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Interactions of *Lycopodium* alkaloids with acetylcholinesterase investigated by ^{1}H NMR relaxation rate

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Abstract

In order to further understand the interaction processes between the *Lycopodium* alkaloids and acetylcholinesterase, the binding properties of *N*-acetyl huperzine A (**1**), huperzine B (**2**) and huperzine F (**3**) with *Torpediniforms Nacline* acetylcholinesterase (TnAchE) were investigated by ^{1}H NMR methods. The nonselective, selective and double-selective spin-lattice relaxation rates were acquired in the absence and presence of TnAchE at a ratio of [ligand]/[protein] = 1:0.005. The selective relaxation rates show protons of **1–3** have dipole–dipole interaction with protons of TnAchE at the binding interface. The molecular rotational correlation time of bound ligands was calculated by double-selective relaxation rate at 298 K, which showed that **1–3** had high affinity with the protein. The results indicate that investigation of ^{1}H NMR relaxation data is a useful method to locate the new *Lycopodium* alkaloids as AchE inhibitors.

Keywords: Acetylcholinesterase; *Lycopodium* alkaloids; ^{1}H spin-lattice relaxation rate; Ligand–macromolecules interaction

1. Introduction

The enzyme acetylcholinesterase (AchE) catalyzes the hydrolysis of the ester bond of acetylcholine (Ach) to terminate the impulse transmitted action of Ach through cholinergic synapses [1]. The Torpedo Acetylcholinesterase contains 14 α-helices and 12 stranded mixed β-sheet. The mixed structures of AchE with its ligands have showed that AchE contains an 'active-site gorge' [2]. When Ach binds to this gorge, it is quickly hydrolyzed into acid and choline. Although the basic reason of Alzheimer's disease (AD) is not clear so far, AD is firmly associated with impairment in cholinergic transmission by present study. A number of AchE inhibitors have been considered as candidates for the symptomatic treatment of AD as the most useful relieving strategy [3]. (−)-huperzine A (**4**) (Fig. 1) is a natural compound first isolated from Chinese medicine *Huperzia serrata* (Thumb.) in 1986 [4]. **4** is a potent, reversible and selective inhibitor of AchE with a rapid absorption and penetration into the brain in animal tests. It exhibits memory-enhancing activities in animal and clinical trials. Compared to tacrine and donepezil, which are also AchE inhibitors used as drugs in the market, **4** possesses a longer duration of action and higher therapeutic index as well as less peripheral cholinergic side effects at therapeutic dose [5]. To find more powerful inhibitors of AchE, many alkaloids have been isolated from *H. serrata*.

Nuclear magnetic resonance (NMR) has been widely used for studying interactions of small molecules (i.e. ligands) with macromolecules (i.e. receptors and enzymes) [6], due to the large number of spectral parameters that can be measured and analyzed (chemical shift [7], relaxation rates and line width [8], diffusion coefficients [9] or intermolecular magnetization transfer techniques such as NOE [10,11]. Among them, the proton spin relaxation rate of the small molecule has proved to be a very suitable parameter in the ligand–macromolecule complex studies.

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In these studies, NMR investigation is based on the comparison of selective (R^{se}) and non-selective (R^{ns}) proton spin-lattice relaxation rate of the ligand in the presence and absence of the macromolecular receptor. The formation of intermolecular adducts affects R^{ns} and R^{se} at different extents, depending on the dynamical parameters (i.e. molecular rotational correlation time t_c), assuming fast chemical exchange between the bound and the free environments. In particular, the slower rotational tumbling of the ligand–macromolecule complex mainly affects R^{se}. Even if the macromolecule concentration is just 0.5% of a ligand, the R^{se} value of the ligand's protons will sensitively change due to the binding process [12,13].

The parameters R^{se} and t_c have been used in evaluating the tacrine derivatives and huperzine A interacting with AchE [14,15]. In this paper, the characterization of the interaction between AchE and three *lycopodium* alkaloids: *N*-acetyl huperzine A (**1**), huperzine B (**2**) and huperzine F (**3**) (Fig. 1) were investigated using NMR spectroscopy.

2. Materials and methods

2.1. Sample preparation and chemical characterization

The huperzine A and three *lycopodium* alkaloids were isolated from the whole plant of *H. serrata* (Thumb.) Trev. (Huperziaceae) by using the same procedure as described previously [16]. Chromatography of the crude alkaloids of the whole plant of *H. serrata* on SiO_2 and elution with $CHCl_3$–Me_2CO followed by MeOH afforded two alkaloid-rich fractions. Repeated chromatography of the MeOH fraction over neutral Al_2O_3 and SiO_2 afforded *N*-acetyl huperzine A, huperzine B and huperzine F, whose structures were identified using various NMR spectroscopy data [4,16]. AchE from *Torpedo californa* was obtained from Sigma and used without further purification. Solutions were prepared in 99.9% deuterium oxide (CIL) buffered at pH 7.0 (phosphate saline buffer). All solutions were carefully deoxygenated by sealing off the NMR tube after filling the nitrogen. In all the experiments ligand concentration was 1.0 mM and the protein concentration was 5.0 μM.

2.2. NMR spectroscopy

All measurements were performed on a Bruker Avance 400 MHz NMR spectrometer operating at 400.13 MHz for hydrogen. All experiments were carried out at a temperature of 298 K. ^{1}H spectra were recorded using a BBO broadband probe. 16 scans were collected into 32 K data points giving a digital resolution of 0.13 Hz/point at a spectral width of 4006 Hz. To evaluate the data and calculate relaxation times, the XWINNMR program package (Version 3.5) was used on a Microsoft Windows PC. Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) through the water resonance calibrated at 298 K. The spin-lattice relaxation rates were measured using the $(180°\text{-}\tau\text{-}90°\text{-}t)_n$ sequence. The τ values used for the selective and nonselective experiments

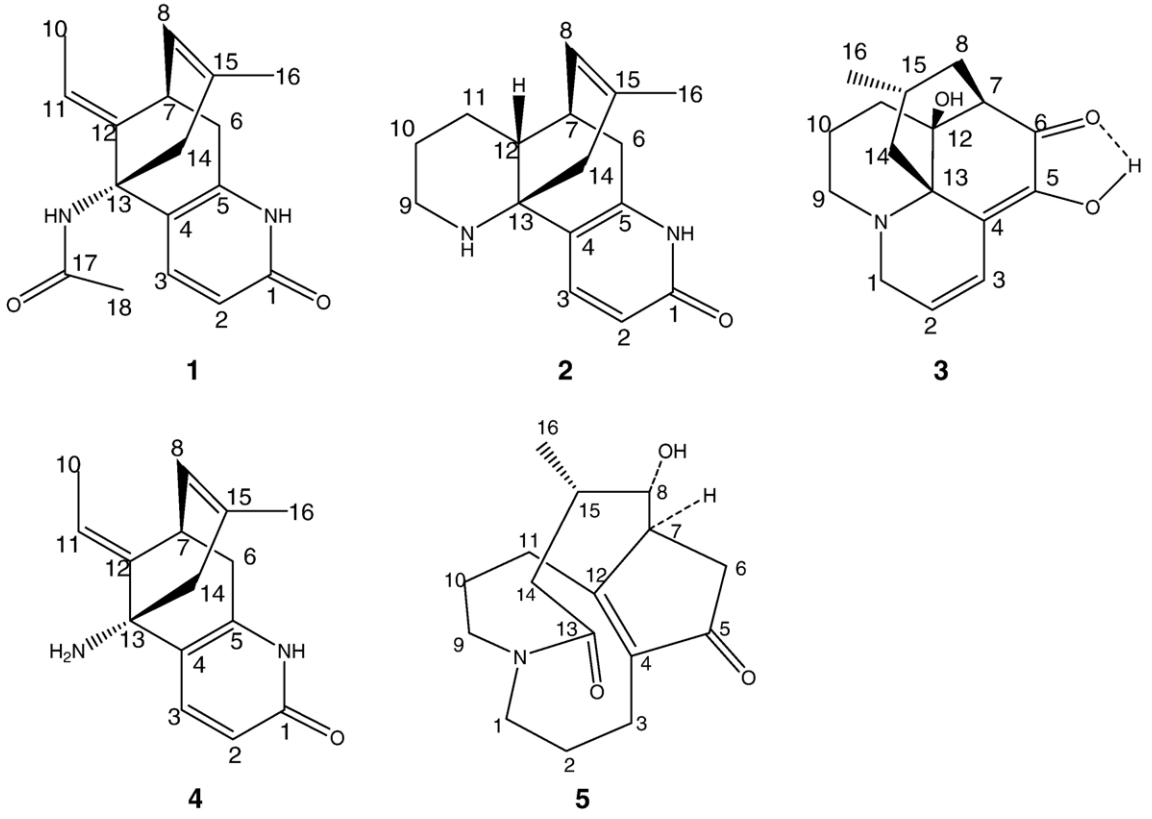

Fig. 1. Chemical structures of **1**–**5**.