Research Article

Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities of β -Carboline and Quinoline Alkaloids Derivatives from the Plants of Genus *Peganum*

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It was reported that the main chemical constituents in plants of genus *Peganum* were a series of β -carboline and quinoline alkaloids. These alkaloids were quantitatively assessed for selective inhibitory activities on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) by *in vitro* Ellman method. The results indicated that harmaline was the most potent selective AChE inhibitor with an IC_{50} of $7.11 \pm 2.00 \mu M$ and AChE selectivity index (SI, IC_{50} of BChE/ IC_{50} of AChE) of 10.82. Vasicine was the most potent BChE inhibitor with feature of dual AChE/BChE inhibitory activity, with an IC_{50} versus AChE/BChE of $13.68 \pm 1.25/2.60 \pm 1.47 \mu M$ and AChE SI of 0.19. By analyzing and comparing the IC_{50} and SI of those chemicals, it was indicated that the β -carboline alkaloids displayed more potent AChE inhibition but less BChE inhibition than quinoline alkaloids. The substituent at the C7 position of the β -carboline alkaloids and C3 and C9 positions of quinoline alkaloids played a critical role in AChE or BChE inhibition. The potent inhibition suggested that those alkaloids may be used as candidates for treatment of Alzheimer's disease. The analysis of the quantitative structure-activity relationship of those compounds investigated might provide guidance for the design and synthesis of AChE and BChE inhibitors.

1. Introduction

Alzheimer's disease (AD) was characterized by dementia that typically begins with subtle recognition failure and poor memory. It slowly becomes more severe and, eventually, incapacitating. The cholinergic system seemed particularly susceptible to synapse loss, especially in cortical regions associated with memory and executive function [1]. Recent studies showed that the main cause of the loss of cognitive functions in AD patients was a continuous decline of the cholinergic neurotransmission in cortical and other regions of the human brain [2]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are hydrolytic enzymes that act on acetylcholine (ACh) to terminate its actions in the

synaptic cleft by cleaving the neurotransmitter to choline and acetate. Both enzymes are present in the brain and detected in neurofibrillary tangles and neuritic plaques. It was suggested that AChE predominates in the healthy brain, with BChE considered to play a minor role in regulating brain ACh levels. However, BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines. Both enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral, and global functioning characteristics of AD [3]. Despite the unknown etiology of AD, those findings supported that it was needed to control the activity of the cholinesterase (ChE) at different stages of AD progression. One of the most effective

treatment strategies was suggested to restrain cholinergic function and elevate ACh level through inhibiting AChE and BChE. Therefore, AChE and BChE inhibitors were developed for the treatment of AD [4, 5].

The history of drug discovery showed that plants were rich sources of new active compounds, and many synthetic drugs owed their origin to plant-based medicine. The genus *Peganum* consists of six species and one subspecies and three of them, that is, *P. harmala* Linn, *P. nigellastrum* Bunge, and *P. multisectum* (Maxim.) Bobr, are mainly distributed in the arid and semiarid areas in the northwest of China [6]. *P. harmala* is a well-known and effective herbal medicine in Turkey, Iran, and China [6–8]. The seeds and whole plants appear to possess medicinal properties and can be used to treat various ailments including rheumatism, hypertension, diabetes, and asthma jaundice and as antiparasitic agents [6, 9–11]. It was reported that the main chemical constituents in the seeds and whole plant of genus *Peganum* were a series of β -carboline and quinoline alkaloids [12, 13].

As part of the same project, we first reported the screening of AChE inhibition activity of seeds extracts from genus *Peganum* with rapid bioautographic assay on TLC plates [14, 15]. It was found that the alkaloids fraction (mainly β -carboline and quinoline alkaloids) showed potential inhibitory effects on the AChE activity. A number of alkaloids including two new compounds nigellastrine I and nigellastrine II, along with eight known alkaloids, vasicinone, vasicine, harmine, deoxyvasicinone, deoxyvasicine, harmaline, harmol, and harmane, were isolated with their structures identified from *P. nigellastrum* Bunge, and their AChE inhibition activity was semiquantitatively evaluated by TLC-bioautographic assay [14]. Subsequently, intrigued by the interesting biological activities, Boerth and Rasapalli developed a synthetic route for nigellastrine, vasicinone, and luotonins [16]. In addition, it was also proved that the β -carboline alkaloids from *P. harmala* exhibited improvement effects on learning and memory of model dementia mice induced by aluminum [17].

The present study was undertaken to quantificationally evaluate the inhibitory activity of those alkaloids against AChE and BChE by *in vitro* Ellman method [18, 19]. Furthermore, by comparing the IC_{50} values and AChE selectivity index (SI, IC_{50} of BChE/ IC_{50} of AChE) and analyzing the structure-activity relationship of those compounds investigated on selective AChE or BChE inhibition, it could provide a direction for the design and synthesis of new AChE and BChE inhibitors.

2. Materials and Methods

2.1. Chemicals and Instruments. The following chemicals were obtained from Sigma-Aldrich (USA): AChE from *Electrophorus electricus*, BChE from equine serum, acetylthiocholine iodide, butyrylthiocholine iodide, 5,5'-dithiobis-bis-nitrobenzoic acid (DTNB), L-glutathione (L-GSH), harmaline, harmine, harmane, 1,2,3,4-tetrahydroharmine-3-carboxylic acid, and galanthamine; harmalol hydrochloride dehydrate and harmol were purchased from Wako

Pure Chemical Industries, Ltd. (Japan); vasicine, vasicinone, deoxyvasicinone, and deoxyvasicine were separated and purified in our laboratory from seeds of *P. harmala*, and nigellastrine I and nigellastrine II were separated and purified in our laboratory from seeds of *P. nigellastrum* ($\geq 98\%$ purity), and their physical and spectral data were in good agreement with the literature data [14]. The structures of those compounds investigated were showed in Figure 1.

Microplate reader (Power wave XS, Bio-Tek Instruments, Winooski, VT, USA), precise pH instrument (PB-10, sartorius, Germany), and Eyela bath SB-9 (NTT-2200, Tokyo Rikakikak Co. Ltd.) were used.

2.2. Preparation of Solutions. The enzymes AChE and BChE were dissolved in 20 mM sodium phosphate buffer (pH 7.6) to make the 3.47 unit/mL stock solution, and the solutions were stored at -80°C before use. The stock solutions of the test compounds (harmaline, harmine, harmalol, harmol, harmane, vasicine, 1,2,3,4-tetrahydroharmine-3-carboxylic acid, vasicinone, deoxyvasicinone, deoxyvasicine, nigellastrine I, nigellastrine II, and galanthamine) were prepared, respectively, by dissolving an adequate quantity of each compound in 0.2% DMSO to obtain 20 mM solutions. All the stock solutions were diluted to a series of concentrations with 20 mM sodium phosphate buffer solution (pH 7.6) before each experiment. DTNB-phosphate-ethanol reagent was prepared as follows: to 12.4 mg of DTNB dissolved in 120 mL of 96% ethanol, 80 mL of distilled water and 50 mL of 0.1 mM phosphate buffer (pH 7.6) were added [20].

2.3. In Vitro Anticholinesterase Assays. The AChE and BChE activity assay was carried out using acetylthiocholine iodide and butyrylthiocholine iodide as substrates, respectively, based on a colorimetric method, as described previously [18–20]. 10 μL of the test compounds solution in 0.2% DMSO, 79 μL of 20 mM sodium phosphate buffer (pH 7.6), and 1 μL enzyme preparation (with final concentrations: 0.087 unit/mL for AChE, or 0.035 unit/mL for BChE, and final concentrations: 1 to 500/1000 μM for compounds tested) were mixed and preincubated for 15 min. To the mixture, 10 μL substrate solution was added (final concentrations 1.5 mM for acetylthiocholine iodide, or 4 mM for butyrylthiocholine iodide) and incubated for 30 min. The reaction was stopped by adding 900 μL DTNB-phosphate-ethanol reagent. The absorption was read immediately at 412 nm on a microplate reader. The concentration of the test compound required to inhibit AChE or BChE activity by 50% (IC_{50}) was calculated using an enzyme inhibition dose response curve, with galanthamine as a positive control and L-GSH as standard to draw the standard curves. AChE SI was calculated by using the following formula: $SI = IC_{50} \text{ of BChE} / IC_{50} \text{ of AChE}$.

2.4. Data Analysis. The assays were conducted in triplicate, and all tabulated results were expressed as means $\pm$ SD, and the IC_{50} values were calculated by Boltzmann's dose response analysis using Origin 8.0 software.

FIGURE 1: Structures of the compounds investigated.

3. Results

The inhibitory activities of the β -carboline and quinoline alkaloids on AChE and BChE were evaluated *in vitro* using an inhibition assay with AChE (electric eel) and BChE (equine serum) and were summarized in Table 1.

First of all, it was observed that the inhibition data of reference compounds galanthamine within the limits of the different enzyme source and assay experimental conditions are in good agreement with the literature data [21].

All tested compounds exhibited some degree of activity on both AChE and BChE, and their IC_{50} values were in the low micromolar range for at least one enzyme, with

the exception of compounds deoxyvasicine and 1,2,3,4-tetrahydroharmine-3-carboxylic acid. Harmane showed the most potent inhibitory activity with IC_{50} value of $7.11 \pm 2.00 \mu M$ against AChE. However, harmane had a low inhibitory activity against BChE (IC_{50} of $76.91 \pm 1.57 \mu M$). Harmine and harmaline significantly inhibited AChE activity with IC_{50} values of $9.05 \pm 1.08 \mu M$ and $10.58 \pm 2.01 \mu M$, but had lower inhibitory activity against BChE with IC_{50} values of $75.07 \pm 1.29 \mu M$ and $101.39 \pm 1.39 \mu M$, respectively. Vasicine exhibited strong inhibition both on AChE and BChE activities with IC_{50} values of 13.68 ± 1.25 and $2.60 \pm 1.47 \mu M$, respectively. As the main metabolites of harmaline and harmine *in vivo* [22, 23], harmalol and harmol exhibited

TABLE 1: Inhibitory activity (IC_{50}) and selectivity index (SI) of the compounds investigated against AChE and BChE.

Compounds	IC_{50} ($\mu M \pm SD$)		SI
	AChE	BChE	
Gаланthamine	1.18 $\pm$ 1.05 0.8 [21]	/ 7.3 [21]	9.13
Harmane	7.11 $\pm$ 2.00	76.91 $\pm$ 1.57	10.82
Harminine	9.05 $\pm$ 1.08	75.07 $\pm$ 1.29	8.30
Harmaline	10.58 $\pm$ 2.01	101.39 $\pm$ 1.39	9.58
Harmol	21.58 $\pm$ 1.39	8.21 $\pm$ 3.65	0.38
Harmalol	27.88 $\pm$ 1.13	9.48 $\pm$ 2.03	0.34
Vasicine	13.68 $\pm$ 1.25	2.60 $\pm$ 1.47	0.19
Nigellastrine II	40.46 $\pm$ 1.09	9.26 $\pm$ 1.47	0.23
Nigellastrine I	70.50 $\pm$ 1.14	18.24 $\pm$ 3.23	0.26
Vasicinone	370.78 $\pm$ 1.07	—	
Deoxyvasicinone	294.44 $\pm$ 1.47	—	
Deoxyvasicine	—	—	
1,2,3,4-tetrahydroharmane-3-carboxylic acid	—	—	

IC_{50} values were determined by regression analyses and expressed as the means $\pm$ SD of three replicate determinations. SI is the AChE selectivity index defined as IC_{50} BChE/ IC_{50} AChE affinity ratio.

—: no inhibitory activity.

moderate inhibitory activity against AChE with IC_{50} values of 27.88 ± 1.13 and $21.58 \pm 1.39 \mu M$ and stronger inhibitory activity against BChE with IC_{50} values of 9.48 ± 2.03 and $8.21 \pm 3.65 \mu M$. Nigellastrine I and nigellastrine II had stronger inhibitory activity against BChE with IC_{50} values of 18.24 ± 3.23 and $9.26 \pm 1.47 \mu M$, but with weaker inhibitory activity against AChE with IC_{50} values of 70.50 ± 1.14 and $40.46 \pm 1.09 \mu M$.

4. Discussion

Results from previous studies showed that the alkaloids fraction containing mainly β -carboline and quinoline alkaloids from the seeds of *P. nigellastrum* exhibited potential inhibitive activity on AChE [14]. In our ongoing studies, the inhibitory activities of those individual β -carboline and quinoline alkaloids separated from genus *Peganum* on AChE and BChE were determined using the *in vitro* Ellman method. Based on the inhibitory potency and selectivity of those alkaloids on AChE and BChE, the structure-activity relationship of those alkaloids could be speculated as follows.

Amongst the series of β -carboline alkaloids (Figure 1), varying the substituent at C7 modulated the ChE inhibitory profile. The presence of an *O*-methyl substituent at C7 (harminine) led to the inhibition and selection on AChE slightly reduce with IC_{50} of $9.05 \pm 1.08 \mu M$ and AChE SI of 8.30, compared to harmane with IC_{50} of $7.11 \pm 2.00 \mu M$ and AChE SI of 9.13. No obvious difference was observed between the inhibitory activity of harmane and harminine

against BChE. When oxidation at C7 occurs, such as harmol, the inhibition against BChE was enhanced ($IC_{50} = 8.21 \pm 3.65 \mu M$) but with reduced activity on AChE ($IC_{50} = 21.58 \pm 1.39 \mu M$). Therefore the hydroxyl substituent at C7 obviously reduced the selectivity for AChE inhibition. According to the above analysis, C7 could be regarded as a possible active site for β -carboline alkaloids, and varying the substituent at C7 could significantly affect the inhibition on AChE and BChE. The double bond at C3-C4 seemed to have a certain degree of influence on the inhibitory activity against BChE, as demonstrated by the IC_{50} values of harmaline and harminine. As the main *O*-demethylation metabolites of harmaline and harminine, harmalol and harmol showed moderate inhibitory potency on AChE with IC_{50} values of 27.88 ± 1.13 and $21.58 \pm 1.39 \mu M$ and stronger inhibitory potency on BChE with IC_{50} values of 9.48 ± 2.03 and $8.21 \pm 3.65 \mu M$. Namely, the selective inhibition of harmalol and harmol versus AChE deduced compared with their parent compound harmaline and harminine. On the contrary, their selective inhibition against BChE enhanced.

Amongst the quinoline alkaloids (Figure 1), vasicine was identified as the potent inhibitor with IC_{50} values of $13.68 \pm 1.25 \mu M$ on AChE and $2.60 \pm 1.47 \mu M$ on BChE, respectively. Selective BChE inhibitors have already been reported to increase the ACh levels in the brain and to also reduce the formation of abnormal amyloid. Therefore, the discovery of potent and highly selective BChE inhibitors and/or of dual AChE/BChE inhibitors is an actively pursued goal in AD treatment drug discovery [21]. Very interestingly, although with poor selectivity, vasicine may be a very potential lead compound with the feature of dual AChE/BChE inhibitory activity.

Vasicinone and deoxyvasicinone possessing a carbonylation at C9 or dehydroxylation at C3 exhibited no much inhibitory activity on either AChE or BChE with IC_{50} values of 370.78 ± 1.07 and $294.44 \pm 1.47 \mu M$ on AChE and with IC_{50} values of above $1000 \mu M$ on BChE. Those data indicated that the inhibitive potency of quinoline alkaloids on both AChE and BChE was affected by the substitution at C3 and C9. Deoxyvasicine, possessing a carbonylation reaction at C9 and dehydroxylation at C3 simultaneously, showed no inhibitory potency on both AChE and BChE with larger IC_{50} values more than $1000 \mu M$.

As seen from Table 1, nigellastrine I and nigellastrine II (Figure 1), two dimers of quinoline (deoxyvasicinone) and β -carboline alkaloids (harmane or dehydroharmane), displayed a stronger activity on BChE inhibition and weaker activity on AChE inhibition. When compared with deoxyvasicinone, those dimers enhanced the inhibitor potency on both AChE and BChE, but more on BChE. On the other hand, by comparison with harmane, the two dimers seemed to have certain inhibitory selectivity for BChE.

From the above analysis, harmane, harminine, and harmaline, the main active constituent in genus *Peganum*, had good selective inhibitory activities against AChE, and the oxidation of the substituent at C7 could significantly modify the inhibitory potency and selectivity for both AChE and BChE. C3 and C9 positions of quinoline alkaloids were inferred to be the active site for both AChE and BChE

inhibition. Moreover, it was found that, by the polymerization of quinoline and carboline alkaloids, the inhibitory activity declined on AChE, but increased on BChE. In the light of those findings, it could be concluded that the alkaloids from the plants of genus *Peganum* showed inhibitory activity against both AChE and BChE. In addition, those findings may provide some guidance for the design and synthesis or semisynthesis of potential inhibitors on AChE or BChE.

Conflict of Interests

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

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