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Indole alkaloids from *Ervatamia hainanensis* with potent acetylcholinesterase inhibition activities

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ABSTRACT

Through bioassay-guided fractionation and chromatography technique, eight indole alkaloids were furnished from the stems of *Ervatamia hainanensis*. All isolates were evaluated for acetylcholinesterase (AChE) inhibition activities, in which compounds **1** and **3** exhibited the same level of activities as galantamine, a marketed cholinesterase inhibitor for the treatment of Alzheimer's disease. Discussion about the relationships between structure and activity of these alkaloids was also presented.

Alzheimer's disease (AD), a progressive and degenerative disorder of the brain, is believed to be the most common cause of dementia among the elderly. AD is associated with a loss of the presynaptic markers of the cholinergic system in the brain areas related to memory and learning, and is characterized by the presence of amyloid deposits and neurofibrillary tangles in the brain of afflicted individuals.[1,2] Now, the most widely accepted biochemical theory of the disease, known as the cholinergic hypothesis,[3–6] is that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. The enzyme acetylcholinesterase, one of the well-known enzymes and catalyzes the cleavage of acetylcholine in the synaptic cleft after depolarization, plays an important role in the central nervous system.[7] As a result, several acetylcholinesterase inhibitors (AChEI) have been approved for AD treatment, which can enhance cholinergic neurotransmission by increasing acetylcholine (ACh) availability in the synaptic cleft.

An enormous source of structural diversity from natural products is extremely valuable for the lead-finding process.[8] As far, lots of natural product-derived AChEIs have been reported,[9] some of which have been approved for AD treatment, such as galantamine and huperzine A (Fig. 1).

There are about 120 plant species in the genus *Ervatamia* (Apocynaceae) distributed in the tropical and subtropical areas of Asia and Australia. Fifteen plant species and five varieties of this genus grow in the south of China, and many of them have been administered in traditional Chinese medicine or folklore medicine.[10] *Ervatamia hainanensis*, its root has been used for the treatment of stomachache, dysentery, rheumatic arthritis, hypertension, and virus hepatitis in traditional Chinese medicine for a long time.[10] Several indole alkaloids[11–15] and other constituents[16] have been reported from this plant in the previous studies. In our continuing search for the AChE inhibitor,[17] eight known indole alkaloids were isolated from the stems of *E. hainanensis*, some of which showed potent AChE inhibitor activities. We report herein the isolation,

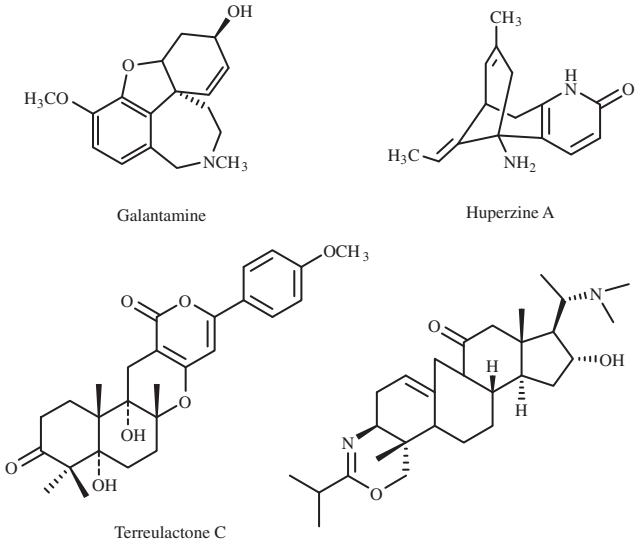

Figure 1. Representative natural product-derived AChEIs.

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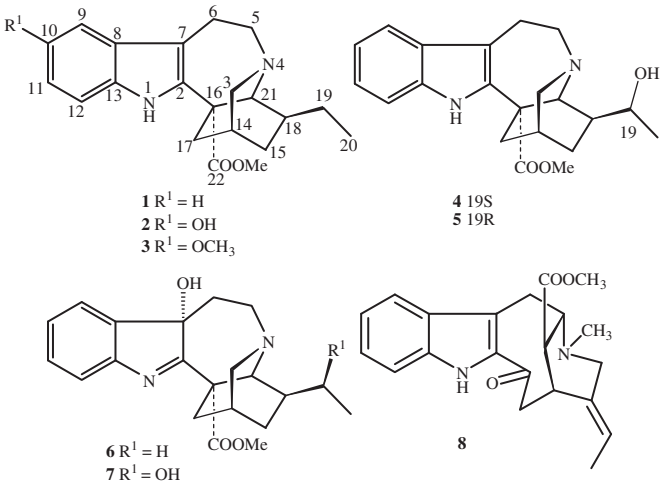

Figure 2. Indole alkaloids from *E. hainanensis*.

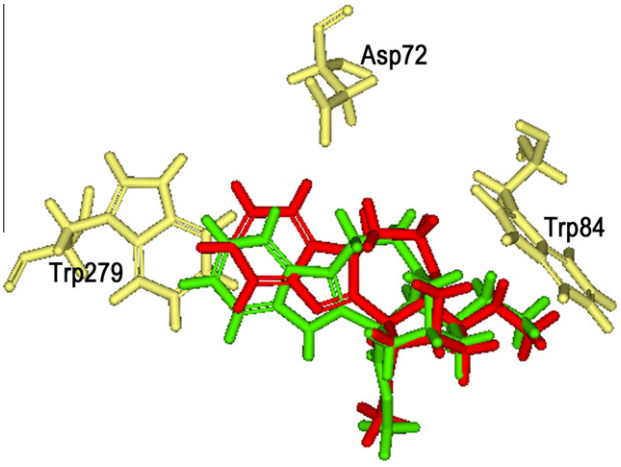

Figure 3. Docking model of **1** (green) and **6** (red) in the binding domain of AChE.

structural determination, and bioassay of these alkaloids from the stems of *E. hainanensis*.

In the preliminary experiments, we found the crude alkaloids from the stems of *E. hainanensis* exhibited AChE inhibition activity. Bioassay-guided investigation of the bioactive components led to the isolation of eight known indole alkaloids (**1–8**, Fig. 2). The known alkaloids were identified as coronaridine (**1**), 10-hydroxycoronaridine (**2**), voacangine (**3**), 19(*S*)-heyneanine (**4**),[18] 19(*R*)-heyneanine (**5**),[19] coronaridine hydroxyindolenine (**6**),[20] heyneanine hydroxyindolenine (**7**),[20] and vobasine (**8**)[15] by comparison their MS and NMR data with those reported in the literature, respectively. The purity of all alkaloids except **6–7** (93.8 and 93.0, respectively) was more than 95%, as determined by HPLC analysis. The except all these alkaloids except **8** were iboga-type indole alkaloids, in which **4** and **5** were a pair of diastereoisomer, and **6** and **7** had 3*H*-indole moiety.

To determine the inhibition potency of AChE of these indole alkaloids from the stems of *E. hainanensis*, their effect on AChE was assayed according to spectroscopic Ellmann's method,[21] and galantamine, an acetylcholinesterase inhibitor approved for AD treatment, was used as positive control. AChE from electric eel (EC 3.1.1.7) was commercially available,[22] and acetylthiocholine was used as the substrate. Inhibition of AChE activity of these indole alkaloids was shown in Table 1.

As shown in Table 1, some of isolates showed inhibition against AChE, especially **1** and **3** exhibiting the same level of activities as galantamine. It is interesting that compound **2** with one more hydroxyl at phenyl group compared to **1** reduced apparently its AChE potency. In contrast, voacangine (**3**), an analog of **1** with a methoxyl at phenyl group displayed nearly twofold improvement in AChE potency compared to **1**. The result indicated that hydrophobic and electron-donor substituents at phenyl group were better choices for the retention or improvement of AChE inhibition potency. Alkaloids **4** and **5**, two derivatives with one more hydroxyl at C-19 almost lost its inhibition against AChE, and only gave marginal activities. So the non-polar substituent at ethyl group may be a good choice for the retention of bioactivity. Compounds **6** and **7** bearing 3*H*-indole moiety, showed no inhibition against AChE, which showed that the indole moiety in these type alkaloids was an important factor for the retention of bioactivity.

To assess H-bonding and other binding interaction between these compounds and AChE, compounds **1** and **6** and *Torpedo californica* AChE (TcAChE, EC 3.1.1.7) were modeled (Fig. 3). The X-ray crystal structure of AChE (PDB code: 1EVE) was retrieved from the Protein Data Bank. Binding sphere was selected from the active site using the binding site tools. For all tested compounds, hydrogens were added and CHARMm force fields were employed. Each of the compounds was minimized by Dreiding Minimize tool. CDOCKER (Discovery Studio 2.1) was used for the docking simulation.

The docking studies of compounds **1** and **6** were performed using Discovery Studio/CDOCKER program. As shown in Figure 3, the results indicated that a stable p–π interaction between N_4 atom and TRP 279 as well as π–π interaction between indole moiety and TRP 84 were observed in the complex of compound **1** and AChE proposed by docking studies, leading to a good AChE inhibitory activity of **1** (IC_{50} = 8.6 μM). In addition, the proposed docking conformation of **6** was slightly different from **1**, due to the introduction of hydroxyl group on C-7 position of **1**. Loosing of π–π interaction between compound **6** and AChE, the value of CDOCKER_energy score of compound **6** increased to 16.07 which was much higher than that of **1** (4.49), which showed that the indole moiety in these alkaloids was essential pharmacophore for AChE inhibitory activities. The data mentioned above indicated that the molecular modeling results were consistent with experimental assay against AChE, suggesting its further application value in development of potent indole alkaloids as AChE inhibitors.

The isolates in the research are ascribed to the monoterpenoid indole alkaloids. These type alkaloids which derivate from the condensation of tryptophan and secologanin, are outstanding among secondary plant metabolites for their structural intricacy and a wide variety of pharmacological effects.[20,23] Up to date, more than 2000 different compounds of this class have been isolated, and the best known are the Vinca alkaloids for their potent anti-cancer activity. Besides the Vinca alkaloids, coronaridine and ibogaine are one of the most studied indole alkaloids due to their activity in the CNS and their potential use as anti-addiction agents.[24] To our best

Table 1
50% inhibitory concentration of compounds against AChE

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Galantamine[a]
IC_{50} (μM)	8.6	29	4.4	420	730	>1000	>1000	>1000	3.2

[a] Galantamine was used as positive control.

knowledge, only monoterpenoid indole alkaloids from *Haplophyton crooksii*[25] and *Tabernaemontana australis*[26] have been assayed as AChE inhibitors. The isolation of monoterpenoid indole alkaloids from the genus of *Ervatamia* is reported for the first time.

In this study, the bioassay-guided investigation of the stems of *E. hainanensis* resulted in the isolation of eight indole alkaloids. The anticholinesterase evaluation of these compounds revealed that **1** and **3** showed potent inhibition of AChE activity in vitro. Compounds **1** and **3** exhibited the same level of activities with positive control, galantamine. Herein, compounds **1** and **3** can be used as a lead compound for further optimization for the potential use of treatment of AD. Further investigations are undergoing and the details will be reported in due course.

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Supplementary data

Supplementary data including the NMR data and the experimental procedure associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.123.

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