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Clerodane diterpenes from *Polyalthia longifolia* var. *pendula* protect SK-N-MC human neuroblastoma cells from *β*-Amyloid insult

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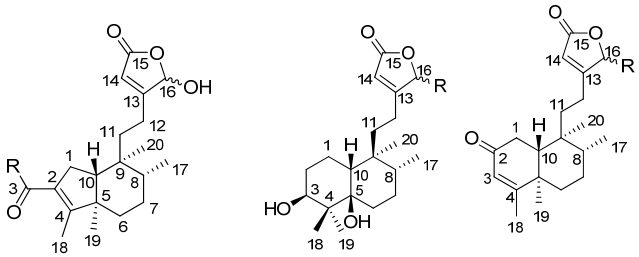
Three new diterpenes, polylongifoliaic A (**1**), polylongifoliaon A (**4**), and B (**5**), together with nine known diterpenes, were isolated from the unripe fruit of *Polyalthia longifolia* var. *pendula*. The structures of the new isolates were determined by extensive spectroscopic analysis. The effect of all isolated compounds on the viability of human neuroblastoma SK-N-MC cells under *β*-amyloid (A*β*)-induced neurotoxicity was evaluated. Polylongifoliaic A (**1**), polylongifoliaon B (**5**), and **8**-**10** improved the viability of human neuroblastoma cells (SK-N-MC cells) under A*β*-induced neurotoxicity. Among the active compounds, polylongifoliaic A (**1**) and polylongifoliaon B (**5**) exhibited the most potent activity toward SK-N-MC cells with IC_{50} 1.64 μM and 3.75 μM, respectively. Additionally, the isolated diterpenes were found to possess potent promising acetylcholinesterase inhibitory activity which was revealed by the TLC bioautographic assay.

Introduction

Polyalthia longifolia var. *pendula* known as "Indian Mast Tree" is an evergreen plant which is native to India.[1] It is cultivated in the tropical and subtropical regions of the world as ornamental or avenue trees. Since antiquity, it has been used by traditional healers in Asian folk medicine. Its name gives a clear indication of such therapeutic potential. *Polyalthia* is the Greek word for poly, meaning much or many and althia from àltheo, meaning to cure.[2] The plant has been used as anthelmintic and germicide as well as in the treatment of pyrexia, skin diseases, diabetes and helminthiasis. Its methanolic extract showed potent activity in regulating high blood pressure.[3] A series of clerodane diterpenoids were isolated from this plant with a wide range of biological activities such as cytotoxic,[4, 5] anti-bacterial,[6, 7] anti-fungal,[6] anti-inflammatory activities.[8, 9] Interestingly, recent studies have reported the potent neuroprotective activity of certain clerodane diterpenoids demonstrating their potentiating effect on nerve growth factor (NGF) activity[10] and their inhibitory effect on A*β* fibril accumulation.[11] However, the effect of this plant or its active secondary metabolites on acetylcholinesterase (AChE) has never been studied. AChE does not only play an important role in ailments related to cholinergic dysfunction, but it can also accelerate the aggregation of A*β* into amyloid fibril acting as an amyloid promoting factor.[12-14] In the present study, the TLC bioautographic assay was utilized to evaluate the AChE inhibitory activity of different extracts of *P. longifolia* var. *pendula* unripe fruits at the concentration of 10 μg/mL (Fig. S14). The preliminary bioassay results revealed the potent inhibitory effect of the methanolic extract on AChE activity. This active extract was further purified resulting in the isolation of three new diterpenes, polylongifoliaic A (**1**), polylongifoliaon A (**4**), and B (**5**), together with nine known diterpenes (**2**, **3**, **6**-**12**). The structural elucidation of the new isolates was achieved utilizing extensive analysis of their NMR and MS spectroscopic data. The relative configuration of polylongifoliaon A (**4**) and B (**5**) was determined by CD experiments. The neuroprotective activity of the isolated compounds was evaluated through examining their effect on AChE activity and on the viability of human neuroblastoma SK-N-MC cells under A*β*-insult.

Results and discussion

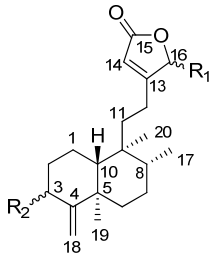
The methanolic extract of *P. longifolia* var. *pendula* unripe fruits was fractioned using different organic solvents. Bioassay-guided fractionation utilizing TLC bioautographic assay[15] to detect the AChE inhibitory activity indicated that the *n*-hexane layer is the most active layer and was subjected to further purification. From this layer, three new diterpenes, including one (4→2)-*abeo*-clerodane diterpene: polylongifoliaic A (**1**), and two 2-oxo-clerodane diterpenes: polylongifoliaon A (**4**), and B (**5**), along with nine known compounds including: 3*β*,5*β*-dihydroxy-16*α*-methoxy-halima-13*Z*-en-15,16-olide (**2**),[16] 3*β*,5*β*-dihydroxy-16*β*-methoxy-halima-13*Z*-en-15,16-olide (**3**),[16] (4→2)-*abeo*-16-hydroxy-cleroda-2(4)*E*,13*Z*-dien-15,16-olide-2-al (**6**),[17] 3*α*,16-dihydroxy-cleroda-4(18),13*Z*-dien-15,16-olide (**7**),[18] 3*β*,16-dihydroxy-cleroda-4(18),13*Z*-dien-15,16-olide (**8**),[19] 16(*R*&*S*)-hydroxy-3,13*Z*-kolavadien-15,16-olide-2-one (**9**),[17] 2-oxokolavenic acid (**10**),[20, 21] 16-methoxy-cleroda-3,13*Z*-dien-15,16-olide (**11**),[22] and 16-oxo-cleroda-3*Z*,13*E*-dien-15-oic acid methyl ester (**12**),[23] were isolated (Fig. 1). Among the known isolates, this is the first report for **11** to be isolated from natural sources.



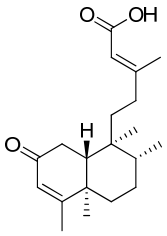
1 R = OH
6 R = H

2 R = *α*-OCH_3
3 R = *β*-OCH_3

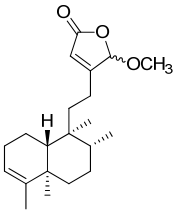
4 R = *α*-OCH_3
5 R = *β*-OCH_3
9 R = OH



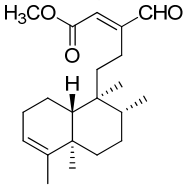
7 R_1 = OH, R_2 = *β*-OH
8 R_1 = OH, R_2 = *α*-OH



10



11



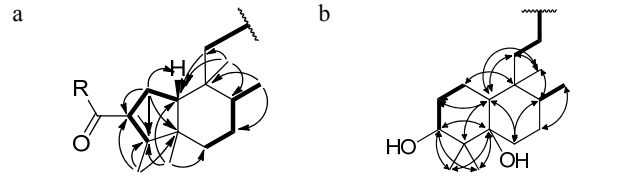
12

Fig. 1. Isolated compounds from *Polyalthia longifolia* var. *pendula*

Polylongifoliaic A (**1**) was isolated as white wax. The molecular formula of **1** was established as $C_{20}H_{28}O_5$ by its ^{13}C NMR and HRFABMS data, representing seven indices of hydrogen deficiency (IHD). The UV absorption band at 216 nm and the IR absorption bands at 1749 and 1685 cm^{-1} suggested the presence of *α*,*β*-unsaturated *γ*-lactone and *α*,*β*-unsaturated carboxylic acid, respectively. The ^{13}C NMR experiment revealed the presence of 20 carbons including four methyls, five methylenes, three methines and eight quaternary carbons (Table S1). The ^1H NMR spectrum indicated the presence of four methyl groups, including one olefinic methyl at δ_H 1.97 (3H, s, CH_3-18), two tertiary methyl groups with overlapped signals at δ_H 0.93 (6H, s, CH_3-19/CH_3-20), and a secondary methyl group at δ_H 0.86 (3H, d, J = 6.5 Hz, CH_3-17) (Table S1). Two signals at δ_H 6.04 (1H, s, H-16) and 5.90 (1H, s, H-14) which are characteristics to the *γ*-hydroxyl *α*,*β*-unsaturated-*γ*-lactone moiety were also detected, supporting the data from the UV and IR experiments (Table S1). Comparing the spectral data of **1** with other known diterpenes such as (4→2)-*abeo*-16-hydroxy-cleroda-2,13*Z*-dien-15,16-olide-2-al (**6**),[17] revealed that **1** possesses a clerodane-type skeleton with (4→2) rearranged ring A. This suggestion was further supported by the HMBC correlations between δ_H 2.33 and 2.27 (m, 2H, H_2-1) to δ_C 128.9 (C-2), 165.2 (C-4), 51.5 (C-5), and 55.5 (C-10) (Fig. S1). Additionally, the ^{13}C NMR data revealed that the aldehyde group (δ_C 189.3) attached to C-3 in **6** is replaced by a carboxylic group in **1** (δ_C 173.7). The A/B ring junction in **1** was deduced to be *trans* due to the lack of a NOESY correlation between δ_H 0.93 (CH_3-19) and δ_H 1.67 (H-10). The key correlations were shown in Fig. 2. Therefore, the structure of **1** was assigned as (4→2)-*abeo*-16-hydroxy-cleroda-2(4)*E*,13*Z*-dien-15,16-olide-2-oic acid.

Compounds **2** and **3** were isolated as a pair of diterpene enantiomers and their stereochemistry was deduced utilizing simulations of circular dichroism spectra by a time-dependent density functional theory.[16] The isolation of these compounds (**2** and **3**) was previously reported but without showing any spectral data.[16] Compound **2** was obtained as white wax. Its molecular formula was calculated as $C_{21}H_{34}O_5$ from the analysis of its positive HRFABMS data. The UV and IR absorption bands of **2** indicated the presence of a hydroxyl (IR: 3483 cm^{-1}), *α*,*β*-unsaturated lactone (UV: λ_{max} 207 nm; IR: 1758 cm^{-1}) functionalities. The ^1H and ^{13}C NMR signals of **2** (Table S1) indicated the presence of four methyl protons [δ_H 1.27 (3H, s, CH_3-18), 1.14 (3H, s, CH_3-19), 0.79 (3H, s, CH_3-20), 0.78 (3H, m, CH_3-17)], one methoxy [δ_H 3.57 (3H, s, CH_3-16)], one oxymethine proton [δ_H 3.59 (1H, t, J = 2.5 Hz, H-3)]. The presence of the *α*,*β*-unsaturated lactone was supported by two characteristic proton signals [δ_H 5.87 (1H, s, H-14), 5.65 (1H, s, H-16)], as well as by certain typical carbon resonances [δ_C 104.4 (C-16), 170.8 (C-15), 117.6 (C-14), 168.5 (C-13)]. The HMBC correlations [δ_H 3.59 (H-3)/δ_C 76.3 (C-5); δ_H 1.27 (H_3-18), 1.14 (H_3-19)/δ_C 76.3 (C-5), 76.3 (C-3), 41.3 (C-4); δ_H 1.75 (H-10*β*)/δ_C 76.3 (C-5)] confirmed the location of the quaternary hydroxylated carbon at C-5, which is next to the quaternary C-4 carbon with the geminal methyl groups (Fig. S1). The detailed analysis of the NMR spectral data of **2** revealed similarity to those of halimane-type diterpene such as 3*β*,5*β*,16*α*-trihydroxy-halima-13*Z*-en-15,16-olide except for an additional methoxy group in **2**.[5] The methoxy group was

assigned at C-16 based on the key HMBC correlation between δ_H 3.57 (CH_3-16) and δ_C 104.4 (C-16). To determine the stereochemistry of compound **2**, its NOESY and CD spectra were analyzed (Figs. 2 and 3). The NOESY correlation between δ_H 1.14 (CH_3-19) and 0.79 (CH_3-20) confirmed the A/B ring junction of this bicyclic diterpene as *cis* suggesting a *β*-orientation for H-10 and 5-OH (Fig. 2). Additionally, a smaller coupling constant and the NOESY correlations (Fig. 2) confirmed the *β*-orientation of 3-OH. The CD spectrum of **2** (Fig. 3) demonstrated a positive Cotton Effect due to n-*π** transition (235-250 nm) and a negative Cotton Effect due *π*-*π** (200-220 nm) transition suggesting an *S* configuration at C-16.[16] Thus, the structure of **2** was assigned as 3*β*,5*β*-dihydroxy-16*α*-methoxy-halima-13*Z*-en-15,16-olide.



The main fragment of *abeo*-clerodane **1** | The main fragment of **2**-**3**

Fig. 2. Selected HMBC and COSY correlations of **1**-**3**.

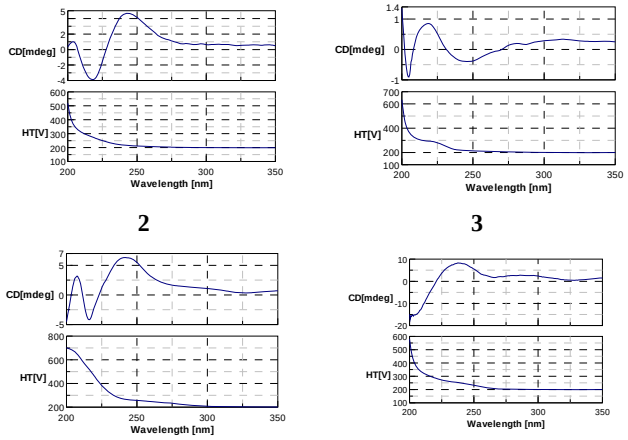


Fig. 3. The CD spectra of **2**, **3**, **4**, and **5**

Compound **3** was isolated as white wax, and was assigned the molecular formula of $C_{21}H_{34}O_5$ based on its HRFABMS. The UV, IR, 1D and 2D NMR spectroscopic data of **3** were identical to those of **2** (Table S1), except for the CD spectrum (Fig. 3). The detected negative Cotton Effect due to n-*π** transition (235-250 nm) and the positive Cotton Effect due to *π*-*π** transition (200-220 nm) were opposite to those of **2**, suggesting that **2** and **3** are epimers with **3** possessing *R* configuration at C-16 (Fig. 3).[16] Therefore, the structure of **3** was assigned as 3*β*,5*β*-dihydroxy-16*β*-methoxy-halima-13*Z*-en-15,16-olide. The experimental CD data of **2** and **3** were in agreement with the *ab initio* calculations of CD using Gaussian TDDFT (B3LYP) with the 6-31+G(d) and 6-311++G(d,p) basis.[16]

Polylongifoliaon A (**4**), and B (**5**) were both isolated as white waxes. They possess the same molecular formula ($C_{21}H_{30}O_4$) as revealed by their HRFABMS data, which indicated seven IHD. The IR spectra of both **4** and **5** showed characteristic absorption bands suggesting the presence of a hydroxyl (3449 cm^{-1}), *α*,*β*-unsaturated *γ*-lactone (1757 cm^{-1}) and conjugated carbonyl moieties (1681 cm^{-1}). The ^1H and ^{13}C NMR spectra of **4** showed signals attributable to a clerodane diterpene with an *α*,*β*-unsaturated *γ*-lactone including: four methyl groups [δ_H 1.95 (3H, d *J* = 1.0 Hz, CH_3-18)/δ_C 19.3, δ_H 1.18 (3H, s, CH_3-19)/δ_C 18.6, δ_H 0.90 (3H, s, CH_3-20)/δ_C 18.1, δ_H 0.87 (3H, d *J* = 6.2 Hz, CH_3-18)/δ_C 16.0], olefinic and oxymethine protons [δ_H 5.98 (1H, s, H-14)/δ_C 118.6, δ_H 5.82 (1H, s, H-16)/δ_C 106.3] with three quaternary carbons [δ_C 173.0, 170.4] (Table S2). A proton signal [δ_H 5.72 (1H, s, H-3), a tertiary carbon [δ_C 125.8 (C-3)] and two quaternary carbons [δ_C 202.8 (C-2), 176.4, (C-4)] suggested that the C-2 position in **4** should be oxidized, which was further supported by the HMBC correlations [δ_H 5.72 (H-3)/δ_C 41.3 (C-5), 19.2 (C-18); δ_H 2.48, 2.29 (H_2-1)/δ_C 202.8 (C-2), 47.0 (C-10)]. The detailed analysis of the NMR spectral data of **4** revealed its similarity to 16(*R*&*S*)-hydroxy-3,13*Z*-kolavadien-15,16-olide-2-one (**9**),[17] expect for an additional methoxy group [δ_H 3.55 (3H, s, CH_3-16) and δ_C 57.6]. The HMBC correlation between δ_H 5.82 (H-16) and δ_C 57.6 (CH_3-16) further confirmed the location of the methoxy group at C-16 in **4**, instead of the hydroxyl group (16-OH) in **9**.

The 1D and 2D NMR spectra of **4** and **5** were almost identical (Table 2), but they showed different CD absorption (Fig. 3). Compound **4** displayed a positive Cotton effect at 291 (shoulder) and 240 nm due to n-π* excitation and a negative Cotton effect at 214 nm due to π-π* transition. On the other hand, **5** showed a positive Cotton effect at 291 (shoulder) and 240 (weak) nm for n-π* band and a strong negative Cotton effect at 203 nm for π-π* transition. Through comparing these data with the CD data of known butenolides and applying the octant rule,[24] the orientation of the methyl groups at C-19 in **4** and **5** were suggested as *α*. However, the orientation of the methoxy group at C-16 in **4** was assigned as *α*, and as *β* in **5**.[16] Finally, the structures of **4** and **5** were elucidated as 16*α*-methoxy-3,13*Z*-kolavadien-15,16-olide-2-one and 16*β*-methoxy-3,13*Z*-kolavadien-15,16-olide-2-one, respectively.

The nine known compounds were identified by comparing their UV, IR, ^1H NMR, ^{13}C NMR and MS data with those reported in literature.

The recent findings on the neuroprotective activity of certain clerodane diterpenoids have encouraged us to evaluate the activity of the isolated compounds on the viability of human neuroblastoma SK-N-MC cells under A*β*-induced neurotoxicity (Fig. 4). The results indicated that the isolates did not exhibit cytotoxicity toward SK-N-MC cells except for **6** and **12**, the derivatives with an aldehyde group. In the Fig. 4, **1** and **5** showed significant protective activity (65-66%) against $A\beta_{1-42}$ insult at 10 μM. These compounds, **1** and **5**, demonstrated their neuroprotective effect in a dose dependent manner with IC_{50}

values of 1.64 μM and 3.75 μM, respectively (Fig. 5). On the other hand, compounds **8-10**, exhibited moderate protection (37.7-42%) (Fig. 4). The activity was comparable to epigallocatechin gallate (EGCG) (48.5%), which is a well-known neuroprotective agent. Many studies have demonstrated the neuroprotective activity of EGCG and revealed its mechanism.[25]

To further understand the activity of the isolated diterpenoids, we sought to study the structure activity relationship (SAR) of these compounds. It was found that the substituent nature as well as its stereoconfiguration at C-16 in the 2-furanone ring significantly affects the neuroprotective activity of the isolated diterpenes. Compound **8** with α hydroxyl group (electron withdrawing group) at C-16 exhibited more potent activity compared to **7** with β hydroxyl group. On the other hand, **5** with β methoxy group (electron donating group) was more active than **4** α methoxy group. Additionally, it was found that the opening of ring C as in compound **12**, did not significantly reduce the activity when compared to compounds with closed C ring (**11**).

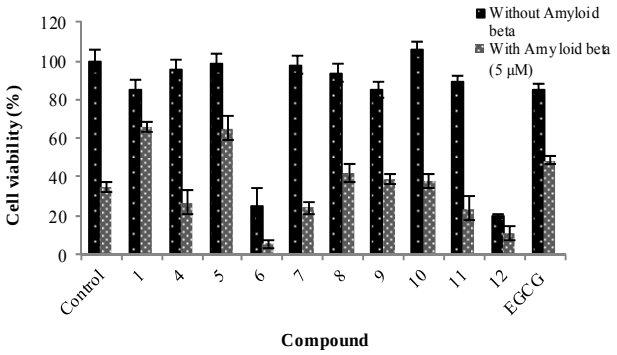


Fig. 4. The effect of the selected diterpenes isolated from *P. longifolia* var. *pedula* on SK-N-MC cell viability. Results are presented as mean ± S.E.M. (n = 3)

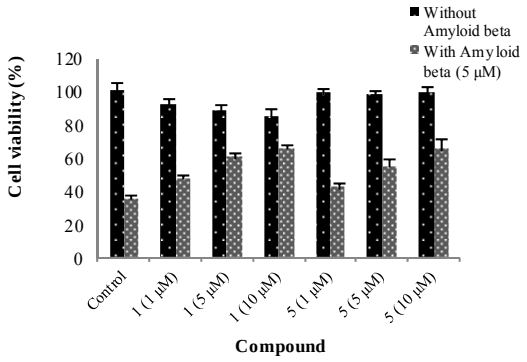


Fig. 5. The effect of **1** and **5** in different dosages were tested on SK-N-MC cell viability.

Additionally, the inhibitory effect of the isolated compounds on AChE was examined. Compounds **1**, **4**, **5**, **6**, **9**, **10** and **11** exhibited potent inhibitory effects on acetylcholinesterase (AChE) at the concentration of 20 μg/mL (Fig. S15). The effect of the active compounds on AChE was comparable to galantamine, a marketed second generation acetylcholinesterase inhibitors.[26] It is known that the accumulation of beta-amyloid peptides and the reduction in the activity of the cholinergic neurons are one of the well-known features of Alzheimer's disease (AD). However, no medication has been discovered to effectively delay or halt the progression of AD.[27] The potent neuroprotective activity of the isolated compounds warrants further investigation to determine the mechanism of action of *Polyalthia longifolia* diterpenes.

Experimental

Materials and methods

General Experimental Procedures.

Melting points were measured on a Yanaco MP-500D melting point apparatus (Yanaco, Kyoto, Japan) and were used uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on a Hitachi U-2800 UV-Vis spectrophotometer (Hitachi, Tokyo, Japan). IR spectra were taken on a Shimadzu IR Prestige-21 FT-IR spectrometer (Shimadzu, Nakagyo-ku, Japan). CD spectra were obtained on a JASCO J-715 spectropolarimeter (Bruker BioSpin GmbH, Karlsruhe, Germany). 1D and 2D NMR spectra were recorded with Bruker 400 AV, Bruker 500 AVII, and Varian Unity 600 NMR spectrometers (Bruker Daltonics, Bremen, Germany). HRFABMS data were measured with a Finnigan/Thermo Quest MAT 95XL spectrometer (Finnigan MAT LCQ, San Jose, CA, USA) and ESI-MS/MS data were obtained on a Bruker HCT ultra PTM Discovery system (Bruker Daltonics, Bremen, Germany). Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and Silica gel 60 (230-400 mesh or 70-230 mesh, Merck, Darmstadt, Germany) were used for column chromatography; precoated Si gel plates (silica gel 60 F_{254}, Merck, Darmstadt, Germany) were used for analytical TLC. The spots were detected by spraying with 50% H_2SO_4 aqueous solution and then heating on a hot plate. HPLC was performed on a Hitachi L-2130 pump equipped with a Hitachi L-2420 UV-Vis detector (Hitachi, Tokyo, Japan). Discovery® HS C_{18} (5 μm, 250 × 4.6 mm i.d., Supelco, Bellefonte, PA, USA) and semi-preparative Discovery® HS C_{18} (5 μm, 250 × 10 mm i.d., Supelco, Bellefonte, PA, USA) columns were applied for analytical and preparative purpose, respectively.

Reagent and Cell cultures

Amyloid β_{1-42} was purchased from Sigma (München, Germany). The human neuroblastoma SK-N-MC cell line (American Type Culture Collection) were cultured in minimum essential medium (MEM), supplemented with penicillin (50 IU/ml), streptomycin (50 μg/ml), non-essential amino acids, sodium pyruvate (1 mM), and 10% heat-inactivated fetal bovine serum. Cell culture was performed according to manufacturer's instructions.

The Cell viability assay in SK-N-MC cells under Aβ-induced neurotoxicity

For cell viability assay, cells were cultured in 24-well culture dishes, seeded at 2×10^5 cells per well, and maintained in a logarithmic growth phase. The cells were treated with the indicated compounds (10 μM) with or without amyloid $\beta_{1\text{-}42}$ peptide (5 μM) in the culture medium. After incubation for 24 h, cell viability was measured by the modified 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay method using MEM medium without phenol red. Absorbance of the converted dye was measured under the wavelength of 550 nm with a microplate reader. All tested compounds were re-purified by reversed-phase HPLC before the bioassay test (purity > 99%). All experiments were expressed as the mean ± S.E.M. from three different independent experiments. (n = 3) Student's *t*-test was performed for statistical analyses; a value of $P < 0.05$ was considered statistically significant.

Plant Material

The unripe fruits of *P. longifolia* var. *pendula* (500 g) were collected from Kaohsiung City, Taiwan, in September, 2005. A voucher specimen (PLP-F) was deposited in the Department of Marine Biotechnology and Resource, National Sun Yat-sen University, Kaohsiung 804, Taiwan.

Extraction and Isolation

The unripe fruits of *P. longifolia* var. *pendula* (500 g) were extracted with methanol (4L X 4 times). After removing the solvent, the MeOH extract (27.9 g) was partitioned with *n*-hexane and water to yield *n*-hexane (8.0 g) and aqueous layers. The *n*-hexane layer was further separated into four fractions (Ha-Hd) by column chromatography (CC) on silica gel with *n*-hexane-$CHCl_3$ and $CHCl_3$-MeOH as an eluent. Fraction Hb (2.5 g) was applied on a silica gel column eluted with a solvent mixture of increasing polarity (*n*-hexane-$CHCl_3$ and $CHCl_3$-MeOH) to yield 30 fractions (Hb-1~Hb-30). Fraction Hb-17 (254.0 mg) was purified by reversed-phase HPLC (75:25 MeOH:H_2O + 0.05% TFA) to obtain **9** (3.4 mg, t_R 12.4 min), **6** (15.0 mg, t_R 16.0 min), and **1** (4.0 mg, t_R 19.5 min). Fraction Hb-18 (210.0 mg) was separated by reversed-phase HPLC (75:25 MeOH:H_2O + 0.05% TFA) to obtain **10** (4.8 mg, t_R 18.5 min). Compounds **4**, **5** and **11** were isolated from the subfraction Hb-20 (139.0 mg) by reversed-phase HPLC eluted with 75:25 MeOH-H_2O + 0.05% TFA. Fraction Hb-21 (93.0 mg) was separated by reversed-phase HPLC (70:25 MeOH-H_2O + 0.05% TFA) to obtain **7** (1.0 mg, t_R 13.6 min) and **8** (2.4 mg, t_R 15.0 min). Fraction Hb-22 (59.0 mg) was separated by reversed-phase HPLC (73/27 MeOH:H_2O + 0.05% TFA) to obtain **3** (4.0 mg, t_R 29.0 min) and **2** (2.4 mg, t_R 34.0 min).

Characterization of compounds

Polylongifoliaic A (**1**): White wax; $[\alpha]_D^{25}$ +4.0 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (2.82) nm; IR (KBr) ν_{max}: 2961, 2927, 1749, 1685, 1642, 1405, 1384 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table S1; (+)-ESIMS *m/z* 371.2 $[M+Na]^+$ (100), 349.3 $[M+H]^+$ (12.6); (-)-ESIMS *m/z* 695.4 $[2M-H]^-$ (17.1), 461.2 $[M+TFA-H]^-$ (100), 347.3 $[M-H]^-$ (32,7); HRFABMS *m/z* 349.4401 $[M+H]^+$ (calcd for $C_{20}H_{31}O_4^+$, 349.4407).

Polylongifoliaon A (**4**): White wax; $[\alpha]_D^{25}$ -8.6 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 236.0 (3.40), 216.0 (3.50) nm; IR (KBr) ν_{max}: 2959, 2933, 2876, 1763, 1653, 1448, 1383, 1118 cm^{-1}; CD (*c* 0.30×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): 6.29 (244.5), -4.18 (216.0), 3.80 (207.5); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table S2; (+)-ESIMS *m/z* 715.4 $[2M+Na]^+$ (50.7), 369.2 $[M+Na]^+$ (100), 347.2 $[M+H]^+$ (0.2); (-)-ESIMS *m/z* 345.3 $[M-H]^-$ (100); HRFABMS *m/z* 347.2216 $[M+H]^+$ (calcd for $C_{21}H_{31}O_4^+$, 347.2222).

Polylongifoliaon B (**5**): White wax; $[\alpha]_D^{25}$ -18.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 236 (3.21), 215 (3.30) nm; IR (KBr) ν_{max}: 2963, 2938, 1788, 1761, 1668, 1652, 1464, 1377, 1118 cm^{-1}; CD (*c* 0.30×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): 8.27 (238.0), -15.70 (203.0); ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table S2; (+)-ESIMS *m/z* 715.4 $[2M+Na]^+$ (49.3), 369.2 $[M+Na]^+$ (100), 347.2 $[M+H]^+$ (0.8); (-)-ESIMS *m/z* 345.3 $[M-H]^-$ (73.4); *m/z* 346.2; HRFABMS *m/z* 347.2217 $[M+H]^+$ (calcd for $C_{21}H_{31}O_4^+$, 347.2222).

Conclusions

In summary, three new diterpenes (polylongifoliaic A (**1**), polylongifoliaon A (**4**), and B (**5**), together with nine known diterpenes (**2**, **3**, **6-12**), were isolated from the methanolic extract of *Polyalthia longifolia* var. *pendula* unripe fruits. Notably, it is the first report that extracts and active secondary metabolites from this species exert acetylcholinesterase (AChE) activity and protective effect to human neuroblastoma cells (SK-N-MC cells) under Aβ-induced neurotoxicity. Among the separated metabolites, polylongifoliaic A (**1**) and polylongifoliaon B (**5**) exhibited the most potent activity toward SK-N-MC cells with IC_{50}: 1.64 μM and 3.75 μM, respectively. The *in vivo* neuroprotective activity of the active metabolites in under investigation in our laboratory and will be disclosed in due course.

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