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New cholinesterase inhibiting bisbenzylisoquinoline alkaloids from *Abuta grandifolia*

Maria Francesca Cometa [a], Stefano Fortuna [a], Giovanna Palazzino [a], Maria Teresa Volpe [a], Elsa Rengifo Salgado [b], Marcello Nicoletti [c], Lamberto Tomassini [c,*]

[a] *Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299, I-00161 Rome, Italy*
[b] *Instituto de Investigaciones de la Amazonia Peruana (I.I.A.P.), Centro de Investigación Allpahuayo, Carretera Iquitos-Nauta km 26.8. Distrito San Juan Bautista, Maynas, Loreto, Peru*
[c] *Department of Environmental Biology, "La Sapienza" University, P.le Aldo Moro 5, I-00185 Rome, Italy*

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ABSTRACT

The phytochemical study of the stem bark and wood of *Abuta grandifolia* (Mart.) Sandwith led to the identification of four bisbenzylisoquinoline alkaloids (BBIQs), namely (*R,S*)-2 N-norberbamunine (**1**), (*R,R*)-isochondodendrine (**2**), (*S-S*)-O4″-methyl, Nb-nor-O6′-demethyl-(+)-curine (**3**), and (*S-S*)-O4″-methyl, O6′-demethyl-(+)-curine (**4**), together with the aporphine alkaloid *R*-nornuciferine (**5**), all obtained by countercurrent distribution separation (CCD) and identified on the basis of their spectroscopic data. Alkaloids **3** and **4** were new. All the isolated compounds were tested for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities. **1** was the most active against AChE, whereas **3** and **4** were the most potent against BChE. Interestingly, all tested alkaloids are more potent against BChE than against AChE. This selectivity of cholinesterase (ChE) inhibition could be important in order to speculate on their potential therapeutic relevance.

1. Introduction

Bisbenzylisoquinoline alkaloids (BBIQs) are one of the most important classes of natural alkaloids, for their pharmacological activities and taxonomically relevant distribution. Menispermaceae are a good source of BBIQs [1], characterized by different pharmacological activities [2–10], including cholinesterase (ChE) inhibitory activity [11–13]. *Abuta grandifolia* (Mart.) Sandwith is an Amazonian woody climbing vine used in ethnomedicine against various diseases, and, in particular, against malarial fevers [14–16]. This popular use was confirmed by the isolation of BBIQs with antiplasmodial activity [5].

We report the isolation of five alkaloids, 1–5, from stem bark and wood of *A. grandifolia*, as obtained by countercurrent distribution (CCD), including the two new structures **3** and **4** (Fig. 1). Based on several reports concerning the inhibitory activity of ChEs by BBIQs from Menispermaceae [11–13], we also carried out an evaluation of this activity for all the compounds obtained from *A. grandifolia*.

In particular, we assayed **1**–**5** in acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory tests, following recent indications of the different roles of brain AChE and BChE in neurological disease, such as Alzheimer's disease (AD), and of the therapeutic value of selective BChE inhibitory activity.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a JASCO DIP-370 polarimeter. CD spectra were registered on a JASCO 710

Abbreviations: ACh, Acetylcholine;AChE, Acetylcholinesterase;AcOH, Acetic acid;AcThCh, Acetylthiocholine iodide;AD, Alzheimer's disease;BBIQ, Bisbenzylisoquinoline alkaloid;BChE, Butyrylcholinesterase;BW284c51, 1,5-bis (4-allyldimethylammoniumphenyl)pentan-3-one dibromide;CCD, Countercurrent Distribution;CD, Circular Dichroism;CH_2Cl_2, Dichloromethane; ChE, Cholinesterase;DTNB, 5,5′-dithiobis (2-nitrobenzoic acid);ESI, Electrospray Ionization;HR-ESI, High Resolution Electrospray Ionization;iso-OMPA, Tetraisopropyl-pyrophosphoramide;NMR, Nuclear Magnetic Resonance;NOE, Nuclear Overhauser Effect;TLC, Thin Layer Chromatography.

* Corresponding author. Tel.: +39 6 49912198; fax: +39 6 49912195.
E-mail address: lamberto.tomassini@uniroma1.it (L. Tomassini).

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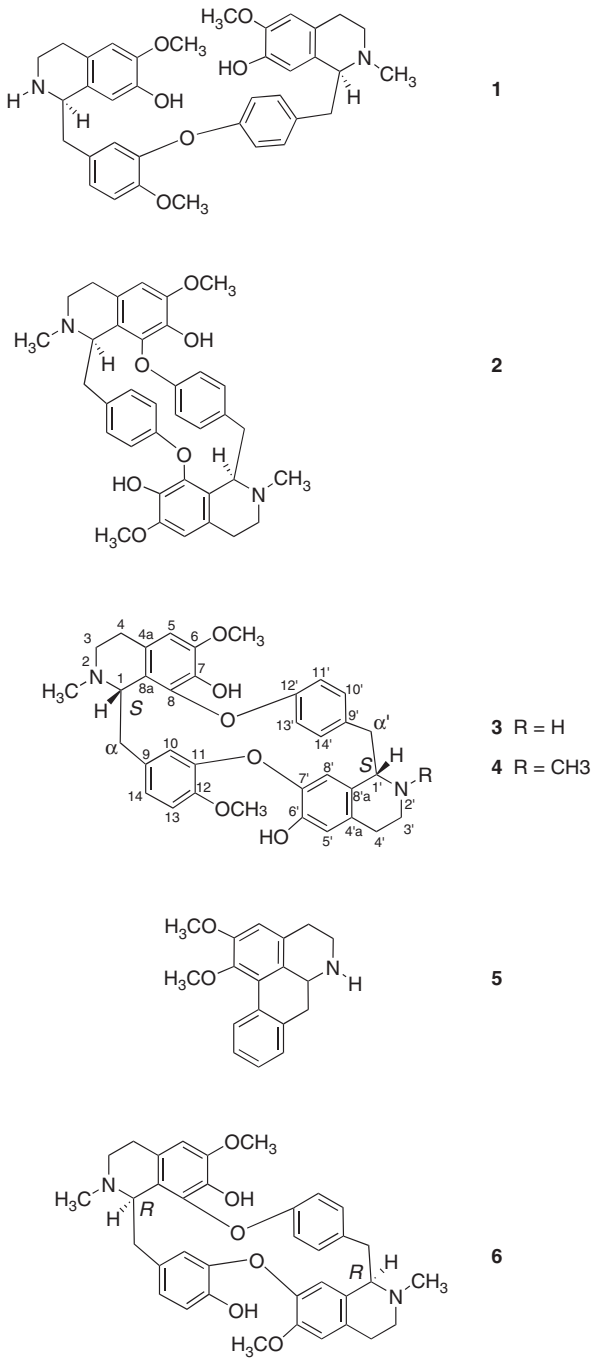

Fig. 1. Alkaloids isolated from *Abuta grandifolia* (**1**–**5**), including the new compounds (**3**–**4**), compared to curine (**6**).

spectropolarimeter. NMR spectra were recorded on a Bruker AM spectrometer, 500 and 125 MHz for ^{1}H-NMR e ^{13}C-NMR, respectively, and on a Bruker Avance spectrometer, 400 and 100 MHz for ^{1}H-NMR e ^{13}C-NMR, respectively. Electrospray ionization (ESI) mass spectra were obtained on a Thermofisher LCQ-Deca XP Plus ion-trap mass spectrometer and HR-ESI mass spectra were obtained on a Thermofisher Orbitrap Exactive spectrometer; both instruments were fitted with an ESI source and a syringe pump. CCD separation was performed in a Craig Post apparatus (200 steps, 10:10 ml upper and lower phases volume) between CH_2Cl_2 and discontinuously decreasing pH buffer solutions.

2.2. Chemicals

For anticholinesterase inhibitory tests, DMSO, 2-propanol, Acetylthiocholine iodide (AcThCh), 1,5-bis (4-allyldimethylammoniumphenyl)pentan-3-one dibromide (BW284c51), Tetraisopropyl-pyrophosphoramide (iso-OMPA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The alkaloid stock solutions were prepared in DMSO (10^{-2} M) diluted in DMSO/distilled water (1:10). The stock solution of BW284c51 and iso-OMPA were prepared in distilled water (10^{-2} M). Control assays were done with the concentration of the vehicle used.

2.3. Animals

Male CD1 mice (weighing 25–30 g) were purchased from Charles River (Calco, Italy). The animals were acclimatized to a 12-hr light/dark cycle at 21 °C with food and water available ad lib.

The directives of the Council of the European Communities (86/609/EEC) on animal care were duly observed.

2.4. Plant material

A large piece of twisted trunk, consisting of wood and bark of *A. grandifolia* (Mart.) Sandwith, was purchased in Iquitos, Peru, from an indigenous herbalist, who stated its popular use as efficient remedy against malaria. The species was identified by E. Rengifo Salgado, at *Centro de Investigación Allpahuayo* (I.I.A.P.), where a voucher specimen (ES-289) is deposited in the local herbarium.

2.5. Extraction and isolation

The dried plant material was ground to a yellowish powder with a strong bitter smell. The powder (470 g) was percolated with 2% aqueous acetic acid (AcOH), overnight for three times. The resulting combined acidic extracts were made alkaline up to pH 8 by Na_2CO_3 and extracted three times with CH_2Cl_2. The combined organic layers were evaporated under vacuum to dryness to afford the crude total tertiary alkaloids as free bases. The remaining aqueous phase, acidified to pH 2 with 4 N HCl and treated with picric acid, did not afford any precipitate of quaternary alkaloid picrates.

The dried organic extract of tertiary alkaloids (about 5 g) was separated by CCD [17] in a Craig–Post apparatus between CH_2Cl_2 (stationary lower phase) and phosphate/citric acid buffer solutions (mobile upper phase) at discontinuously decreasing pH (from 6.5 to 3.0) [18].

The separation by CCD was monitored by TLC plates (silica gel 60 F_{254}; elution with BAW, the upper phase of the system solvent *n*-BuOH/AcOH/H_2O(5:1:4; v/v/v)). From the emerging aqueous phases the alkaloids were extracted by CH_2Cl_2 after alkalinization with Na_2CO_3 up to pH 8–9.5.

With pH 6.4 aqueous phase was first eluted compound **1** (200 mg), at pH 5.8 compound **2** (850 mg), at pH 4.8 compounds **3** (150 mg) and **4** (180 mg), and at pH 3.8 compound **5** (80 mg).

(S-S)-O4″-methyl, Nb-nor-O6′-demethyl-(+)-curine (**3**): light yellow powder; $[\alpha]^{25}{}_{D}$ +243.7 (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.39), 285 (4.02) nm; IR ($CHCl_3$) ν_{max} 3450, 1501, 1458 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz); δ 6.97 (1H, dd, *J* = 8.2, 1.7 Hz, H-10′), 6.95 (1H, dd, *J* = 8.2, 1.8 Hz, H-14), 6.77 (1H, d, *J* = 8.2 Hz, H-13), 6.74 (1H, dd, *J* = 8.1, 2.2 Hz, H-13′), 6.64 (1H, s, H-5′), 6.62 (1H, d, *J* = 1.8 Hz, H-10), 6.61 (1H, dd, *J* = 8.2, 2.2 Hz, H-11′), 6.58 (1H, dd, *J* = 8.1, 1.7 Hz, H-14′), 6.53 (1H, s, H-5), 5.89 (1H, s, H-8′), 3.86 (3H, s, OCH_3-6), 3.82 (1H, dd, *J* = 9.7, 2.5 Hz, H-1′), 3.72 (3H, s, OCH_3-12), 3.49 (1H, dd, *J* = 6.8, 4.2 Hz, H-1), 3.32 (1H, dt, *J* = 12.6, 4.5 Hz, H-3a), 3.20 (1H, m, H-3′a), 3.01 (2H, m, H-3′b and H-α′a), 2.93 (1H, ddd, *J* = 15.3, 6.3, 4.5 Hz, H-4a), 2.85 (1H, dd, *J* = 12.6, 6.3 Hz, H-3b), 2.77 (2H, m, H_2-α), 2.68 (3H, m, H_2-4′ and H-α′b), 2.41 (1H, dd, *J* = 15.3, 4.5 Hz, H-4b), 2.19 (3H, s, NCH_3-2); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; ESIMS *m/z* 581 $[M+H^+]^+$, 564, 550, 533, 298, 284; HR-ESIMS *m/z* 581.2644 $[M+H^+]$ (calcd for $C_{35}H_{36}N_2O_6$, 581.2646).

(S-S)-O4″-methyl, O6′-demethyl-(+)-curine (**4**): light yellow powder; $[\alpha]^{25}{}_{D}$ +213.9 (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.90), 225 (4.61), 282 (3.98) nm; IR ($CHCl_3$) ν_{max} 3465, 1510, 1452 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz); δ 7.02 (1H, dd, *J* = 8.4, 1.8 Hz, H-14), 6.99 (1H, dd, *J* = 8.3, 1.7 Hz, H-10′), 6.77 (1H, d, *J* = 8.4 Hz, H-13), 6.67 (1H, dd, *J* = 8.3, 2.0 Hz, H-11′), 6.66 (1H, s, H-5′), 6.63 (1H, dd, *J* = 8.0, 2.0 Hz, H-13′), 6.62 (1H, d, *J* = 1.8 Hz, H-10), 6.53 (1H, s, H-5), 6.41 (1H, dd, *J* = 8.0, 1.7 Hz, H-14′), 5.75 (1H, s, H-8′), 3.88 (3H, s, OCH_3-6), 3.73 (3H, s, OCH_3-12), 3.58 (1H, dd, *J* = 8.3, 3.0 Hz, H-1), 3.42 (1H, dd, *J* = 11.1, 2.1 Hz, H-1′), 3.34 (1H, dt, *J* = 12.8, 4.5 Hz, H-3a), 3.23 (1H, m, H-3′a), 3.08 (1H, dd, *J* = 11.1, 2.1 Hz, H-α′a), 2.96 (1H, ddd, *J* = 17.2, 6.3, 4.5 Hz, H-4a), 2.88 (1H, dd, *J* = 12.8, 6.3 Hz, H-3b), 2.83 (1H, m, H-4′a), 2.81 (2H, m, H_2-α), 2.78 (1H, dd, *J* = 15.3, 3.2 Hz, H-3′b), 2.62 (1H, dd, *J* = 16.9, 4.5 Hz, H-4′b), 2.46 (3H, s, NCH_3-2′), 2.45 (1H, t, *J* = 11.1 Hz, H-α′b), 2.42 (1H, dd, *J* = 17.2, 4.5 Hz, H-4b), 2.28 (3H, s, NCH_3-2); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; ESIMS *m/z* 595 $[M+H^+]^+$, 564, 552, 533, 298; HR-ESIMS at *m/z* 595.2795 $[M+H^+]$ (calcd for $C_{36}H_{38}N_2O_6$, 595.2803).

2.6. Cholinesterase inhibition assays

Mice were killed by decapitation, and the whole brain minus cerebellum was rapidly removed, weighed and homogenized, using a Polytron apparatus (Kinematica Gmbh) for 1 min, in 20 volumes of cold 0.038 M Tris–HCl buffer, pH 7.6.

Brain homogenates were centrifuged at 1000 *g* at 4 °C for 10 min in a Sorwall RC-5B centrifuge to remove unbroken cells and cellular debris. Aliquots (50 μl) of the supernatants (diluted 1:10 in distilled water) were used as the enzyme source.

Blood samples were centrifuged at 1000 *g* at 4 °C for 5 min in a Sorwall RC-5B centrifuge to separate plasma from red blood cells. Aliquots (25 μl) of the serum (diluted 1:40 in distilled water) were used as the enzyme source.

For enzymatic analysis, ChE activity was determined by a well-known and suitable spectrophotometric method [19], as already described [20,21]. The samples were incubated with the substrate in 0.05 M sodium phosphate buffer, pH 7.2, containing 0.25 mM DTNB in a final volume of 1.4 ml, for 30 min at 25 °C (in duplicate). AcThCh, 0.56 mM/tube, was used as substrate. The hydrolysis of the substrate was measured spectrophotometrically at a wavelength of 412 nm by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of AcThCh. Specificity of the spectrophotometric method was assessed by using specific AChE (BW284c51) and BChE (iso-OMPA) inhibitors.

Brain homogenates were incubated with BW284c51 (0.001–1 μM), or alkaloids (1–100 μM), 10 min before addition of the substrate (0.56 mM). Similarly, serum samples were incubated with iso-OMPA (0.001–10 μM) or alkaloids (0.001–10 μM). The residual enzyme activity for each sample was determined and the percentage inhibition of control activity was calculated.

Table 1
^{13}C-NMR data (100 MHz, $CDCl_3$) (δ_C, mult.) for 3 and 4, in comparison with 6.

Position	**3**		**4**		**6**	
	Part A	Part B	Part A	Part B	Part A	Part B
1	60.5, CH	57.1, CH	60.3, CH	65.1, CH	60.2, CH	65.1, CH
3	43.7, CH_2	39.4, CH_2	43.7, CH_2	45.0, CH_2	43.7, CH_2	45.2, CH_2
4	21.7, CH_2	28.6, CH_2	21.6, CH_2	24.0, CH_2	21.8, CH_2	24.8, CH_2
4a	124.3, C	129.1, C	128.7, C	127.1, C	124.8, C	128.7, C
5	108.3, CH	116.0, CH	107.9, CH	115.6, CH	108.1, CH	112.4, CH
6	147.0, C	146.3, C	147.0, C	146.2, C	146.7, C	148.4, C
7	137.8, C	142.8, C	139.0, C	142.4, C	137.3, C	143.3, C
8	138.8, CH	117.8, CH	137.7, CH	118.7, CH	138.4, C	119.3, CH
8a	124.8, C	130.3, C	124.2, C	124.5, C	124.1, C	128.6, C
α	39.9, CH_2	43.1, CH_2	39.8, CH_2	40.0, CH_2	39.7, CH_2	39.2, CH_2
9	135.0, C	132.4, C	134.9, C	132.1, C	133.5, C	131.7, C
10	122.2, CH	129.7, CH	121.9, CH	129.5, CH	120.9, CH	132.1, CH
11	145.2, C	113.3, CH	145.0, C	113.2, CH	143.6, C	113.1, CH
12	148.9, C	155.5, C	148.8, C	155.4, C	146.2, CH	155.3, C
13	111.4, CH	115.2, CH	111.5, CH	114.8, CH	115.4, CH	115.4, CH
14	126.4, CH	132.0, CH	126.0, CH	131.9, CH	126.6, CH	129.4, CH
CH_3N	41.6, CH_3	…	41.5, CH_3	41.5, CH_3	41.4, CH_3	41.7, CH_3
$CH_3O(6)$	56.1. CH_3	…	55.9, CH_3	…	56.0, CH_3	56.0, CH_3
$CH_3O(12)$	55.9, CH_3	…	55.9, CH_3	…	…	…

2.7. Statistical analysis

IC_{50} values were expressed as the mean ± S.E.M and obtained by extrapolation from the linear regression line of ChE activity as a function of the inhibitor (BW284c51, iso-OMPA or alkaloids) concentration (GraphPad software). The significance of differences between the means was determined by one-way analysis of variance (ANOVA) and post-hoc analysis was performed using Student–Newman–Keul's method for multiple comparison of means.

The value of $p<0.01$ was taken as the cut-off value to consider a difference statistically significant.

3. Results and discussion

The plant material, consisting in ground wood and bark of *A. grandifolia*, was extracted with diluted AcOH, and the filtered solution was basified and re-extracted with CH_2Cl_2. The extract obtained from the organic phase was then dried and subjected to CCD between CH_2Cl_2 and decreasing pH buffer solutions, to yield compounds **1–5**.

The structures of compounds **1** and **2**, elucidated by means of accurate spectroscopic data comparison to literature values, were assigned to two known BBIQs, namely: (*R,S*)-2 N-norberbamunine and (*R,R*)-isochondodendrine (previously isolated from *Berberis stolonifera* cell cultures [22] and from *Chondodendron* species [23], respectively).

Compound **3** was obtained as a pale yellow, amorphous powder. A molecular formula of $C_{35}H_{36}N_2O_6$ was assigned to **3** on the basis of HR-ESIMS peak at *m/z* 581.2644 $[M+H]^+$ (calcd. 581.2646), consistent with a BBIQ containing two diaryl ether bridges and one N-methyl group. Typical fragmentation pattern (protonated ions at 298 and 284, originated by the breaking of C-1−C-α and C-1′−C-α′ bonds) imposed a head to tail structure, in accordance with the structure assigned to **2**.

The NMR spectra of **3** showed a close similarity with the corresponding data of (*R,R*)-curine, **6**, a BBIQ well known for its abundant presence in Menispermaceae and in the tube-curare obtained from *Chondodendron* [24], with three main differences.

- In the ^{13}C NMR spectrum of **3** the lower-field N–Me, present in **6** at δ 42.3, was missing. This absence was easily assigned to the part B of **3**, based on the shift variation of the two carbons next to NH (C-1′ and C-3′, respectively at δ 58.3 and 39.8) (Table 1).
- The NMR spectra of **3** showed the presence of two methoxy groups, as in **6**, but its ^1H NOE-difference spectra allowed to assign both of them to part A, at positions 6 and 12. In particular, an evident positive NOE on H-13 was observed by irradiation of 12-OMe, as well as that on H-5 by irradiation of 6-OMe.
- The CD spectrum of **3**, characterized by positive Cotton effect at 235 and 288 nm, was indicative of a stereochemistry type 1-*S*,1′-*S*, opposite to that of **6**.

On the basis of the aforementioned data, also confirmed by ^{13}C-–^1H HETCOR and COLOC NMR data, the structure of **3** was assigned as (*S-S*)-O4″-methyl, Nb-nor-O6′-demethyl-(+)-curine.

Analogous considerations could be done for compound **4**, whose NMR data were very similar to those of **3**, being almost superimposable. In this regard, it was noteworthy in the proton spectrum of both **3** and **4** the presence of characteristic singlets at δ 5.62 and 5.78, respectively (attributed in both cases to the aromatic proton H-8′). These singlets were strongly shielded, in comparison to the other aromatic signals, due to the effect of the benzylic ring-current, as a consequence of the particular spatial disposition of the molecules.

Besides these similarities, the more evident difference between the two compounds was the presence in **4** of both N-methyl groups (at δ 2.28 and 2.46 in the ^1H NMR, and, coincident, at δ 41.5, in the ^{13}C NMR spectrum). A further confirmation was obtained from the ESIMS spectrum which showed the peak $[M+H]^+$ at *m/z* 595 and one peak at 298, owing to the coincidence of the two peaks caused by the molecule fragmentation at the 1-α bonds.

This evidence, also confirmed by the raw formula $C_{36}H_{38}N_2O_6$, assigned on the basis of HR-ESIMS peak at *m/z* 595.2795 (calcd. 595.2803), and by the CD spectrum (almost identical to the previous one and then corresponding to a 1-*S*, 1′-*S* type configuration), indicated that **4** was the N-methyl derivative of **3**, (*S-S*)-O4″-methyl, O6′-demethyl-(+)-curine.

The last isolated compound, **5**, was different from the others, having a base structure of aporphine type. Its spectroscopic data, compared with the literature, showed that **5** was the known compound *R*-(+)-nor-nuciferine already isolated from *Nelumbo nucifera* [25].

So far, several BBIQs obtained from Menispermaceae have been reported to inhibit both AchE and BChE [11–13]. For many years, brain AChE has been considered as the principal target of cholinesterase inhibitors used for the therapy of AD [26]. AD is a chronic neurological disorder characterized principally by cognitive dysfunction associated with a cholinergic deficit [27,28]. A few AChE inhibitors as tacrine [29], donepezil [30], galanthamine [31], and rivastigmine [32] obtained also a market approval for patients with mild-to moderate AD. However, all of these inhibitors have limited effectiveness and different tolerability profiles mainly due to their differential selectivity for AChE and BChE, mechanisms of enzyme inhibition, and differential selectivity for various molecular forms of each ChE [33–35].

It is well known that in healthy human brain, AChE activity predominates over BChE [36]. In AD, AChE activity falls by up to 85% in specific brain regions, while BChE levels rise with disease progression [37].

Moreover, other studies suggest that AChE and BChE may have roles also in modulating the activity of other proteins, regional cerebral blood flow, tau phosphorylation, and the amyloid cascade that may affect rates of AD progression. In particular, BChE seems to have an important role in the development of the amyloid-rich neuritic plaques which are considered the most important focus of current AD research [33].

These pieces of evidence could modify the normally supportive role of BChE in hydrolyzing excess ACh only, suggesting that a selective BChE inhibition may be useful in inhibiting other pathogenetic factors, which likely worsen in AD due to increased activity of BChE [38,39].

Therefore, the search for the new ChE inhibitors with a different therapeutic balance between AChE and BChE inhibition

Table 2
At concentrations of 1–100 μM, each alkaloid inhibits mouse brain total ChE activity in a concentration-dependent manner. The highly selective AChE inhibitor, BW284c51 (0.001–1 μM), was used as positive control. At concentrations of 0.001–10 μM, each alkaloid inhibits serum ChE activity in a concentration-dependent manner. The highly selective BChE inhibitor, iso-OMPA, was used as positive control (0.001–10 μM). IC_{50} was obtained by extrapolation from linear regression line of the ChE activity as function of each alkaloid and positive controls concentrations.

Compounds	IC $_{50}$ (μM) ± S.E.M.[a]			
	AChE		BChE	
		R^2		R^2
1	34.66 ± 3.02	0.90	9.46 ± 0.49	0.90
2	78.22 ± 2.12	0.92	N.y.	
3	68.32 ± 2.81	0.92	1.90 ± 0.15	0.86
4	44.56 ± 3.50	0.91	1.00 ± 0.44	0.84
5	N.y.		5.60 ± 0.72	0.81
BW284c51	0.02 ± 0.01	0.82		
ISO-oMPA			1.88 ± 0.12	0.95

[a] Standard error of the mean of five assays.

could be considered an important strategy to introduce new drug candidates against AD and also related dementia.

Like in human brain, in the experimental animal models, by using selective inhibitors, it has been found that brain AChE predominates over brain BChE activity while the serum ChE form is predominantly of BChE [36,40].

Based on these observations, all the isolated alkaloids were tested for ChE inhibitory activities using as source of AChE and BChE, mouse brain and serum respectively. The single alkaloid potency of ChE inhibition was also calculated by comparing their inhibitory activities to those of the highly selective AChE and BChE inhibitors (BW284c51 and iso-OMPA respectively).

Interestingly, all tested alkaloids inhibited mouse brain total AChE activity (in the range of 1–100 μM) and serum BChE activity (in the range of 0.001to 10 μM) in a concentration-dependent manner.

For brain AChE inhibitory activity, based on IC_{50} values the order of potency is: **1>4>3>2**, being **1** IC_{50} = 34.66 ± 3.02 (R2 = 0.90) The highly selective AChE inhibitor BW284c51, yielded an IC_{50} of 0.02 ± 0.01 μM (R2 = 0.82). Compound **5** IC_{50} was not yielded (Table 2).

For serum BChE inhibitory activity, based on IC_{50} values the order of potency is: **4>3>5>1**, being IC_{50} values for **4** and **3** equal to 1.00 ± 0.44 (R2 = 0.84) and IC_{50} = 1.90 ± 0.15 (R2 = 0.86), respectively. The highly selective BChE inhibitor iso-OMPA, yielded an IC_{50} of 1.88 ± 0.12 μM (R2 = 0.95). Compound **2** IC_{50} was not yielded (Table 2).

It is noteworthy that, as already described for other BBIQs [13], all tested alkaloids are more potent against BChE than against AChE. Among them, compound **1** is the most active against AChE, whereas the new compounds **3** and **4** are the most potent against BChE activity. In our opinion this different selectivity of ChE inhibition merits to be studied in new investigations on kinetic mechanism of inhibition and has to be confirmed also in *in vivo* studies, in order to speculate on a therapeutic potential clinically relevant.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.fitote.2011.12.015.

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