem. Lett.* **2004**, *15*, 41−42.
(41) Zhang, H. Y.; Zhao, T. Z.; Wei, Y.; Liu, H. M. *J. Asian Nat. Prod. Res.* **2011**, *13*, 304−311.
(42) Das, U. N. *Nutrition* **2003**, *19*, 988−993.
(43) Safer, D. J.; Allen, R. P. *Biol. Psychiatry* **1971**, *3*, 347−355.
(44) Klinkenberg, I.; Blokland, A. *Neurosci. Biobehav. Rev.* **2010**, *34*, 1307−1350.
(45) Yu, H.; Yao, L.; Zhou, H.; Qu, S.; Zeng, X.; Zhou, D.; Zhou, Y.; Li, X.; Liu, Z. *Neurochem. Int.* **2014**, *75*, 89−95.
(46) Kaminsky, Y. G.; Marlatt, M. W.; Smith, M. A.; Kosenko, E. A. *Exp. Neurol.* **2010**, *221*, 26−37.
(47) Gao, X.; Zheng, C. Y.; Qin, G. W.; Tang, X. C.; Zhang, H. Y. *J. Neurosci. Res.* **2012**, *90*, 1981−1988.
(48) Smith, C. R., Jr.; Miller, R. W.; Weisleder, D.; Rohwedder, W. K. *J. Org. Chem.* **1976**, *41*, 3264−3269.
(49) (a) Torres-Romero, D.; Muñoz-Martínez, F.; Jiménez, I. A.; Castanys, S.; Gamarro, F.; Bazzocchi, I. L. *Org. Biomol. Chem.* **2009**, *7*, 5166−5172. (b) Tu, Y.; Wu, D.; Zhou, J.; Chen, Y. *Phytochemistry* **1990**, *29*, 2923−2926.
(50) (a) Liu, J.; Zhang, H. Y.; Tang, X. C.; Wang, B.; He, X. C.; Bai, D. L. *Zhongguo Yao Li Xue Bao* **1998**, *19*, 413−416. (b) Wang, R.; Tang, Y.; Feng, B.; Ye, C.; Fang, L.; Zhang, L.; Li, L. *Neuroscience* **2007**, *149*, 739−746.

DOI: 10.1021/acs.jnatprod.5b00234
J. Nat. Prod. XXXX, XXX, XXX−XXX