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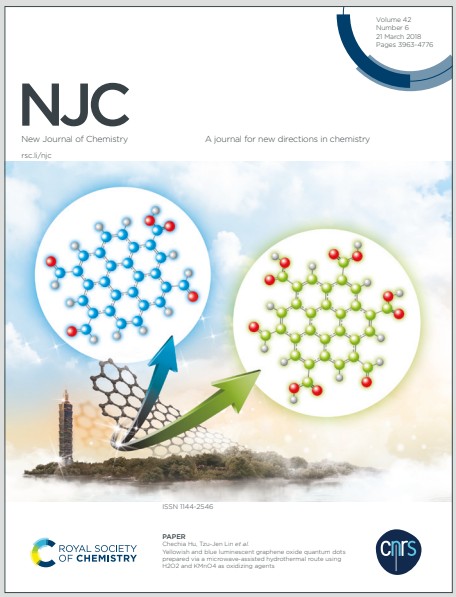

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Alkaloids and lignans with acetylcholinesterase inhibitory activity from the flower buds of *Magnolia biondii* Pamp

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Abstract

Two previously undescribed alkaloids, 4,4'-dihydroxy-3-methoxy-paucine-4'-*O*-*β*-D-glucopyranoside, (*S*)-2-(1,3-propanediol-2-yl)-isococlaurine, and three new lignans, (7*R*,8*S*)-9,9'-dihydroxy-3,4-dimethoxy-7,8-dihydrobenzofuran lignan, (7*R*,8*S*)-4,9,9'-trihydroxy-3,3'-dimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-glucopyranoside, (7*S*,8*R*)-4,9,9'-trihydroxy-3,3',5-trimethoxy-9'-acetoxy-7,8-dihydro-benzofuran lignan-4-*O*-*β*-D-glucopyranoside, together with eleven known compounds, were isolated from the flower buds of *Magnolia biondii* Pamp. Their structures were determined by extensive spectroscopic analysis, and their absolute configurations were deduced by analysis of optical rotation and electronic circular dichroic (ECD) spectra. All the isolated compounds were evaluated *in vitro* for their acetylcholinesterase (AChE) inhibitory activity. As a result, (+)-isococlaurine exhibited strongest AChE inhibitory effect. Molecular docking experiments were carried out to identify the probable binding mode of this compound in the binding sites of AChE and molecular dynamics simulations were performed to evaluate the stability over time of the main interactions observed in docking calculations.

Key words: *Magnolia biondii* Pamp; Magnoliaceae; alkaloids; lignans; acetylcholinesterase inhibitory activity; molecular docking; molecular dynamic

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simulations

Introduction

The plant of *Magnolia biondii* Pamp (family Magnoliaceae) is a perennial herb mainly distributed in Henan, China.[1] Its flower buds, commonly known as Xin-yi in China, have been used in traditional medicine to treat allergic rhinitis, sinusitis, and headache.[2] Previous phytochemical studies on this herb resulted in the identification of variety of constituents including esstenial oils, lignans, neolignans, monoterpends, sesquiterpends, and alkaloids.[3–7]

Alkaloids have been reported as important chemical constituents in *M. biondii*, which possess significant activity, especially antitumor, antibacterial, anti-inflammatory, antioxidative, and anti-neurodegenerative activity.[8,9] In our efforts to discover more bioactive constituents from *M. biondii*, sixteen compounds, including two new alkaloids, 4,4'-dihydroxy-3-methoxy-paucine-4'-*O*-*β*-D-glucopyranoside (**1**), (*S*)-2-(1,3-propanediol-2-yl)-isococlaurine (**2**), and three new lignans, (7*R*,8*S*)-9,9'-dihydroxy-3,4-dimethoxy-7,8-dihydrobenzofuran lignan (**3**), (7*R*,8*S*)-4,9,9'-trihydroxy-3,3'-dimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-glucopyranoside (**4**), (7*S*,8*R*)-4,9,9'-trihydroxy-3,3',5-trimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-glucopyranoside (**5**), together with eleven known compounds (**6**–**16**), were isolated (Fig. 1). Herein, the isolation, structure elucidation, acetylcholinesterase inhibitory activity of all the compounds, and molecular docking experiment, molecular dynamic simulations of **8** with AChE would be described.

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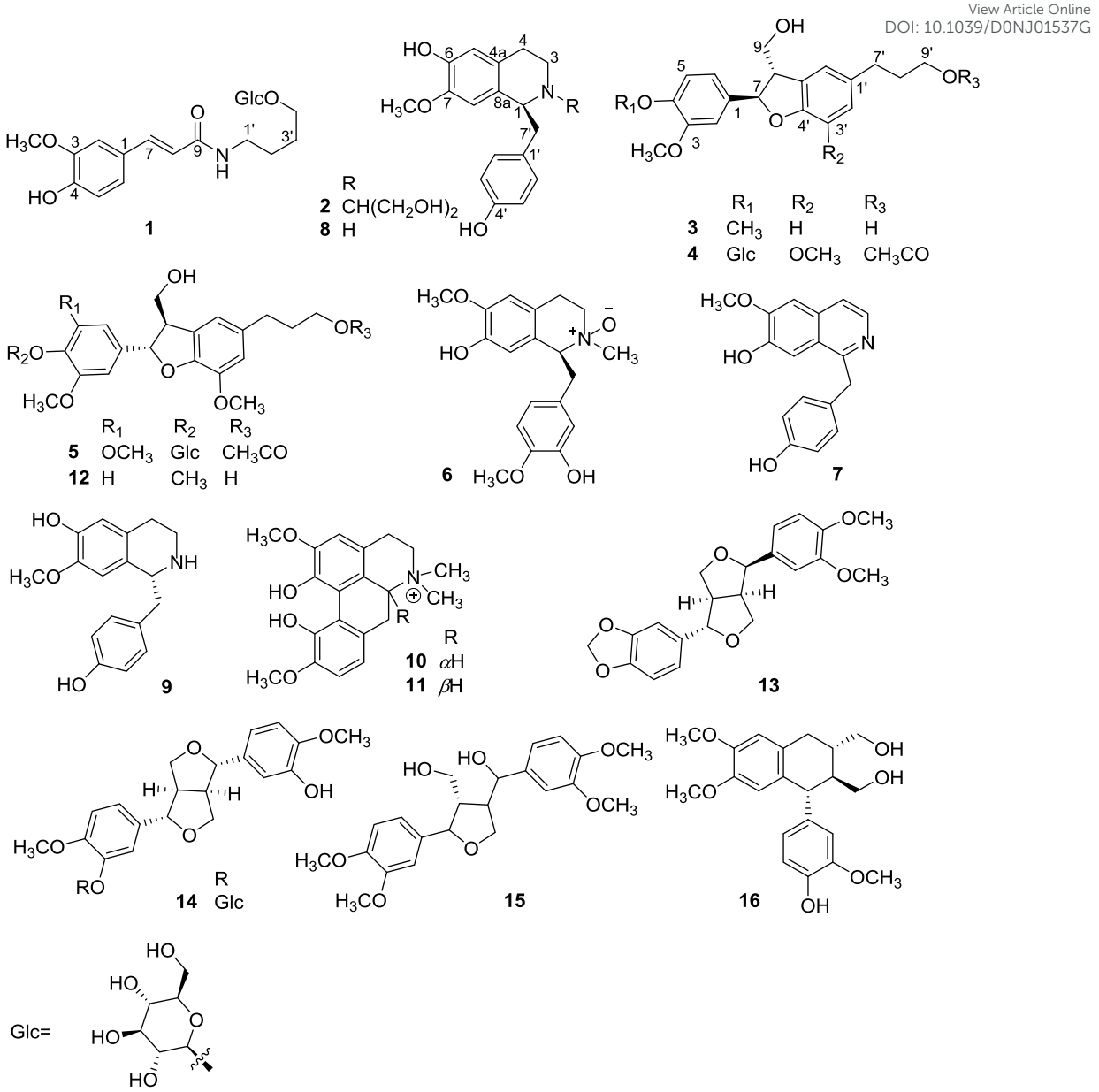

Fig. 1 Structures of compounds **1**–**16**.

Results and Discussion

Compound **1** was isolated as a yellow crystal with a molecular formula of $C_{20}H_{29}NO_9$ established by the HRESIMS and NMR data. The ^{1}H NMR data of **1** (Table 1) revealed signals for an 1,3,4-trisubstituted aromatic ring [δ_H 7.11 (1H, d, J = 1.6 Hz), 7.02 (1H, dd, J = 8.2, 1.6 Hz), and 6.78 (1H, d, J = 8.2 Hz)], a conjugated olefinic group [δ_H 7.43 (1H, d, J = 15.6 Hz) and 6.42 (1H, d, J = 15.6 Hz)], an oxygenated methylene [δ_H 3.94 (1H, m) and 3.59 (1H, m)], a methoxyl group [δ_H 3.88 (s)], and a nitrogenated methylene [δ_H 3.32 (2H, m)]. Furthermore, an anomeric proton resonance was observed at δ_H 4.25 (1H, d, J = 7.8 Hz), while additional sugar signals appeared at δ_H 3.15–3.85.

Its ^{13}C NMR data (Table 1) indicated the presence of a carbonyl carbon (δ_C 169.2), three sp^2 quaternary carbons (δ_C 150.0, 149.3, and 128.2), five sp^2 methine carbons (δ_C 142.0, 123.2, 118.7, 116.5, and 111.5), a sp^3 oxymethlene carbon (δ_C 70.3), a methoxyl group (δ_C 56.4), as well as six monosaccharide carbons (δ_C 104.4, 78.1, 78.0, 75.2, 71.7, and 62.8). The above NMR data resembled those of paucine 3'-β-D-glucopyranoside,[10] but for the replacement of the nitrogenated methylene group by the oxygenated methylene group and the presence of the methoxyl group. Acid hydrolysis of **1** yielded D-glucose, which was detected in the hydrolysate by chiral-phase HPLC analysis. β-configuration of the D-glucose was determined based on coupling constant (J = 7.8 Hz) of the anomeric proton. The D-glucose was attached to C-4' due to the HMBC correlation of H-1" (δ_H 4.25)/C-4' (δ_C 70.3). The methoxyl group was located at C-3 based on the HMBC correlation from protons of the methoxyl group (δ_H 3.88) to C-3 (δ_C 149.3) (Fig. 2). Therefore, the structure of **1** (Fig. 1) was elucidated as 4,4'-dihydroxy-3-methoxy-paucine-4'-*O*-β-D-glucopyranoside.

Table 1 ^1H and ^{13}C NMR Spectroscopic Data for **1** in CD_3OD (500 MHz for ^1H, 125 MHz for ^{13}C).

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1		128.2	3'	1.67, m	27.2
2	7.11, d (1.6)	111.5	4'α	3.94, m	70.3
3		149.3	4'β	3.59, m	
4		150.0	1"	4.25, d (7.8)	104.4
5	6.78, d (8.2)	116.5	2"	3.17, dd (8.8, 8.0)	75.2
6	7.02, dd (8.2, 1.6)	123.2	3"	3.26[a]	78.0
7	7.43, d (15.6)	142.0	4"	3.27[a]	71.7
8	6.42, d (15.6)	118.7	5"	3.34, m	78.1
9		169.2	6"α	3.85[a]	62.8
1'	3.32, m	40.2	6"β	3.66, dd (11.8, 5.2)	
2'	1.68, m	28.1	3-OCH_3	3.88, s	56.4

[a] overlapped signals.

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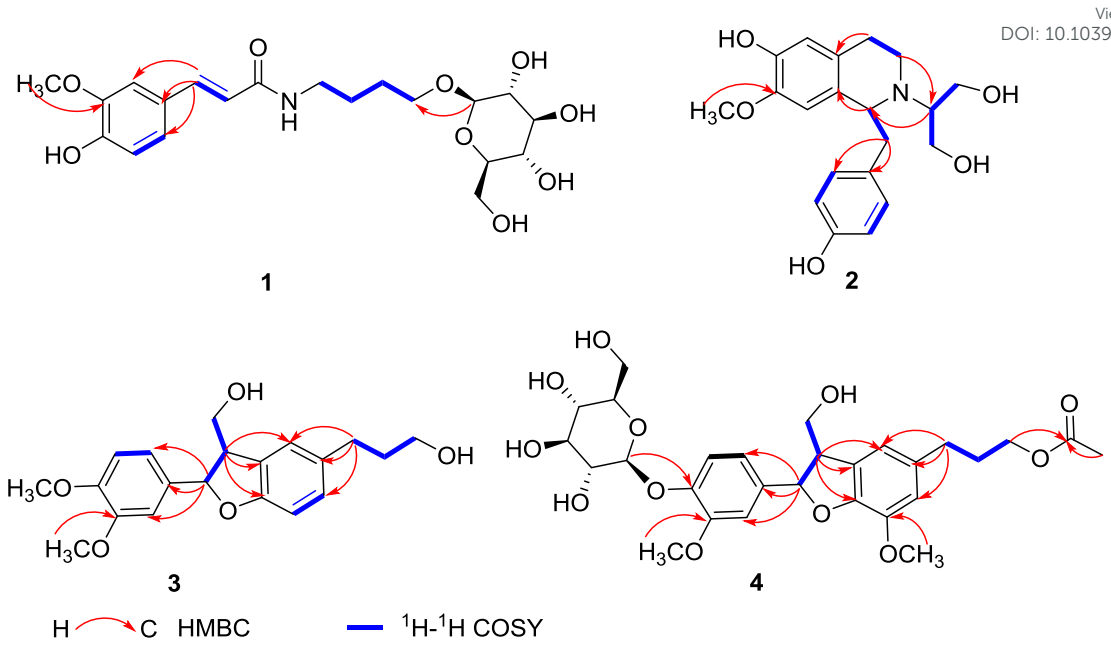

Fig. 2 Key HMBC and ^{1}H-^{1}H COSY correlations of **1**–**4**.

Compound **2** was isolated as a yellow crystal. Its molecular formula was determined to be $C_{20}H_{25}NO_5$ by the HRESIMS and NMR data. The ^{1}H NMR spectra of **2** (Table 2) displayed resonances for an 1,4-disubstituted aromatic ring [δ_H 7.12, (2H, d, J = 8.0 Hz) and 6.80 (2H, d, J = 8.0 Hz)], an 1,2,4,5,-tetrasubsituted aromatic ring [δ_H 6.77 (1H, s) and 6.64 (1H, s)], two nitrogenated methines [δ_H 4.60 (1H, dd, J = 8.6, 5.6 Hz) and 3.65 (1H, m)], two oxygenated methylenes [δ_H 3.58 (2H, dd, J = 11.1, 4.7 Hz) and 3.51 (2H, dd, J = 11.1, 5.8 Hz)], a nitrogenated methylene [δ_H 3.48 (1H, m) and 3.25 (1H, m)], and a methoxyl group [δ_H 3.84 (3H, s)]. Twenty carbon signals in its ^{13}C NMR (Table 2) and DEPT135 spectra were attributed to six sp^2 quaternary carbons (δ_C 158.3, 149.3, 146.9, 126.9, 125.1, and 123.6), six sp^2 methine carbons [δ_C 131.7 (2 × C), 117.0 (2 × C), 114.2, and 112.6], two sp^3 nitrogenated methine carbons (δ_C 73.8 and 57.9), two sp^3 oxymethylene carbons (δ_C 64.4), a sp^3 nitrogenated methylene carbon (δ_C 40.8), and a methoxyl group (δ_C 56.4). The 1D NMR data of **2** were similar to those of isococlaurine,[11] with the main difference being the presence of an 1,3-propanediol-2-yl moiety attached to the N-2, which was determined by the HMBC correlation of H-1'' (δ_H 3.65) with C-1 (δ_C 57.9) (Fig. 2).

Table 2 ^{1}H and ^{13}C NMR Spectroscopic Data for **2** in CD_3OD (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1	4.60, dd, (8.6, 5.6)	57.9	3'	6.80, d (8.0)	117.0

3α	3.48, m	40.8	4'		158.3
3β	3.25, m		5'	6.80, d (8.0)	117.0
4	3.03, m	25.9	6'	7.12, d (8.0)	131.7
			7'α	3.39[a]	40.4
4a		123.6	7'β	2.98[a]	
5	6.64, s	114.2	1"	3.65, m	73.8
6		146.9	2"α	3.58, dd (11.1, 4.7)	64.4
7		149.3	2"β	3.51, dd (11.1, 5.8)	
8	6.77, s	112.6	3"α	3.58, dd (11.1, 4.7)	64.4
8a		125.1	3"β	3.51, dd (11.1, 5.8)	
1'		126.9	7-OCH_3	3.84, s	56.4
2'	7.12, d (8.0)	131.7			

[a] overlapped signals.

The (*S*) absolute configuration of **2** was determined by comparing its optical rotation $[\alpha]_D^{20}$ –15.7 (c 0.08; MeOH) with that of the similar published compound (+)-isococlaurine.[12] Thus, the structure of compound **2** (Fig. 1) was defined as (*S*)-2-(1,3-propanediol-2-yl)-isococlaurine.

Compound **3** was isolated as a pale yellow solid with a molecular formula of $C_{20}H_{24}O_5$ determined by the HRESIMS and NMR data. In its ^{1}H NMR spectrum (Table 3), the signals indicated the presence of two 1,3,4-trisubstituted benzene rings [δ_H 7.08 (1H, s), 7.00 (1H, d, J = 8.2 Hz), 6.95 (1H, s), 6.92 (1H, d, J = 8.5 Hz), 6.90 (1H, d, J = 8.5 Hz), and 6.72 (1H, d, J = 8.2 Hz)], an oxymethine [δ_H 5.48 (1H, d, J = 5.9 Hz)], two oxymethylenes [δ_H 3.83 (1H, dd, J = 13.4, 8.0 Hz), 3.76 (1H, dd, J = 13.4, 4.4 Hz), and 3.55 (2H, t, J = 6.5 Hz)], and two methoxyl groups [δ_H 3.80 (3H, s) and 3.78 (3H, s)]. In the ^{13}C NMR spectrum of **3** (Table 3), 20 carbon signals, including six sp^2 quaternary carbons (δ_C 159.4, 150.6, 150.2, 136.5, 135.7, and 128.7), six sp^2 methine carbons (δ_C 129.7, 125.9, 119.4, 112.9, 110.6, and 109.8), a sp^3 oxygenated methine carbon (δ_C 88.2), two sp^3 oxygenated methylene carbons (δ_C 65.2 and 62.2), and two methoxyl groups (δ_C 56.5 and 56.4) were observed. These NMR data mentioned above was similar to those of 4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol,[13] except for an extra methoxyl group attached to C-3, which was confirmed by the HMBC correlation of methoxyl group protons (δ_H 3.80) with C-3 (δ_C 150.2) (Fig. 2).

Table 3 ^{1}H and ^{13}C NMR Spectroscopic Data for **3**–**5** in CD_3OD (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	**3**		**4**		**5**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		135.7		136.3		135.9
2	6.95, s	110.6	7.02, s	111.2	6.73, s	104.4
3		150.2		151.0		154.4
4		150.6		147.6		136.4
5	6.92, d (8.5)	112.9	7.13, d (8.4)	118.1		154.4
6	6.90, d (8.5)	119.4	6.92, d (8.4)	119.4	6.73, s	104.4
7	5.48, d (5.9)	88.2	5.55, d (5.6)	88.5	5.56, d (5.6)	88.5
8	3.44, m	55.2	3.45, m	55.7	3.46, m	55.7
9α	3.83, dd (13.4, 8.0)	65.2	3.85[a]	65.0	3.85, dd (12.1, 6.1)	65.0
9β	3.76, dd (13.4, 4.4)		3.74[a]		3.77, dd (12.1, 4.8)	
1'		136.5		137.1		136.4
2'	7.00, d (8.2)	129.7	6.71, s	114.2	6.73, s	114.3
3'	6.72, d (8.2)	109.8		138.3		140.3
4'		159.4		145.3		145.4
5'		128.7		129.6		129.6
6'	7.08, s	125.9	6.70, s	118.0	6.70, s	118.0
7'	2.61, t (7.6)	32.6	2.63, dd (14.6, 7.0)	32.9	2.63, dd (14.8, 7.1)	32.9
8'	1.79, m	35.9	1.92, m	31.7	1.91, m	31.7
9'	3.55, t (6.5)	62.2	4.05, t (6.3)	65.1	4.07, t (6.5)	65.1
1"				173.1		173.1
2"			2.02, s	20.8	2.01, s	20.8
1'"			4.89, d (7.8)	102.8	4.88, d (7.8)	105.3
2'"			3.48[a]	74.9	3.86[a]	75.7
3'"			3.46[a]	77.9	3.84[a]	77.8
4'"			3.37[a]	71.4	3.85[a]	71.3
5'"			3.38, m	78.2	3.19, m	78.3
6'"α			3.67, br d (10.4)	62.5	3.65, dd (9.8, 4.6)	62.6
6'"β			3.57, t (6.4)		3.45, d (9.8)	
3-OCH_3	3.80, s	56.5	3.82, s	56.8	3.81, s	57.0
4-OCH_3	3.78, s	56.4				
5-OCH_3					3.81, s	57.0
3'-OCH_3			3.86, s	56.7	3.87, s	56.8

[a] overlapped signals.

The relative-*trans* configuration of **3** was established on the basis of the coupling constant $J_{7,8}$ (5.9 Hz) in accordance with literature reports.[9] The (7*R*,8*S*) absolute configuration was determined by the negative Cotton effect at 291 nm ($\Delta\varepsilon$ – 1.7) observed in its electronic circular dichroic (ECD) spectrum (Fig. 3).[14] Thus, the

structure of **3** was elucidated as (7*R*,8*S*)-9,9'-dihydroxy-3,4-dimethoxy-7,8-dihydrobenzofuran lignan.

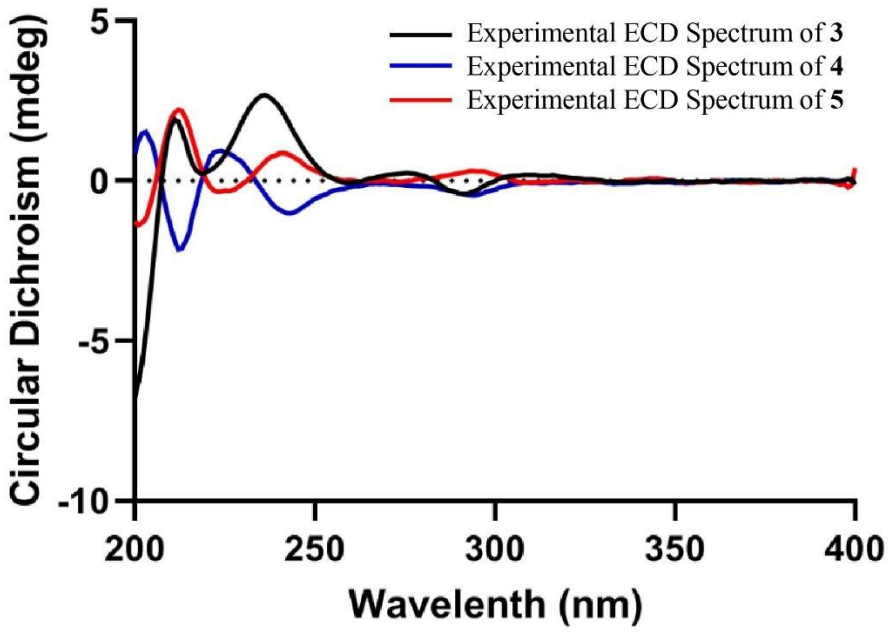

Fig. 3 Experimental ECD spectra of **3**–**5**.

Compound **4** was isolated as a pale yellow solid with a molecular formula of $C_{28}H_{36}O_{12}$ established by the HRESIMS and NMR data. The 1D NMR data of **4** (Table 3) revealed similarities with those of **3**, with the main difference being the presence of an acetyl group and *β*-glucopyranosyl moiety. The acetyl group was linked to C-9' based on the correlation from H-9' (δ_H 4.05) to C-1'' (δ_C 173.1) in the HMBC spectrum. The methoxyl groups was located at C-3 and C-3', respectively, based on the HMBC correlations from the protons of 3-OCH_3 (δ_H 3.82) to C-3 (δ_C 151.0) and from the protons of 3'-OCH_3 (δ_H 3.86) to C-3' (δ_C 138.3). The hexose moiety was identified as D-glucose by chiral-phase HPLC analysis of the acid hydrolysate of **4**, which was attached to C-4 due to the HMBC correlation of H-1''' (δ_H 4.89)/C-4 (δ_C 147.6). The relative-*trans* configuration of **4** was also established on the basis of the coupling constant $J_{7,8}$ (5.6 Hz) in accordance with **3** and literature report.[9] The negative Cotton effect at 294 nm (Δε – 1.7) observed in its ECD spectrum (Fig. 3), indicating the (7*R*,8*S*) absolute configuration of **4**.[14] Therefore, the structure of **4** was defined as (7*R*,8*S*)-4,9,9'-trihydroxy-3,3'-dimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-

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glucopyranoside.

Compound **5** was isolated as a pale yellow solid with a molecular formula of $C_{29}H_{38}O_{13}$ deduced from the HRESIMS and NMR data. The 1D NMR data of **5** (Table 3) resembled to those of **4** and differed only in the methoxyl group. The connection locations of methoxyl groups and *β*-glucopyranosyl moiety were determined based on the HMBC correlations. The coupling constant $J_{7,8}$ (5.7 Hz) suggested the relative-*trans* configuration of **5**. The (7*S*,8*R*) absolute configurations of **5** were determined due to the positive Cotton effect at 294 nm (Δε + 1.6) observed in its ECD spectrum (Fig. 3).[15–17] Therefore, the structure of **5** was defined as (7*S*,8*R*)-4,9,9'-trihydroxy-3,3',5-trimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-glucopyranoside.

By comparison of the NMR data with those reported in the literatures, eleven known compounds were identified as (+)-reticuline N-oxide (**6**),[18] 4'-hydroxybenzyl-6-methoxy-7-hydroxyisoquinoline (**7**),[19] (+)-isococlaurine (**8**),[11,12] (–)-isococlaurine (**9**),[11,12] *α*-magnoflorine (**10**),[20] *β*-magnoflorine (**11**),[20] 3',4-*O*-dimethylcedrusin (**12**),[21] (+)-fargesin (**13**),[22] 4,4'-dimethoxy-3'-hydroxy-7,9':7',9-diepoxy lignan-3-*O*-*β*-D-glucopyranoside (**14**),[23] fargesol (**15**),[24] scaphopetalone (**16**).[25]

All the isolated compounds (**1**–**16**) were evaluated *in vitro* for their AChE inhibitory activity. As a result, compounds **1**, **6**, and **8** exhibited moderate AChE inhibitory effect with IC_{50} values of 12.5 ± 2.4, 10.4 ± 2.5, and 8.2 ± 1.8 *μ*M (Table 4).

Table 4 Acetylcholinesterase inhibitory effects of compound **1**–**16**.

Compounds	IC_{50} (*μ*M)
1	12.5 ± 2.5
2	26.1 ± 2.8
3	30.4 ± 3.2
4	>50
5	>50
6	10.4 ± 2.4
7	29.9 ± 3.7
8	8.2 ± 1.8
9	25.7 ± 2.9
10	>50
11	>50
12	45.2 ± 3.6
13	>50
14	>50

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15	34.7 ±3.2
16	42.6 ±2.7
tacrine[a]	7.7 ±2.3 × 10^{-3}

[a]. Positive control compound.

The docking results (Fig. 4.) indicated that two conventional hydrogen bonds between 6-OH and residue SER203 and between 7-OCH_3 and residue GLY122, a carbon hydrogen bond between H-3 and residue TYR337, four π-π stacked interactions between 4-hydroxy-benzyl moiety and residue TRP286 and between benzene ring of the isoquinoline moiety and residue TRP86, and a π-π T-shaped interaction between benzene ring of the isoquinoline moiety and residue TYR337 were observed in the complexes of compound **8** and AChE proposed by docking studies.

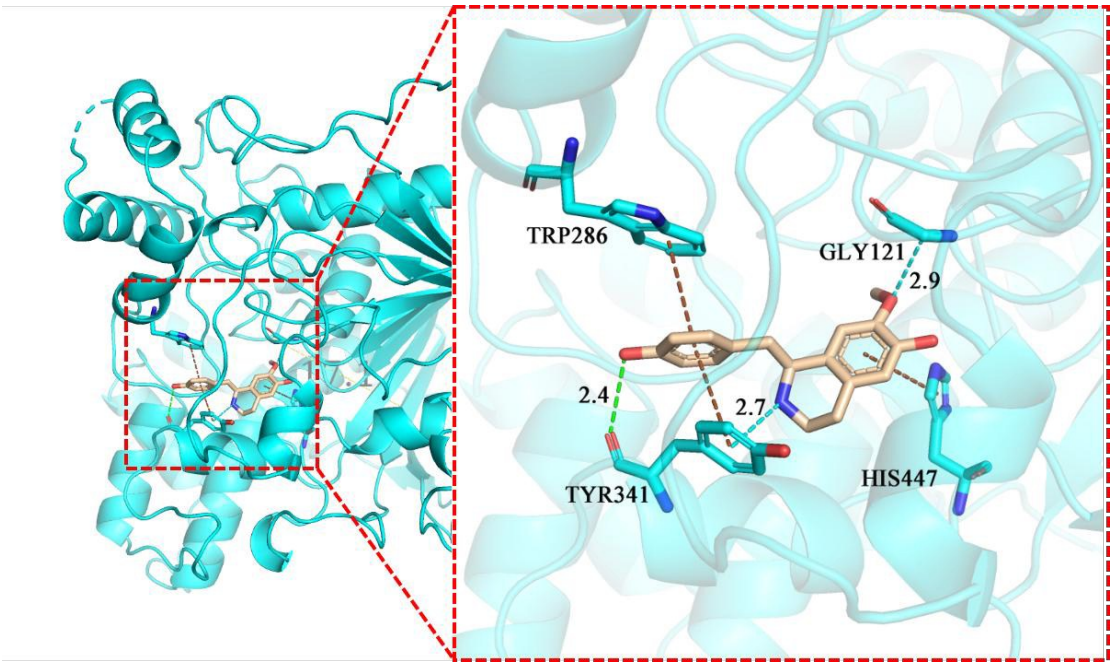

Fig. 4 The receptor-ligand interaction of **8** with AChE.

In order to evaluate the stability over time of the main interactions observed in docking calculations, molecular dynamics simulations (1000 ps, 300 K) of the docked complexes (**8** with AChE) were performed. The root mean square deviation (RMSD) data suggested that the binding of **8** stabilized AChE and lead to less structural deviations from its first frame (Fig. 5). Comparison between initial and final pose of each accommodated ligand confirmed the essential role of the conserved triad TRP^{86}-SER^{203}-TRP^{286} for carbohydrate binding (Fig. 6).

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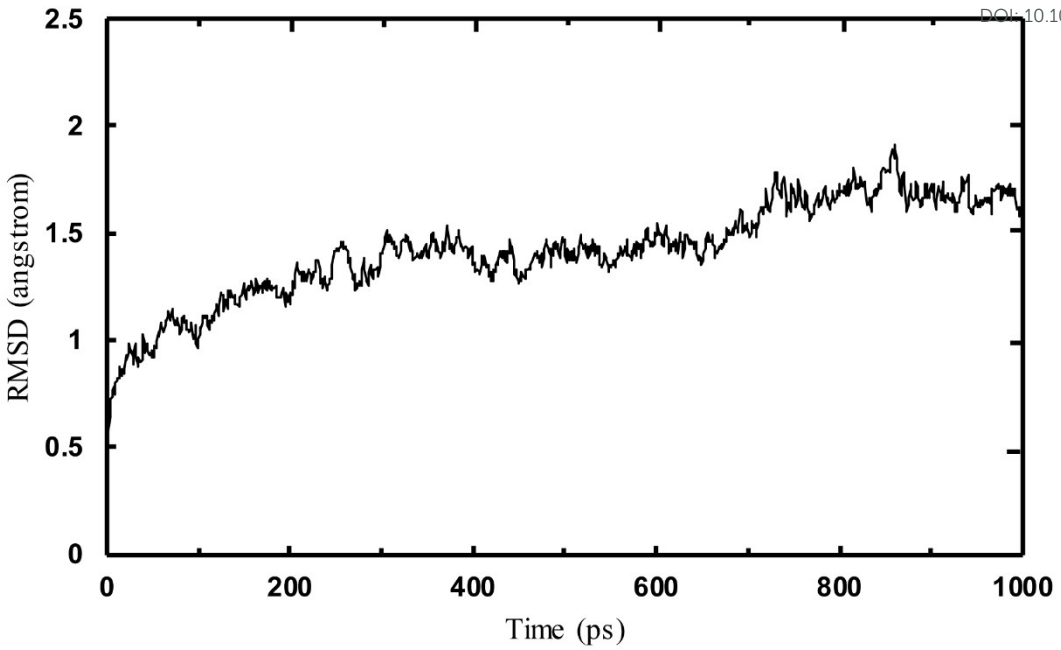

Fig. 5 RMSD plot of AChE complexed with 8 during MD simulations (1000 ps).

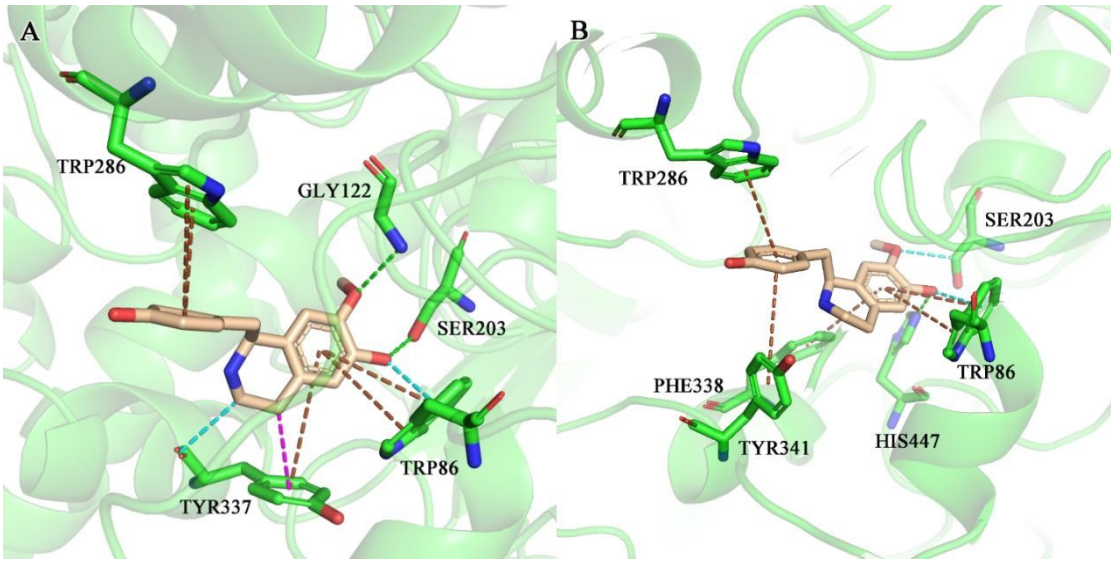

Fig. 6 The initial (A) and final (B) pose of **8** docked in the AChE during the MD simulations.

Conclusion

In this study, two new alkaloids, 4,4'-dihydroxy-3-methoxy-paucine-4"-*O*-*β*-D-glucopyranoside, (*S*)-2-(1,3-propanediol-2-yl)-isococlaurine, and three new lignans, (7*R*,8*S*)-9,9'-dihydroxy-3,4-dimethoxy-7,8-dihydrobenzofuran lignan, (7*R*,8*S*)-4,9,9'-trihydroxy-3,3'-dimethoxy-9'-acetoxy-7,8-dihydrobenzofuran lignan-4-*O*-*β*-D-glucopyranoside, (7*S*,8*R*)-4,9,9'-trihydroxy-3,3',5-trimethoxy-9'-acetoxy-7,8-dihydro-benzofuran lignan-4-*O*-*β*-D-glucopyranoside, as well as eleven known compounds,

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were isolated from flower buds of *M. biondii*. Biologically, all the isolated compounds were evaluated for their inhibitory effects against AChE. Among them, compound **8** exhibited a moderate inhibitory AChE activity with an IC_{50} value of 8.2 ± 1.8 μM. The results of molecular docking and molecular dynamics simulations experiments suggested that compound **8** bound to AChE stably.

Experimental

General experimental procedures

NMR spectra were measured by a Bruker Avance III 500 spectrometer (Bruker, Germany). MS spectra were acquired using a Bruker maXis HD mass spectrometer (Bruker, Germany). UV spectra were measured by a Thermo EVO 300 spectrometer (Thermo, Waltham, MA, USA). IR spectra were recorded on a Thermo Nicolet IS 10 spectrometer (Thermo, Waltham, MA, USA). Optical rotations were record by a Rudolph AP-Ⅳ polarimeter (Rudolph, Hackettstown, NJ, USA). ECD spectra were obtained using an Applied Photophysics Chirascan qCD spectropolarimeter (AppliedPhotophysics, Leatherhead, Surrey, UK). Semisemipreparative HPLC separations were conducted on a Saipuruisi LC 50 HPLC system, equipped with an UV/VIS 50 detector (Saipuruisi, Beijing, China). Monosaccharide elucidation was performed on a Waters 2695 separation module equiped with an evaporative light scattering detector (ELSD) (Waters, Milford, MA, USA) using a CHIRALPAK AD-H column (250 × 4.6 mm) (Daicel Chiral Technologies Co., Ltd., China). Column chromatographies were performed using MCI gel CHP-20 (TOSOH Corp., Tokyo, Japan), ODS gel (50 μm) (YMC Group, Kyoto, Japan), Sephadex LH-20 (40–70 μm) (Amersham Pharmacia Biotech AB, Uppsala, Sweden), Toyopearl HW-40C (TOSOH Corp., Tokyo, Japan), and silica gel (160–200 mesh and 200–300 mesh) (Marine Chemical Industry, Qingdao, China).

Plant material

The dry flower buds of *M. biondii* collected in Nanzhao, Henan province, China, was identified by Professor Chengming Dong, School of Pharmacy, Henan University

of Chinese Medicine, Zhengzhou, China. A voucher specimen (20140609) was deposited at the Department of Pharmaceutical Chemistry, Henan University of Chinese Medicine.

Extraction and isolation

The powder flower buds (5.0 kg) were extracted with 50% aqueous acetone three times (20 L, smashed tissue extraction) at room temperature. The combined solutions were evaporated under vacuum to give a crude extract (463 g). The crude extract was suspended in H_2O (2 L) and then successively extracted five times with petroleum ether, EtOAc and *n*-BuOH, respectively. The *n*-BuOH fraction (60.0 g) was subjected to Diaion HP-20 column chromatography (CC) eluted with an EtOH-H_2O (0:100, 20:80, 40:60, 80:20, 95:5) gradient to give five subfractions (B1–B5). B2 (14.0 g) was then applied to ODS CC eluted with a MeOH-H_2O (0:100, 5:95, 10:90, 15:85, 20:80, 25:75, 35:65,45:55, 100:0) gradient to give nine subfractions (B2-1–B2-9). B2-3 (1.9 g) was subjected to MCI gel CHP-20 CC eluted with a MeOH-H_2O (10:100, 30:70, 50:50, 70:30, 100:0) gradient to yield 5 subfractions (B2-3-1–B2-3-5). B2-3-4 (241.3 mg) was purified by semipreparative HPLC eluted with MeOH-H_2O (50:50) to give **7** (2.7 mg, t_R = 20.0 min). B2-4 (3.2 g) was subjected to silica gel CC eluted with a CH_2Cl_2-MeOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) gradient to yield 5 subfractions (B2-4-1–B2-4-5). B2-4-4 (102.4 mg) was chromatographed by Toyopearl HW-40C CC eluted with MeOH-H_2O (30:70) to give 3 subfractions (B2-4-4-1–B2-4-4-3). B2-4-4-1 (24.3 mg) was purified by semipreparative HPLC eluted by MeOH-H_2O (20:80) to give **2** (4.1 mg, t_R = 48.1 min). B2-4-4-3 (50.8 mg) was purified by semipreparative HPLC eluted with MeOH-H_2O (30:70) to give **8** (35.2 mg, t_R = 17.6 min), **9** (7.8 mg, t_R = 23.8 min), and **10** (16.1 mg, t_R = 32.4 min). B2-4-6 (982.4 mg) was applied to Sephadex LH-20 CC eluted by MeOH to give 4 subfractions (B2-4-6-1–B2-4-6-4). B2-4-6-3 (46.1 mg) was purified by semipreparative HPLC eluted with MeOH-H_2O (35:65) to give **6** (3.3 mg, t_R = 28.5 min) and **1** (6.6 mg, t_R = 30.3 min). B3 (14.5 g) was subjected to silica gel CC eluted with a CH_2Cl_2-MeOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) gradient to yield 9 subfractions (B3-1–B3-9). B3-6 (1.4 g) was applied to Sephadex LH-20 CC

eluted with MeOH to give 3 subfractions (B3-6-1–B3-6-3). B3-6-2 (122.4 mg) was purified by semipreparative HPLC eluted by MeOH-H_2O (50:50) to give **5** (66.6 mg, t_R = 18.1 min) and **4** (19.3 mg, t_R = 40.2 min). B4 (1.1 g) was subjected to silica gel CC eluted with a CH_2Cl_2-MeOH (100:0, 80:20, 60:40, 40:60 0:100) gradient to yield 8 subfractions (B4-1–B4-8). B4-2 (114.4 mg) was applied to Toyopearl HW-40C CC eluted with MeOH-H_2O (70:30) to give 2 subfractions (B4-2-1–B4-2-2). B4-2-2 (35.2 mg) was chromatographed by semipreparative HPLC eluted by MeOH-H_2O (45:55) to give **14** (4.7 mg, t_R = 25.2 min). B4-3 (278.1 mg) was applied to Sephadex LH-20 CC eluted with MeOH-H_2O (70:30) to give 4 subfractions (B4-3-1–B4-3-4). B4-3-2 (56.3 mg) was chromatographed by semipreparative HPLC eluted by MeOH-H_2O (40:60) to give **12** (16.5 mg, t_R = 19.3 min) and **16** (6.3 mg, t_R = 23.7 min). B4-3-4 (47.8 mg) was chromatographed by semipreparative HPLC eluted by MeOH-H_2O (40:60) to give **3** (7.4 mg, t_R = 19.3 min), **15** (27.2 mg, t_R = 23.7 min), and **13** (3.6 mg, t_R = 40.7 min).

Compound **1**: yellow crystal; $[\alpha]_D^{20}$ –20.9 (c 0.13, MeOH); UV (MeOH) λ_{max} 219, 234, 294, 319 nm; IR (iTR) ν_{max}: 3365, 2940, 2881, 2844, 1653, 1594, 1517, 1456, 1429, 1274, 1073, 1032 cm^{-1}; HRESIMS *m/z* 450.1737 $[M + Na]^+$ (calcd for 450.1734, $C_{20}H_{29}NO_9Na$); ^1H NMR (500MHz, CD_3OD) and ^{13}C NMR (125MHz, CD_3OD) data see Table 1.

Compound **2**: yellow crystal; $[\alpha]_D^{20}$ –15.7 (c 0.08, MeOH); UV (MeOH) λ_{max} 206, 281 nm; IR (iTR) ν_{max}: 3244, 2950, 2839, 1675, 1518, 1455, 1203, 1136, 1067 cm^{-1}; HRESIMS *m/z* 360.1803 $[M + H]^+$ (calcd for 360.1805, $C_{20}H_{26}NO_5$); ^1H NMR (500MHz, CD_3OD) and ^{13}C NMR (125MHz, CD_3OD) data see Table 2.

Compound **3**: pale yellow solid; $[\alpha]_D^{20}$ –13.6 (c 0.09, MeOH); UV (MeOH) λ_{max} 203, 226, 284 nm; IR (iTR) ν_{max}: 3352, 2936, 1652, 1595, 1515, 1488, 1453, 1262, 1234, 1022 cm^{-1}; HRESIMS *m/z* 367.1537 $[M + Na]^+$ (calcd for 367.1515, $C_{20}H_{24}O_5Na$); ^1H NMR (500MHz, CD_3OD) and ^{13}C NMR (125MHz, CD_3OD) data see Table 3.

Compound **4**: pale yellow solid; $[\alpha]_D^{20}$ –9.4 (c 0.07, MeOH); UV (MeOH) λ_{max} 205, 281 nm; IR (iTR) ν_{max}: 3368, 1683, 1207, 1140, 1073, 1036 cm^{-1}; HRESIMS *m/z* 599.1874 $[M + Cl]^-$ (calcd for 599.1889, $C_{28}H_{36}O_{12}Cl$); ^1H NMR (500MHz, CD_3OD) and ^{13}C NMR (125MHz, CD_3OD) data see Table 3.

Compound **5**: pale yellow solid; $[\alpha]_D^{20}$ –55.5 (c 0.08, MeOH); UV (MeOH) λ_{max} 207, 280, 318 nm; IR (iTR) ν_{max}: 3369, 2942, 1678, 1455, 1205, 1137, 1070, 1038 cm^{-1}; HRESIMS *m/z* 629.1981 $[M + Cl]^-$ (calcd for 629.1995, $C_{29}H_{38}O_{13}Cl$); ^1H NMR (500MHz, CD_3OD) and ^{13}C NMR (125MHz, CD_3OD) data see Table 3.

Acid hydrolysis of compounds 1, 4, and 5

Compounds **1** (1.1 mg), **4** (0.9 mg) and **5** (1.0 mg) were hydrolyzed with 2 M HCl (2.5 mL) (80 °C, 3 h). For each compound, the mixture was dried and the residue was dissolved in H_2O (2 mL) and then extracted with EtOAc (3 × 2 mL).[26] The dry aqueous was subjected to chiral-phase HPLC equipped with a Chiralpack AD-H column (250 × 4.6 mm) and an evaporative light scattering detection (ELSD), using hexane:alcohol:trifluoroacetic acid mixture (750:250:0.25) as the mobile phase (0.5 $mL \cdot min^{-1}$).[27] The retention time of the sugar from hydrolysate was similar to that of D-glucose (t_R = 16.9 min), so the sugar was identified as D-glucose.

AChE inhibition assays

AChE inhibition was determined using a modified Ellman's method.[28] 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB, Ellman's reagent), *Electrophorus electricus* acetylcholinesterase (EeAChE), and acetylcholine iodide were purchased from Sigma Chemical. The assay solution, containing 100 μL of phosphate buffer PH 8.0, 20 μL of DTNB (0.625 mM), 40 μL of EeAChE (0.02 $U \cdot mL^{-1}$), and 20 μL of test compound in DMSO, was taken in a 96-well microtiter plate and pre-incubated at 37 ºC for 20 min. Then, 20 μL of acetylcholine iodide (0.625 mM) was added into each well. The hydrolysis of acetylthiocholine was monitored at 412 nm after 30 min using a microplate reader. Tacrine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = $(E–S)/E \times 100$ (*E* is the activity of the enzyme without test compound and *S* is the activity of enzyme with test compound).

Molecular modeling and molecular dynamic simulations

Molecular docking studies were conducted using Discovery Studio 2018 program (Acclrys Inc., San Diego, USA). The X-ray crystal structure of the recombinant human AChE in complex with donepezil was obtained from the RCSB protein data bank with PDB ID: 4EY7.[29] All the water molecules and ligands in the PDB file were removed and hydrogen atoms along with Gasteiger-Marsili charges were added. The energy was minimized with CHARMm force field. The dock ligands in the set parameters are as follows: top hit 10, pose cluster radius parameter value of 0.1. Evaluation of the molecular docking was performed according to the scores in order to obtain the best pose of compound **8**.[30]

Molecular dynamic simulations for 1000 ps were performed on AChE with **8** at 300 K using the CHARMm force field in Discovery Studio 2018 program (Acclrys Inc., San Diego, USA). The system was minimized using 2000 steps of steepest descent and 2000 steps of conjugate gradient for energy minimization. The system was slowly driven from an initial temperature of 50 K to the target temperature of 300 K for 20 ps and equilibration simulations were run for 20 ps. The molecular dynamic simulations (production) were performed for 1000 ps with NPT system at a constant temperature of 300 K.[31]

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Conflicts of interest

The authors have declared that there is no conflict of interest.

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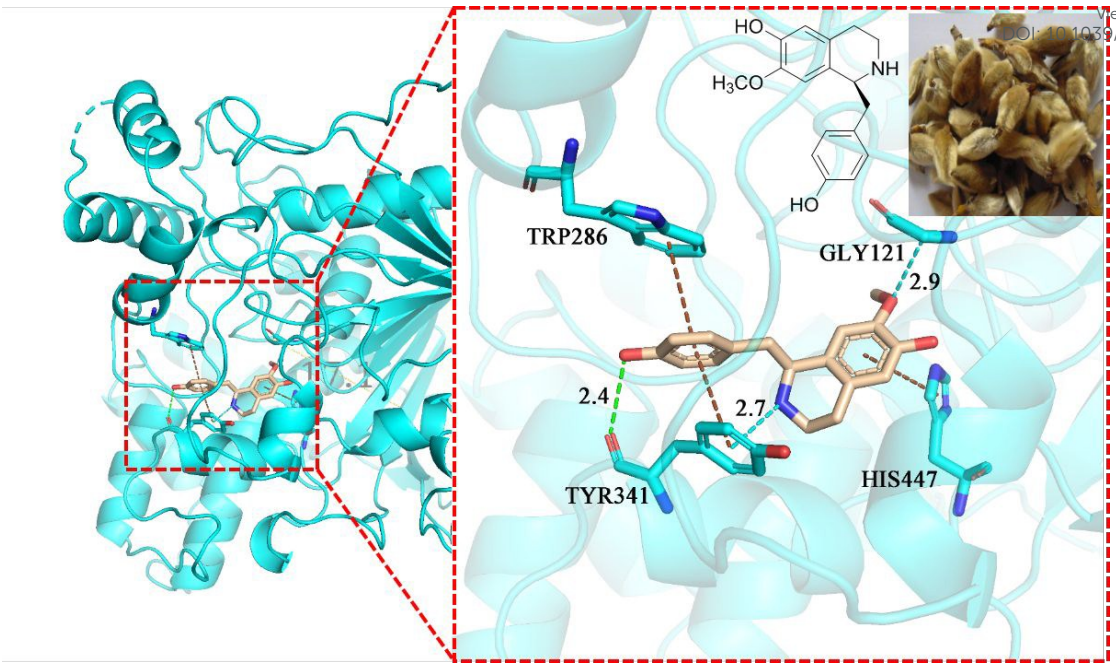

Sixteen compounds, including two new alkaloids and three new lignans, were isolated and their AChE inhibitory activities were evaluated.