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4-Phenylcoumarins from *Mesua elegans* with acetylcholinesterase inhibitory activity

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ABSTRACT

A significant acetylcholinesterase (AChE) inhibitory activity was observed for the hexane extract from the bark of *Mesua elegans* (Clusiaceae). Thus, the hexane extract was subjected to chemical investigation, which led to the isolation of nine 4-phenylcoumarins, in which three are new; mesuagenin A (**1**), mesuagenin C (**3**), mesuagenin D (**4**) and one new natural product; mesuagenin B (**2**). The structures of the isolated compounds were characterized by spectroscopic data interpretation, especially 1D and 2D NMR. Four compounds showed significant AChE inhibitory activity, with mesuagenin B (**2**) being the most potent (IC_{50} = 0.7 μM).

1. Introduction

Alzheimer's disease (AD), the most common form of dementia in the elderly, is a neurodegenerative disease characterized by progressive memory impairment, cognitive deficits and behavioural changes. Currently, cholinesterase (ChE) inhibition represents the most efficacious treatment approach for AD.[1] Two types of ChE have been characterized in vertebrate tissues, acetylcholinesterase (AChE) and butylcholinesterase (BChE). Therefore, under the framework of French–Malaysian collaboration program,[2–4] a survey on extracts inhibiting AChE was conducted on plants from the Malaysian flora and it was found that the hexane extract from the bark of *Mesua elegans* exhibited a strong AChE inhibitory activity with 90% inhibition at 10 μg/mL. Hence, in search for the compound/s responsible for the activity, our team has embarked on a bioassay-guided study of the active extract.

The genus *Mesua* (Clusiaceae) is distributed along Ceylon, India, Indo-China, Thailand, Malaysia and Queensland, centred strongly in West Malaysia with more than 40 species.[5] The plants from this genus were known to be prolific source of phloroglucinols, xanthones, neoflavonoids and coumarins, which exhibit various biological activities including antibacterial, antibiotic, neuromodulator, antitumor, antiviral and cytotoxic effects.[6–8] This communication is the first report on AChE activity of *Mesua* compounds. The chemical investigation has led to the isolation and identification of nine 4-phenylcoumarins in which three are new; mesuagenin A (**1**), mesuagenin C (**3**), mesuagenin D (**4**) and one new natural product; mesuagenin B (**2**), along with isomammeigin[9] (**5**), 5,7-dihydroxy-8-(2-methylbutanoyl)-6-[(*E*)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one[6] (**6**), 5,7-dihydroxy-8-(3-methylbutanoyl)-6-[(*E*)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one[6] (**7**), mammea A/BA cyclo F[10] (**8**) and mammea A/BA[11] (**9**). Herein, we report the structural elucidation of the new coumarins and the AChE inhibitory activities of all the isolated compounds. Mesuagenin B (**2**) exhibited the most potent inhibitory activity with an IC_{50} of 0.7 μM.

2. Results and discussion

2.1. Structural elucidation

The hexane extract from the bark of *M. elegans* gave 90% inhibition of acetyl cholinesterase (AChE) at 10 μg/mL, and was then subjected for fractionation via silica gel column chromatography (CC). Eight fractions were obtained and fraction 1 (hexane–ethyl acetate 95:5) showed the most potent activity (100% of inhibition at 10 μg/mL). Purification of compounds from this fraction gave nine 4-phenylcoumarins; mesuagenin A (**1**), mesuagenin B (**2**), mesuagenin C (**3**), mesuagenin D (**4**), isomammeigin (**5**), 5,7-dihydroxy-8-(2-methylbutanoyl)-6-[(*E*)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one (**6**), 5,7-dihydroxy-8-(3-methylbutanoyl)-6-[(*E*)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one (**7**), mammea A/BA cyclo F (**8**) and mammea A/BA (**9**).

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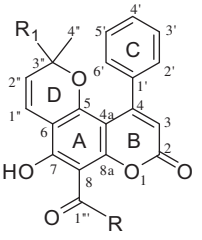
Compounds **1**, **3** and **4** are new 4-phenylcoumarins whilst **2** is a new natural product.

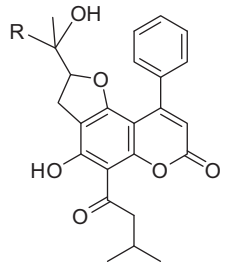
Mesuagenin A (**1**) was isolated as yellowish oil. The HRESIMS spectrum revealed a pseudomolecular ion peak at *m*/*z* 495.2128 $[M+Na]^+$ (calcd 495.2147), which corresponded to the molecular formula of $C_{30}H_{32}O_5$. The UV spectrum supported an 8-acyl-5,7-dioxycoumarin type, with absorptions at λ_{max} 272, 313 nm.[12] The IR spectrum showed absorptions at ν_{max} 1382 cm^{-1}, due to a *gem*-dimethyl, and 1612 cm^{-1} belonging to a chelated acyl group.[13,14] The IR spectrum also exhibited strong peak at 1741 cm^{-1} indicating the presence of α-pyrone and a broad peak at 3461 cm^{-1} due to a chelated OH stretching.[13,14]

The ^{1}H NMR spectrum revealed a singlet belonging to H-3 at δ 5.99, which is characteristic of a 4-substituted coumarin skeleton.[6] The presence of a monosubstituted phenyl group at C-4 was deduced by the presence of two sets of multiplets, centred at δ 7.22 (2H's) and δ 7.38 (3H's), corresponding to aromatic protons H-2′, H-6′ and H-3′, H-4′, H-5′, respectively. Furthermore, the ^{1}H NMR spectrum also supported the presence of a chelated hydroxyl by revealing a deshielded proton singlet at δ 14.64.[6] All these observations suggested the presence of an 8-acyl-5,7-dioxy-4-phenylcoumarin type.

In addition, the ^{1}H NMR spectrum showed a set of doublets, belonging to the olefinic protons H-1″ and H-2″ at δ 6.65 (J = 10.4 Hz) and 5.32 (J = 10.4 Hz), respectively. A methyl singlet was also apparent at δ 0.96 (H-4″). All these protons correlated with the quaternary carbon C-3″ (δ 81.9) in the HMBC spectrum (Fig. 1) and the olefinic protons correlated with the quaternary C-6 (δ 105.4). These observations suggested the presence of a cyclised prenyl group attached to C-6. In addition, the methylene proton signals of C-5″ (δ 1.21) and C-6″ (δ 1.67), which were observed as multiplets, correlated with C-7″ and C-8″ in the HMBC spectrum. H-7″, in return, correlated with two *gem*-dimethyl singlets (H-9″, δ 1.63 and H-10″, δ 1.50), thus implying the existence of another prenyl group linked to C-5″. All these information established the presence of a 3″-methyl-5″-prenyl-$\Delta^{1''}$-pyran ring system in **1**.

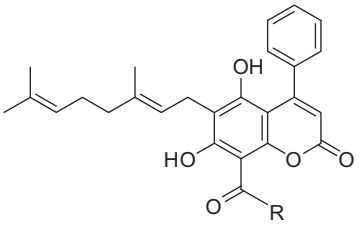
The COSY spectrum revealed cross correlations identifying the presence of an *iso*-butyryl spin system with signals at δ 3.16 (2H, d, J = 6.7 Hz, H-2‴), δ 2.31 (1H, m, H-3‴) and δ 1.04 (6H, d, J = 6.7 Hz, H-4‴ and H-5‴); H-2‴ with H-3‴ and H-3‴ with H-4‴

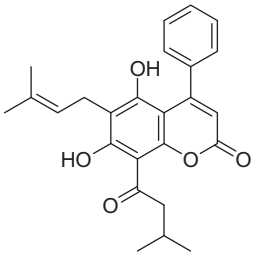




1 R = $CH_2CH(CH_3)_2$ R_1 = $(CH_2)_2CHC(CH_3)_2$
2 R = $CH(CH_3)CH_2CH_3$ R_1 = $(CH_2)_2CHC(CH_3)_2$
5 R = $CH_2CH(CH_3)_2$ R_1 = CH_3

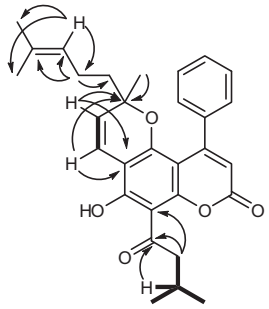
4 R = $(CH_2)_2CHC(CH_3)_2$
8 R = CH_3





3 R = $CH(CH_3)_2$
6 R = $CH_2CH(CH_3)_2$
7 R = $CH(CH_3)CH_2CH_3$

9



COSY

HMBC

Figure 1. Key 2D NMR correlations of mesuagenin A (**1**).

and H-5‴, as shown in Figure 1. In the HMBC spectrum, the protons of C-2‴ and C-3‴ correlated with a carbonyl (C-1‴), thus confirming that C-2‴ is attached to the latter. Furthermore, H-2‴ correlated with C-8 in the HMBC spectrum, hence confirming the attachment of the *oxo*-butyryl group at position C-8 in the coumarin skeleton. Finally, the full proton and carbon assignments of mesuagenin A (**1**) were established by thorough analysis of COSY, HMQC and HMBC spectra (Table 1).

Mesuagenin B (**2**) was isolated as yellowish oil. The HRESIMS spectrum revealed a pseudomolecular ion peak at *m*/*z* 495.2128 $[M+Na]^+$ (calcd 495.2147), which corresponded to the molecular formula of $C_{30}H_{32}O_5$, which is the same as that of **1**. Therefore, both compounds **1** and **2** are structural isomers. The UV spectrum supported an 8-acyl-5,7-dioxycoumarin type, with absorptions at λ_{max} 272, 313 nm.[12] The IR spectrum exhibited strong absorptions at ν_{max} 1384 cm^{-1} due to a *gem*-dimethyl and another peak at 1614 cm^{-1} belonging to a chelated acyl group.[13,14] The IR spectrum also showed strong peak at 1742 cm^{-1} indicating the presence of α-pyrone and a broad peak at 3475 cm^{-1} due to the stretching of chelated hydroxyl group.[13,14]

The ^1H and ^{13}C NMR spectra of **2** is very similar to those of **1** except for some notable differences. The ^1H NMR spectrum in **2** showed two methyls resonating as a doublet and a triplet respectively, where as in **1** both methyls appeared as a doublet. These observations indicated the presence of a 2-methylbutanoyl moiety in **2** instead of a 3-methylbutanoyl moiety in **1**. Therefore, **2** is a new 4-phenylcoumarin natural product named mesuagenin B. It was first reported as a synthetic compound by Verotta et al.,[6] synthesized from 5,7-dihydroxy-8-(2-methylbutanoyl)-6-[(*E*)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one (**7**).

Mesuagenin C (**3**) was obtained as colourless oil. The molecular formula of $C_{29}H_{32}O_5$ was established from the HRESIMS measurement that revealed an $[M+Na]^+$ pseudomolecular ion peak at *m*/*z* 483.2132 (calcd 483.2147). The UV spectrum supported an 8-acyl-5,7-dihydroxycoumarin type, with absorptions at λ_{max} 226, 295 and 331 nm.[12] The IR spectrum showed absorptions at ν_{max} 3470 (chelated OH), 1741 (α-pyrone), 1602 (chelated acyl group) and 1383 cm^{-1} (*gem*-dimethyl).[13,14]

The ^1H NMR of **3** is reminiscent of **1** except that it showed signals of a free geranyl group by showing two broad triplets at δ 5.08 (1H, *J* = 7.4 Hz, H-2″) and δ 4.99 (1H, *J* = 7.4 Hz, H-7″). Another difference from **1** is the apparent signals of an *iso*-propyl group attached to carbonyl C-1‴ (δ 210.7) in place of an *iso*-butyryl moiety in **1**; a multiplet at δ 4.11 (H-2‴) and a doublet at δ 1.28 (H-3‴ and H-4‴) correlating with the quaternary carbon C-1‴ in the HMBC spectrum. Extensive spectroscopic analysis by using COSY, HSQC and HMBC NMR spectra confirmed the structure of **3** as mesuagenin C.

Mesuagenin D (**4**) was isolated as white amorphous powder. The HRESIMS measurement revealed a pseudomolecular ion peak at *m*/*z* 513.2253 $[M+Na]^+$ (calcd 513.2253), which was associated with the molecular formula of $C_{30}H_{34}O_6$. The ^1H and ^{13}C NMR spectra of **4** exhibited similar features as those of **1**, which suggested a close structural relationship between these two compounds. Indeed, the presence of the same substituents at positions C-4, C-7 and C-8 for the coumarin skeleton could be detected through the correlations in COSY, HMQC and HMBC spectra, namely a monosubstituted phenyl, a chelated hydroxyl and a 3-methylbutanoyl chain.

However, one distinct difference was apparent in the ^1H NMR spectrum. The signals corresponding to the C-5 and C-6 substituents were different from those of **1**. The ^1H NMR spectrum showed a triplet at δ 4.53 (1H, *J* = 9.8 Hz, H-2″) and two doublet of doublets at δ 3.09 (1H, *J* = 9.8, 15.9 Hz, H-1″α) and δ 2.49 (1H, *J* = 9.8, 15.9 Hz, H-1″β) respectively, thus indicating the presence of a dihydrofuran moiety. The ^1H NMR resonances at δ 1.24 (2H, m,

Table 1
^1H [δ_H (*J* in Hz)] and ^{13}C (δ_C) NMR spectroscopic data of **1**, **2**, **3** and **4** in $CDCl_3$

Position	**1**		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		158.8		159.0		158.9		159.2
3	5.99 s	111.9	5.99 s	112.0	6.00 s	112.2	6.04 s	111.1
4		157.0		156.2		157.3		155.0
4a		102.0		102.1		100.7		98.8
5		156.6		156.6	5.98 s	154.5		162.0
6		105.4		105.7		112.6		110.2
7-OH	14.64 s	163.7	14.63 s	164.0	14.54 s	167.1	14.29 s	163.8
8		104.0		103.7		103.8		105.1
8a		156.0		156.9		155.8		157.4
1′		140.1		140.2		137.1		138.2
2′	7.22 m	127.1	7.22 m	127.2	7.40 m	127.6	7.29 m	127.5
3′	7.38 m	127.6	7.38 m	127.6	7.52 m	129.6	7.41 m	128.9
4′		127.8		127.9		130.2		128.0
5′		127.6		127.6		129.6		128.9
6′	7.22 m	127.1	7.22 m	127.2	7.40 m	127.6	7.29 m	127.5
1″α	6.65 d (10.4)	115.9	6.66 d (9.7)	116.1	3.30 d (6.7)	21.6	3.09 dd (9.8, 15.9)	26.5
1″β	–	–	–	–	–	–	2.49 dd (9.8, 15.9)	
2″	5.32 d (10.4)	125.3	5.32 d (9.7)	125.5	5.08 t (7.4)	120.6	4.53 t (9.8)	92.4
3″		81.9		82.0		138.2		73.4
4″	0.96 s	26.8	0.96 s	26.9	1.68 s	16.4	0.87 s	21.9
5″	1.21 m	40.9	1.20 m	41.0	2.03–1.91 m	39.7	1.24 m	36.1
6″	1.67 m	22.8	1.67 m	22.9		26.6	1.94 m	21.8
7″	4.87 br t (6.7)	123.5	4.87 br t (6.7)	123.6	4.99 t (7.4)	124.1	5.01 br t (7.3)	123.9
8″		131.8		131.9		131.7		132.4
9″	1.63 s	25.7	1.63 s	25.7	1.59 s	25.8	1.67 s	25.8
10″	1.50 s	17.7	1.50 s	17.8	1.53 s	17.6	1.59 s	17.7
1‴		206.0		210.6		210.7		206.1
2‴	3.16 d (6.7)	53.5	3.96 m	47.0	4.11 m	40.5	3.16 d (6.7)	53.5
3‴	2.31 m	25.6	1.27 d (6.7)	16.7	1.28 d (7.3)	19.4	2.30 m	25.7
4‴α		22.7	1.47 m	27.3		19.4	1.04 d (7.3)	22.8
4‴β	1.04 d (6.7)		1.94 m					
5‴		22.7	1.01 t (7.3)	11.9				22.8

H-6″), 5.01 (1H, br t, J = 7.3, H-7″), 1.67 (3H, s, H-9″) and 1.59 (3H, s, H-10″) together with the ^{13}C signals at δ 21.8 (C-6″), 123.9 (C-7″), 132.3 (C-8″), 25.8 (C-9″) and 17.7 (C-10″) implied the existence of an isoprenyl group attached to C-5″ (δ 36.1). In addition, the HMBC correlations clearly proved that the substituted dihydrofuran ring is located between C-5 (δ 162.0) and C-6 (δ 110.2) by exhibiting the following correlation; H-1″α,β/C-5, C-6, C-2″ and C-4″. Finally, the complete structural elucidation of **4** as mesuagenin D was made possible with the aid of COSY, HMQC and HMBC spectra.

2.2. AChE inhibitory activity

The AChE inhibitory activity was assayed by the method of Ellman[15] using AChE from *Electrophorus electricus* with tacrine as reference compound (Table 2). Compounds **1**, **2**, **4** and **7** exhibited significant inhibition on AChE with compound **2** being the most potent (IC_{50} = 0.70 μM). It could be suggested from these results that the prenyl side chain at C-3″ plays an important role in the AChE inhibiting activity (**1** and **2** vs **5**) for compounds having a dimethylpyran ring at C-5/C-6. On the other hand, for the 6-geranylated coumarins (**3**, **6** and **7**), the presence of a 2-methylbutanoyl moiety at C-8 (**7**) instead of a 2-methylpropanoyl (**3**) or 3-methylbutanoyl moiety (**6**) is more favourable for a significant AChE inhibiting activity.

3. Conclusion

This bioassay-guided study on the bark of *M. elegans* has led to the discovery of four 4-phenylcoumarins that are responsible for the AChE activity with mesuagenin B (**2**) being the most potent (IC_{50} = 0.7 μM). To our knowledge this is the first report of inhibition activity on AChE by 4-phenylcoumarins. In addition, this study is the first communication on the chemical constituents of *M. elegans* in which three of the compounds isolated are new; mesuagenin A (**1**), mesuagenin C (**3**) and mesuagenin D (**4**), and one is a newly reported natural product; mesuagenin B (**2**). Thus, 4-phenylcoumarins can be considered as a new class of compounds that can be potential leads in the search of new therapies for curing Alzheimer's disease (AD).

4. Experimental

4.1. General methods

NMR spectra were obtained using JEOL LA400 FT-NMR and JEOL ECA400 FT-NMR Spectrometer System (400 MHz) with $CDCl_3$ as solvent. UV spectra were recorded on a Shimadzu UV–vis Recording Spectrophotometer using HPLC grade ethanol as solvent with mirror UV cell. The IR spectra were obtained through Perkin–Elmer FT-IR Spectrometer Spectrum RX1 with $CHCl_3$ as solvent. Mass spectra was carried out on Agilent Technologies 6530 Accurate-Mass Q-TOF LC–MS, with ZORBAX Eclipse XDB-C18 Rapid Resolution HT 4.6 mm id × 50 mm × 1.8 μm column. Merck silica gel 60 (230–400 mesh) was used for column chromatography separations and silica gel 60 F_{254} plates for TLC and PTLC separations. Waters autopurification system was used for HPLC separation, equipped with Binary Gradient Module (Waters 2545), System Fluidics Organizer (Waters SFO), and Photodiode Array Detector (190–600 nm, Waters 2998) and Sample Manager (Waters 2767). HPLC analysis and separations were performed on ZORBAX Eclipse Plus C18 (4.6 mm id × 150 mm × 3.5 μm), ZORBAX Eclipse Plus C18 (9.4 mm id × 250 mm × 3.5 μm), Chromolith Performance RP-18 (4.6 mm id × 100 mm) and Chromolith RP-18 endcapped (10 mm id × 100 mm).

Table 2
Inhibition percentage and IC_{50} values of **1**–**9**

Compound	IC_{50} (μM)
1	1.06 ± 0.04
2	0.70 ± 0.10
3	>20
4	8.73 ± 0.25
5	>20
6	>20
7	3.06 ± 0.04
8	>20
9	>20
Tacrine	0.074 ± 0.012

4.2. Plant material

The bark of *M. elegans* (King) Kosterm was collected in Sungai Badak Forest Reserve, Sintok, Kedah, Malaysia on April 2006. The sample with voucher specimen number KL 5232 was identified by Mr. Teo Leong Eng and deposited in the herbarium of the Department of Chemistry, Faculty of Science, University of Malaya.

4.3. Extraction and isolation

Dried ground bark of *M. elegans* (1.5 kg) was macerated with hexane (3 × 4 L, each 48 h) at room temperature. The hexane extract was then dried off by using rotary-evaporator and a yellow gummy residue (120.3 g) was obtained. The plant material was then subjected for ethyl acetate and methanol extraction successively (3 × 4 L, each 48 h, respectively). Both of the extracts were then evaporated off to dryness by using rotary-evaporator. The ethyl acetate crude (81.1 g) was obtained as brown gummy residue while the methanol extract (93.2 g) was obtained as brown amorphous powder. Hexane crude exhibited the most potent AChE inhibitory activity. Thus, the hexane crude was subjected for further chromatographic analysis. Both ethyl acetate and methanol crude was kept for future use and study.

The hexane crude (13.0 g) was subjected to column chromatography fractionation over silica gel (230–400 mesh) and eluted with hexane–EtOAc (from 9.5 to 0) and EtOAc–MeOH (from 5 to 0) to give fractions A–H. Fraction A showed the most potent AChE inhibitory activity. Fraction A was then subjected to silica gel chromatography and eluted with hexane–EtOAc (from 9.7 to 9.5) to produce subfractions A1–A4. Fraction A1 was purified by repeated PTLC using hexane–EtOAc (9.8:0.2) to afford compounds **1** (114 mg), **2** (129 mg) and **5** (15 mg). Fraction A2 was separated by ZORBAX Eclipse Plus C18, 9.4 mm id × 250 mm × 3.5 μm HPLC column (3 mL/min), eluted as a gradient system with H_2O–MeOH (+0.1% formic acid in both solvents), 50–100% MeOH in 35 min to afford compounds **6** and **7**. Separation of fraction A4 with Chromolith RP-18 endcapped, 10 mm id × 100 mm HPLC column (4.7 mL/min), eluted as a gradient system with H_2O–MeOH (+0.1% formic acid in both solvents), 50–100% MeOH in 25 min to afford compounds **3**, **4**, **6**, **8** and **9**.

4.3.1. Mesuagenin A (1)

Yellowish oil, $[\alpha]_D^{26}$ +10 (c 0.0002, EtOH); HRESIMS m/z 495.2128 $[M+Na]^+$ (calcd for $C_{30}H_{32}O_5Na$, 495.2147); UV (EtOH) λ_{max} (log ε): 272 (4.5) and 313 (4.5) nm; IR (NaCl) ν_{max}, cm^{-1}: 3461, 1741, 1612 and 1382; ^1H and ^{13}C NMR: Table 1.

4.3.2. Mesuagenin B (2)

Yellowish oil, $[\alpha]_D^{26}$ +16 (c 0.0003, EtOH); HRESIMS m/z 495.2128 $[M+Na]^+$ (calcd for $C_{30}H_{32}O_5Na$, 495.2147); UV (EtOH) λ_{max} (log ε): 272 (4.5) and 313 (4.5) nm; IR (NaCl) ν_{max}, cm^{-1}: 3475, 1742, 1614 and 1384; ^1H and ^{13}C NMR: Table 1.

4.3.3. Mesuagenin C (3)

Colourless oil; HRESIMS m/z 483.2132 $[M+Na]^+$ (calcd for $C_{29}H_{32}O_5Na$, 483.2147); UV (EtOH) λ_{max} (log ε): 226 (3.8), 295 (4.5) and 331 (4.5) nm; IR (NaCl) ν_{max}, cm^{-1}: 3470, 1741, 1602 and 1383; ^1H and ^{13}C NMR: Table 1.

4.3.4. Mesuagenin D (4)

White amorphous powder, $[\alpha]_D^{26}$ +7.7 (c 0.0001, EtOH); HRESIMS m/z 513.2253 $[M+Na]^+$ (calcd for $C_{30}H_{34}O_6Na$, 513.2253); UV (EtOH) λ_{max} (log ε): 226 (4.6) and 298 (4.5) nm; IR (NaCl) ν_{max}, cm^{-1}: 3447, 1717, 1604 and 1385; ^1H and ^{13}C NMR: Table 1.

4.4. Acetyl cholinesterase inhibitory activity testing

Acetylcholinesterase (AChE) from *E. electricus* (C2888) was purchased from Sigma. Inhibition of AChE activity was determined by the spectroscopic method of Ellman, using acetylthiocholine iodide as substrate, in 96-well microtiter plates. All solutions were brought to room temperature prior to use. Aliquots of 200 μL of a solution containing 640 μL of 10 mM of 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.1 M sodium phosphate, pH 8.0, 19.2 mL of the same buffer, and 13 μL of a solution of AChE (100 U/mL) in water, were added to each well, followed by 2 μL of a DMSO solution of the inhibitor (0.9% final volume). The reaction was initiated by adding 20 μL of acetylthiocholine iodide (7.5 mM) to each well and was followed by monitoring the appearance of the thiolate dianion produced by the reduction of DTNB at 412 nm every 13 s for 120 s at 25 °C in a Molecular Devices spectra Max 384 Plus plate reader. Each inhibitor was evaluated at ten concentrations (ranging from 1 mg/mL to 0.05 μg/mL by diluting by a factor of 3). Percentage inhibition was calculated relative to a control sample of DMSO (% inhibition = $[1 - (\text{slope cpd/slope DMSO})] \times 100$) with SoftMax® Pro software. IC_{50} values displayed represent the mean ± standard deviation for six assays ($SD = [\sum(x - x)^2/n]^{1/2}$). Tacrine hydrochloride (Sigma, purity >99%) was used as reference compound.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.044.

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