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Acetylcholinesterase Inhibitory Activity of Chemical Constituents Isolated from *Miliusa thorelii*

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Abstract: Two new dihydrooxoprotoberberine alkaloids; miliusathorines A (**1**) and B (**2**), a new natural flavone, miliusathorone (**3**), together with twenty-two known compounds (**4**-**25**) were isolated from the combined stem and root extract and the leaf extract of *Miliusa thorelii*. The structures of all isolated compounds were elucidated by spectroscopic methods and mass spectrometry. All compounds were evaluated for their acetylcholinesterase activities. Miliusathorine A (**1**) and norushisunine had the best AChE inhibitory activities, however these were weak inhibitors when compared to the standard galantamine.

Key words: *Miliusa thorelii*, oxoprotoberberine alkaloid, flavone, acetylcholinesterase inhibition

1. Introduction

Miliusa thorelii, which has the local name in Thailand as "Maa-Dam", belongs to the Annonaceae family. This plant is used as a traditional medicine as an analgesic and as an aphrodisiac (Anderson, 1993) in tribal communities of the North of Thailand. Previous phytochemical studies of plants from the *Miliusa* genus have reported many types of secondary metabolites, including, alkaloids (Chen et al., 2003; Hasan et al., 2000; Promchai et al., 2016), flavonoids (Huong et al., 2005; Naphong et al., 2013; Sawasdee et al., 2014; Thuy et al., 2011), homogentisic acid derivatives (Huong et al., 2004; Kamperdick et al., 2002; Sawasdee et al., 2014), styryl derivatives (Huong et al., 2008; Kamperdick et al., 2002) and lignans (Sawasdee et al., 2010, 2013). The phytochemical constituents from this genus also exhibited significant biological activities such as anticancer (Naphong et al., 2013; Promchai et al., 2016; Sawasdee et al., 2013; Thuy et al., 2011), antimalarial (Promchai et al., 2016), and anti-herpes simplex virus types 1 and 2 activities (Sawasdee et al., 2013, 2014). No extracts or phytochemicals from plants of this genus have been tested for their acetylcholinesterase inhibitory activities. Herein, we report the first isolation and structural elucidation of three new natural compounds (**1**-**3**) as well as 22 known compounds (**4**-**25**), from the extract of the combined stems and roots and the extract of the leaves of *M. thorelii*. The structures of the isolated compound were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopy and mass spectrometry. The NMR spectroscopic data of compounds **3** and **4** had not been previously reported and are therefore provided herein. The isolated compounds were evaluated for their acetylcholinesterase inhibitory activities.

2. Results and discussion

Purification of the combined acetone extracts of the stems, roots and leaves of *M. thorelii* by chromatographic techniques gave 25 compounds, two new

dihydrooxoprotoberberine alkaloids, miliusathorines A (**1**) and B (**2**), a new natural flavone, miliusathorone (**3**) as well as 22 known compounds (**4**-**25**), quercetagetin-3,5,7-trimethyl ether (**4**) (El-Negoumy et al., 1982), (–)-norushisunine (**5**) (Chen et al., 1997), 5,3´,4´-trihydroxy-3,7-dimethoxyflavone (**6**) (Xu et al., 2012), quercetagetin-3,5,7,3´-tetramethyl ether (**7**) (Ahmed et al., 1994), 6,4´-dihydroxy-3,5,7-trimethoxyflavone (**8**) (Ahmed et al., 1994), retusin (**9**) (Citoğlu et al., 2004), 5-hydroxy-3,6,7,4´-tetramethoxyflavone (**10**) (Paula and Cruz, 2006), dimethylmikanin (**11**) (Mai et al., 2015), 3,5,7,3´,4´-pentamethoxyflavone (**12**) (Sutthanut et al., 2007), 3-*O*-methylkaemferol (**13**) (Nakatani et al., 1991), quercetin-3-*O*-methyl ether (**14**) (Krenn et al., 2003), quercetin-3,5,3´-trimethyl ether (**15**) (Vogt et al., 1988), 4´-hydroxy-3,5,6,7-tetramethoxyflavone (**16**) (Al-Dabbas et al., 2011), 5-hydroxy-3,7-dimethoxy-3´,4´-methylene-dioxyflavone (**17**) (Promchai et al., 2016), melisimplexin (**18**) (Jong et al., 1989), *N-trans*-feruloyltyramine (**19**) (Promchai et al., 2016), *N-trans*-caffeoyltyramine (**20**) (Promchai et al., 2016), melisimplin (**21**) (Calvert et al., 1979), isokanugin (**22**) (Higa et al., 1987), pachypodol (**23**) (Promchai et al., 2016), 3,5,6,7,3´,4´-hexamethoxyflavone (**24**) (Li et al., 2006), and artemetin (**25**) (Yoshioka et al., 2004) (Fig. 1).

Miliusathorine A (**1**) was obtained as a brown gum with a molecular formula of $C_{19}H_{17}NO_5$ from the HRESI-MS ion at *m/z* 362.1004 $[M+Na]^+$ (calcd 362.1004). The UV spectrum displayed the maximum absorption bands at λ_{max} 225, 261, 337 and 360 nm consistant with an oxoprotoberberine core structure (Costa et al., 2010; González et al., 1997). The IR spectrum showed absorption bands of a hydroxy group (3386 cm^{-1}), a conjugated amide group (1617 cm^{-1}) and an aromatic C=C (1510 cm^{-1}). The ^{1}H-NMR spectrum of compound **1** (Table 1) showed resonances for four aromatic protons (δ_H 7.70, 7.39, 7.04, 6.73, each *s*, 1H), one olefinic proton (δ_H 7.05, *s*, 1H), a set of coupled methylene protons (δ_H 4.29, 2.88, each *t*, *J* = 4.7 Hz, 2H) and two methoxy groups (δ_H

3.98, 3.96, each *s*, 3H). The ^{13}C-NMR and DEPT spectra displayed resonances for an amide carbonyl carbon (δ_C 163.3), nine quaternary carbons (δ_C 153.4, 149.7, 149.4, 148.8, 137.5, 134.8, 130.1, 122.7 and 118.3), five methines (δ_C 115.3, 111.1, 109.4, 108.2 and 102.9), two methylenes (δ_C 41.3 and 28.7) and two methoxyl carbons (δ_C 56.8 and 56.4) (Table 1). A lower field singlet aromatic proton resonance at δ_H 7.70 was assigned as proton at H-9 due to the anisotropic effect of the amide carbonyl group and the correlations of H-9 to C-8 (δ_C 163.3), C-10 (δ_C 149.4), C-11 (δ_C 153.4) and C-12a (δ_C 134.8) in HMBC spectrum. The methoxy group was located at C-10 based on the HMBC correlation from the methoxy protons (δ_H 3.98) to this carbon and the signal enhancement after irradiation of δ_H 7.70 (H-9) in the 1D-NOE spectrum. The singlet aromatic proton resonance at δ_H 7.04 was assigned to H-12 (C-12: δ_C 111.1) on the basis of the HMBC correlations of H-12 with C-8a (δ_C 118.3), C-10, C-11 and C-13 (δ_C 102.9). Based on the HMQC cross peak, the singlet aromatic proton resonance at δ_H 7.05 was assigned as H-13 which showed cross peaks with C-8a, C-12 and C-13a (δ_C 137.5) in the HMBC spectrum. The substituent at C-11 (δ_C 153.4) was identified as a hydroxy group due to its relatively low field ^{13}C NMR chemical shift. From this information, the 2,3-disubstitued-6-hydroxy-7-methoxyisoquinolinone substructure of **1** was deduced. The aromatic proton with a singlet resonance at δ_H 7.39 was attributed to H-1 according to its HSQC correlation to C-1 (δ_C 109.4) and its HMBC correlations with C-3 (δ_C 149.7), C-4a (δ_C 130.1) and C-13a. The remaining methoxy group resonating at δ_H 3.96 was at C-2 (δ_C 148.8), as determined from a 1D NOEDIFF experiment. This experiment showed an enhancement of the signal intensity of this methoxy resonance upon irradiation of H-1 (δ_H 7.39). The substituent at C-3 (δ_C 149.7) was identified as a hydroxy group due to its relatively low field ^{13}C NMR chemical shift. The aromatic proton resonating at δ_H 6.73 was assigned to H-4 based on its HMQC correlation to C-4 (δ_C 115.3) and its HMBC correlations with C-1a (δ_C 122.7),

C-2 and C-5 (δ_C 28.7). The methylene proton resonances at δ_H 2.88 and δ_H 4.29 were assigned to CH_2-5 and CH_2-6, respectively. Their respective chemical shifts and the HMBC cross peaks between CH_2-5 (δ_H 2.88) and C-1a, C-4, C-4a and C-6 (δ_C 41.3) as well as between CH_2-6 (δ_H 4.29) and C-4a and C-5 (δ_C 28.7) supported these assignments. Thus, miliusathorine A (**1**) was elucidated as 8-oxo-3,11-dihydroxy-2,10-dimethoxy-5,6-dihydrooxoprotoberberine.

Miliusathorine B (**2**) was isolated as an orange gum with a molecular formula of $C_{19}H_{17}NO_5$ from the HRESI-MS ion at *m/z* 362.1004 $[M+Na]^+$ (calcd 362.1004). This molecule had the same molecular formula as **1** and a very similar mobility to **1** by TLC analysis (The R_f values of **1** and **2** were 0.46 and 0.27, respectively using 2:98 MeOH-CH_2Cl_2 as the mobile phase). The spectroscopic data (UV, IR and NMR) of **2** were almost the same as those of compound **1**, except for the chemical shifts of H-9 (δ_H 7.64), H-12 (δ_H 7.16) and H-13 (δ_H 7.12), and those of the two methoxy groups (δ_H 4.02 and 3.98) (Table 1). The methoxy group (δ_H 4.02) was located at C-11 (δ_C 154.6) from its HMBC correlation. Moreover, an 1D-NOE difference experiment, involving irradiation of the methoxy group resonance at δ_H 4.02, resulted in the enhancement of the aromatic proton resonance for H-12 (δ_H 7.16). This indicated that the positions of the methoxy and hydroxy groups at C-10 and C-11 of compound **1** were interchanged in compound **2**.

Miliusathorone (**3**) was isolated as a yellow viscous oil with a molecular formula of $C_{19}H_{16}O_8$ by the HRESI-MS ion at *m/z* 373.0925 $[M+H]^+$ (calcd 373.0923). The ^1H and ^{13}C-NMR spectroscopic data of compound **3** (Table 2) were similar to those of melisimplin (**21**) (Calvert et al., 1979), except compound **21** displayed a resonance for a H-bonded hydroxy proton at δ_H 12.56 while in compound **3** this signal was not observed. This result indicated that two methoxy groups, δ_H 4.04 and 4.01, of **3** were located on C-5 and C-7, respectively. The structure of **3** was further confimed from the

HMBC correlations as shown in Table 2. Therefore, miliusathorone (**3**) was identified as the flavone derivative previously synthesized (Briggs and Locker, 1950). This is the first report of its NMR spectroscopic data.

Compound **4** was isolated as a yellow viscous oil with a molecular formula of $C_{18}H_{16}O_8$ from the HRESI-MS ion showed the peak at *m/z* 383.0749 $[M+Na]^+$ (calcd 383.0743). The ^1H and ^{13}C-NMR spectroscopic data of compound **4** were similar to those of compound **3**, except for the lack of resonances for a methylenedioxy moiety in compound **4**. The substituents at C-3´ (δ_C 146.5) and C-4´ (δ_C 149.6) in compound **4** were hydroxy groups based on the chemical shifts of these carbons. Therefore, compound **4** was elucidated to be the quercetagetin-3,5,7-trimethyl ether which was isolated from the leaves and flowers of *Pulicaria arabica* (El-Negoumy et al., 1982). This is the first report on the NMR spectroscopic data of this compound (Table 2).

The isolated compounds were evaluated for their acetylcholinesterase (AChE) inhibitory activities at 100 µM (Table 3). The aporphine alkaloid **5** gave the best result exhibiting 50.17 ± 0.07% inhibition, while the oxoprotoberberines (**1** and **2**) were less active showing 40.70 ± 0.70% and 27.93 ± 2.50% enzyme inhibition, respectively. These differences most likely being attributed to the differences in the positioning of the methoxy and hydroxy groups in these compounds, however these alkaloids were weak inhibitors when compared to the standard galantamine. The flavones (**3**, **4**, **6**-**18** and **21**-**24**) showed AChE inhibition percentages ranging from <10 − 38.68 ± 1.54% which were less than that of the alkaloids **1**, **2** and **5**. Compounds **19** and **20** (amides) were inactive in this assay.

3. Conclusion

The main secondary metabolites from *Miliusa thorelii* are polyoxygenated flavones which were commonly found in plants from the *Miliusa* genus. Two new

dihydrooxoprotoberberine alkaloids (miliusathorines A and B) and a new natural flavone (miliusathorone) were isolated from this plant, together with 22 known natural products. The isolated compounds exhibited weak acetylcholinesterase inhibitory activities.

4. Experimental

4.1 General

Melting points are uncorrected and were measured on a SANYO Gallenkamp melting point apparatus. The UV spectra were determined with a Perkin-Elmer UV-vis spectrometer in CH_3OH. The IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer. The NMR spectra were recorded with a 400 MHz Bruker Ultra Shield FT-NMR spectrometer and 500 MHz Varian Unity INOVA NMR spectrometer. Chemical shifts (δ) are recorded in parts per million in $CDCl_3$ (δ_H 7.26 and δ_C 77.2 ppm), or methanol-d_4 (δ_H 3.31 and 4.78 and δ_C 49.1 ppm) with TMS as an internal standard. Mass spectrometric data were obtained on a Micro TOF, Bruker Daltonics mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was performed on silica gel (Merck) type 100 (63-200 µm), type 60 (5-40 µm for quick column chromatography (QCC)), (Salicycle) type 60 (40-63 µm for flash column chromatography (FCC)) and Sephadex LH-20. All solvents for extraction and chromatography were distilled prior to use.

4.2 Plant material

The stems, roots and leaves of *M. thorelii* were collected from Jaeson National Park Lampang province, Thailand, in November 2014. The plant was identified by Mr. James F. Maxwell and the voucher specimen was deposited at the CMUB Herbarium, Chiang Mai University (Specimen no. T. Ritthiwigrom1).

4.3 Extraction and isolation

An initial study of the acetone extract of the individual stems and roots showed the same components by TLC analysis. Therefore, these plant parts were combined for further analysis. The air-dried stems and roots (6.0 kg) of *M. thorelii* were combined and extracted with acetone (15 L x 3) at room temperature. The filtrate was evaporated under reduced pressure to give the acetone crude extract (77.42 g) as dark brown gum. The crude extract was fractionated by QCC over silica gel and eluted with a gradient system of hexanes-acetone-methanol to give 15 fractions (A-O). Fraction L (5.89 g) was further separated by QCC over silica gel and eluted with the gradient systems of hexanes-CH_2Cl_2-EtOAc to give 17 subfractions (L1-L17). Subfraction L11 (212.0 mg) was further fractionated by CC over silica gel with MeOH-CH_2Cl_2 (2:98, v/v) to provide 12 subfractions (L11A-L11L). Subfraction L11G (15.3 mg) was separated over Sephadex LH-20 with MeOH to give compound **3** (4.0 mg) as a yellow viscous oil. Fraction M (1.01 g) was separated over Sephadex LH-20 with MeOH to give eight subfractions (M1-M8). Subfraction M6 (22.7 mg) was separated over Sephadex LH-20 with MeOH to give five subfractions (M6A-M6E). Subfraction M6D (11.8 mg) was further separated by CC with MeOH-CH_2Cl_2 (2:98 to 5:95, v/v) to provide compound **1** (2.0 mg) as brown gum and **2** (2.2 mg) as an orange gum. Twenty compounds (**1-20**) were purified from the combined stems and roots of the acetone extract. While the air-dried leaves of *M. thorelii* (0.7 kg) were extracted with acetone (15 L x 3). The organic solvent was removed under reduced pressure to provide 32.52 g of the acetone extract as a black green gum. Eigth known compounds (**12**, **17**, **18** and **21-25**) were purified from the leaves acetone extract. Full details of all isolated known compounds are provided in the Supplementary material.

4.3.1 Miliusathorine A (**1**)

Brown gum; UV (MeOH) λ_{max} nm (log ε): 225 (3.6), 261 (3.4), 337 (3.3), 360 (3.1); IR (neat) ν_{max}: 3386, 2923, 1617, 1510 cm^{-1}; ^{1}H and ^{13}C-NMR data see Table 1; HRESI-MS *m/z* $[M+Na]^+$ 362.1004 (calcd for $C_{19}H_{17}NO_5Na$, 362.1004).

4.3.2 Miliusathorine B (**2**)

Orange gum; UV (MeOH) λ_{max} nm (log ε): 228 (4.3), 261 (4.2), 335 (4.1), 365 (3.9); IR (neat) ν_{max}: 3422, 2920, 1637, 1510 cm^{-1}; ^{1}H and ^{13}C-NMR data see Table 1; HRESI-MS *m/z* $[M+Na]^+$ 362.1004 (calcd for $C_{19}H_{17}NO_5Na$, 362.1004).

4.3.3 Miliusathorone (**3**)

Yellow viscous oil; UV (MeOH) λ_{max} nm (log ε): 218 (4.3), 247 (4.1), 337 (4.2); IR (neat) ν_{max}: 3443, 1611, 1492 cm^{-1}; ^{1}H and ^{13}C-NMR data see Table 2; HRESI-MS *m/z* 373.0925 $[M+H]^+$ (calcd for $C_{19}H_{17}O_8$, 373.0923).

4.3.4 Quercetagetin 3,5,7-trimethyl ether (**4**)

Yellow viscous oil; UV (MeOH) λ_{max} nm (log ε): 215 (4.2), 239 (3.9), 339 (4.0); IR (neat) ν_{max}: 3385, 1603, 1515 cm^{-1}; ^{1}H and ^{13}C-NMR data see Table 2; HRESI-MS *m/z* 383.0749 $[M+Na]^+$ (calcd for $C_{18}H_{16}O_8Na$ 383.0743).

4.4 AChE inhibitory activity

Acetylcholinesterase (AChE) inhibitory activity was measured by a spectrophotometric method using Ellman's reagent (Ellman et al., 1961; Ingkaninan et al., 2006). Briefly, 25 µL of 1.5 mM ATCI, 50 µL of 50 mM Tris–HCl buffer (pH 8.0), 125 µL of 3 mM DTNB, and 25 µL of sample dissolved in buffer containing MeOH (not more than 10%) were added to the wells followed by 25 µL of 0.51 U/ml AChE. The reaction was detected at 405 nm, every 17 s. for 2 min, using a microplate reader, in triplicate experiments. Enzyme activity was calculated as a percentage of the velocities of sample compared to the negative control. The inhibitory activity was calculated from

one hundred percentage subtracted by the percentage of enzyme activity. Galantamine was used as the positive control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at

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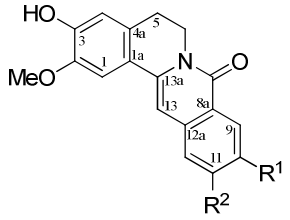
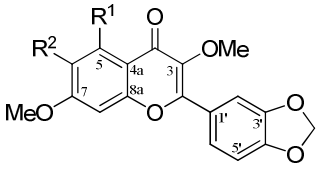
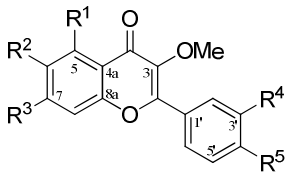


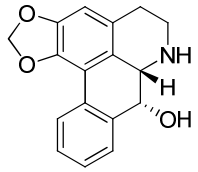
	R^1	R^2
1:	OMe	OH
2:	OH	OMe



	R^1	R^2
3:	OMe	OH
17:	OH	H
18:	OMe	OMe
21:	OH	OMe
22:	OMe	H



	R^1	R^2	R^3	R^4	R^5
4:	OMe	OH	OMe	OH	OH
6:	OH	H	OMe	OH	OH
7:	OMe	OH	OMe	OMe	OH
8:	OMe	OH	OMe	H	OH
9:	OH	H	OMe	OMe	OMe
10:	OH	OMe	OMe	H	OMe
11:	OMe	OMe	OMe	H	OMe
12:	OMe	H	OMe	OMe	OMe
13:	OH	H	OH	H	OH
14:	OH	H	OH	OH	OH
15:	OMe	H	OH	OMe	OH
16:	OMe	OMe	OMe	H	OH
23:	OH	H	OMe	OMe	OH
24:	OMe	OMe	OMe	OMe	OMe
25:	OH	OMe	OMe	OMe	OMe



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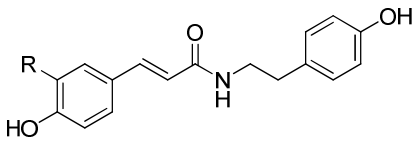

19: **R** = OMe
20: **R** = OH

Fig. 1. Structures of isolated compounds (**1**-**25**) from the stem and root, and leaves extracts of *Miliusa thorelii*.

Table 1

NMR data (500 MHz) of miliusathorine A (**1**) and miliusathorine B (**2**) in CD_3OD.

Position	**1**			**2**		
	δ_C, type	δ_H, (*J* in Hz)	HMBC	δ_C, type	δ_H, (*J* in Hz)	HMBC
1	109.4, CH	7.39, *s*	3, 4a, 13a	109.4, CH	7.40, *s*	1a, 3, 4a, 13a
1a	122.7, C			122.7, C		
2	148.8, C			148.8, C		
3	149.7, C			149.3, C		
4	115.3, CH	6.73, *s*	1a, 2, 5	115.4, CH	6.75, *s*	1a, 2, 5
4a	130.1, C			130.0, C		
5	28.7, CH_2	2.88, *t* (4.7)	1a, 4, 4a, 6	28.6, CH_2	2.88, *t* (4.0)	1a, 4, 4a, 6
6	41.3, CH_2	4.29, *t* (4.7)	4a, 5	41.3, CH_2	4.28, *t* (4.0)	4a, 5
8	163.3, C			163.2, C		
8a	118.3, C			119.3, C		
9	108.2, CH	7.70, *s*	8, 10, 11, 12a	112.0, CH	7.64, *s*	8, 8a, 10, 11, 12a
10	149.4, C			148.0, C		
11	153.4, C			154.6, C		
12	111.1, CH	7.04, *s*	8a, 10, 11, 13	107.5, CH	7.16, *s*	8a, 10, 11, 13
12a	134.8, C			133.7, C		
13	102.9, CH	7.05, *s*	8a, 12, 13a	103.3, CH	7.12, *s*	8a, 12, 13a
13a	137.5, C			136.9, C		
OCH_3 (2)	56.8, CH_3	3.96, *s*	2	56.5, CH_3	3.98, *s*	2
OCH_3 (10)	56.4, CH_3	3.98, *s*	10	-	-	-
OCH_3 (11)	-	-	-	56.8, CH_3	4.02, *s*	11

Table 2

NMR data (400 MHz) of miliusathorone **3** ($CDCl_3$) and compound **4** (CD_3OD).

Position	**3**			**4**		
	δ_C, type	δ_H, (*J* in Hz)	HMBC	δ_C, type	δ_H, (*J* in Hz)	HMBC
2	153.5[a], C			156.3, C		
3	140.3, C			141.5, C		
4	178.3, C			175.8, C		
4a	112.7, C			113.4, C		
5	143.9, C			145.3, C		
6	136.6, C			139.0, C		
7	152.8, C			155.5, C		
8	96.1, CH	6.78, *s*	4a, 6, 8a	97.0, CH	6.99, *s*	4a, 6, 8a
8a	151.2, C			152.3, C		
1´	124.4, C			123.2, C		
2´	108.6, CH	7.60, *d* (1.6)	2, 3´, 4´, 6´	116.5, CH	7.66, *d* (2.2)	2, 4´, 6´
3´	147.6, C			146.5, C		
4´	149.3, C			149.6, C		
5´	108.6, CH	6.94, *d* (8.3)	1´, 3´, 4´	116.4, CH	6.91, *d* (8.4)	1´, 3´
6´	123.4, CH	7.68, *dd* (8.3, 1.6)	2, 2´, 4´	122.0, CH	7.54, *dd* (8.4, 2.2)	2, 2´, 4´
–OCH_2O–	101.8, CH_2	6.06, *s*	3´, 4´	-	-	-
OCH_3 (3)	60.2, CH_3	3.86, *s*	3	60.3, CH_3	3.77, *s*	3
OCH_3 (5)	62.8, CH_3	4.04, *s*	5	62.2, CH_3	3.90, *s*	5
OCH_3 (7)	56.7, CH_3	4.01, *s*	7	57.0, CH_3	4.01, *s*	7

[a] Observed from HMBC spectrum

Table 3

Acetylcholinesterase inhibition (AChE) of isolated compounds (**1**-**25**) at 100 µM in the microplate assay.

Compounds	% AChE inhibition	Compounds	% AChE inhibition
1	40.70 ± 0.70	**14**	26.62 ± 3.65
2	27.93 ± 2.50	**15**	15.78 ± 3.47
3	13.60 ± 2.08	**16**	16.15 ± 3.52
4	23.73 ± 1.94	**17**	21.91 ± 2.33
5	50.17 ± 0.07	**18**	<10
6	<10	**19**	<10
7	35.60 ± 1.04	**20**	<10
8	16.53 ± 1.37	**21**	10.72 ± 1.98
9	<10	**22**	10.16 ± 2.25
10	<10	**23**	16.89 ± 3.03
11	<10	**24**	38.68 ± 1.54
12	15.92 ± 7.37	**25**[b]	-
13	14.82 ± 3.23	Galantamine[a]	98.44 ± 0.59

[a] Positive control: IC_{50} against AChE was 0.15 µM

[b] Not test due to less amount