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Structural basis of acetylcholinesterase inhibition by triterpenoidal alkaloids ☆

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Abstract

Acetylcholinesterase plays a crucial role in the metabolism of neurotransmitter, acetylcholine. Inhibition of *Torpedo californica* acetylcholinesterase by triterpenoidal alkaloids buxamine-B (**1**) and buxamine-C (**2**) has been studied by enzyme kinetics and molecular docking experiments. Buxamine-C (**2**) has been found to be 20-fold potent than buxamine-B (**1**) ($K_i = 5.5$ and 110 μM, respectively). The ligand docking experiments predicted that the cyclopentanophenanthrene skeleton of both inhibitors properly fits into the aromatic gorge of the enzyme. The C-3 and C-20 amino groups of both alkaloids mimic the well-known bis-quaternary ammonium inhibitors such as decamethonium and interact with Trp84 and Trp279 residues of the enzyme, respectively. The C-3 amino group in buxamine-C (**2**) appears to be better positioned at the bottom of the aromatic gorge and thus seems to be crucial for the inhibitory activity of such inhibitors.

Keywords: Molecular docking; FlexX; Hydrophobic interactions; Cation–π interactions; Natural product inhibitors

The acetylcholinesterase (AChE, EC 3.1.1.7) catalyzes the hydrolysis of a neurotransmitter acetylcholine (ACh) in the cholinergic synapses [1]. The enzyme has been an attractive target for rational drug design and discovery of mechanism-based inhibitors for the treatment of Alzheimer's disease and related neurodegenerative disorders [2].

The co-crystallizations of the enzyme with different inhibitors have provided valuable information about the binding modes of substrate/inhibitor. More than 60 sets of crystal-structural coordinates of AChE have been deposited so far with PDB [3]. Many of these structures are complexes of AChE with synthetic and natural inhibitors. The AChE structure has α/β hydrolase protein fold [4,5]. The active site is buried near the bottom of a deep and narrow gorge that penetrates halfway into the enzyme and widens out close to its base. This gorge is named as the 'aromatic gorge' since it is lined by 14 conserved aromatic amino acids. The active site consists of esteratic and anionic subsites. Esteratic subsite contains catalytic triad (Ser200, His440, and Glu327) [4] and oxyanion hole forming residues (Gly118, Gly119, and Ala201) [6]. The anionic subsite (Trp84, Phe330, and Glu199), also termed as quaternary ammonium-binding site, is responsible for binding the quaternary trimethylammonium tail group of ACh by cation–π interaction [7]. The aromatic residues lining the gorge and the residues located at the outer rim of the gorge (Tyr70 and Trp279) have been postulated to

☆ *Abbreviations:* ACh, acetylcholine; AChE, acetylcholinesterase; PDB, Protein Data Bank.

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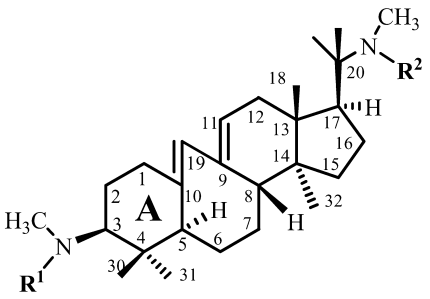

Buxamine-B (**1**) R^1= H, R^2= CH_3

Buxamine-C (**2**) R^1= CH_3, R^2= H

Fig. 1. Structures of buxamine-B (**1**) and buxamine-C (**2**).

be involved in the initial binding and the subsequent guiding of the substrate towards the active site [8].

Since the discovery of physostigmine [9] as a natural AChE inhibitor, several other natural inhibitors have attracted the attention of neuropharmacologists [10]. We have previously reported a number of novel natural inhibitors of cholinesterases (acetylcholinesterase and butyrylcholinesterase) isolated from various medicinal plants [11–14]. Inhibition kinetics, SAR and CoMFA studies have also been conducted for a good number of these inhibitors [15–17]. We present here the AChE inhibiting properties of natural products buxamine-B (**1**) and buxamine-C (**2**) (Fig. 1), isolated from *Buxus papillosa* and *Buxus hyrcana* species.

Materials and methods

Acetylcholinesterase inhibition assays. Electric eel (*Torpedo californica*) AChE (type VI-S, Sigma) activity was measured in vitro by a modified spectrophotometric method developed by Ellman et al. [18]. All the inhibition studies were performed in 96-well microtiter plates, using SpectraMax microplate spectrophotometer (Molecular Devices, CA, USA) as reported previously [13].

K_i values were determined by the interpretation of Dixon plot, Lineweaver–Burk plot, and its secondary replots by using initial velocities [19]. These velocities were obtained over a range of substrate concentrations between 0.1 and 0.4 mM. The assay conditions for measurement of the residual activities of all inhibitors were identical to the aforementioned spectrophotometric assay procedure except that fixed concentrations of inhibiting compounds were used in the assay medium.

Molecular docking. The three-dimensional structures of buxamine-B (**1**) and buxamine-C (**2**) were constructed using the SYBYL program [20]. Energy minimization was carried out using the tripos force field with a distance gradient algorithm with a convergence criterion of 0.05 kCal/(mol Å) and maximum 10,000 iterations, respectively. The docking studies were carried out using FlexX [21] docking software. Buxamine-B (**1**) and buxamine-C (**2**) models were docked in the aromatic gorge of AChE (PDB id; 1ACL). FlexX software is a fast and flexible algorithm for docking small ligands into binding sites of the enzymes, using an incremental construction algorithm that actually builds the ligands in the binding site [21]. The software incorporates protein–ligand interactions, placement of the ligand core, and rebuilding of the complete ligand. Docking results were analyzed by LIGPLOT [22] and WebLab ViewerPro [23].

Results and discussion

The buxamine-B (**1**) and buxamine-C (**2**) have been found to inhibit AChE noncompetitively in a concentration dependent fashion (Fig. 2). The IC_{50} values of buxamine-B (**1**) and buxamine-C (**2**) were 74 and 7.5 μM, respectively. The K_i values of AChE inhibition by buxamine-B (**1**) and buxamine-C (**2**) were 110 and 5.5 μM, respectively, which indicated that buxamine-C (**2**) is 20 times more potent as compared to buxamine B (**1**). Table 1 shows a comparison of K_i values of representative reversible AChE inhibitors with buxamine-B (**1**) and buxamine-C (**2**). This includes the bis-quaternary ligands, decamethonium and BW284C51, that span the distance between the active center and peripheral anionic site and the cationic active center ligands, adrophonium and galanthamine.

The *Torpedo californica* AChE crystal structure, complexed with decamethonium inhibitor (PDB id; 1ACL), (which shares 70% sequence homology with the human enzyme) has been utilized for the molecular docking experiments. The decamethonium contains two cationic trimethylammonium groups at both ends of the molecule connected by a 10-membered aliphatic chain. One quaternary ammonium group involves in cation–π interactions with the indole ring of Trp84 at the bottom of the gorge and the other with that of Trp279, near the entrance of the gorge [5,24,25] (Figs. 3C and F). The

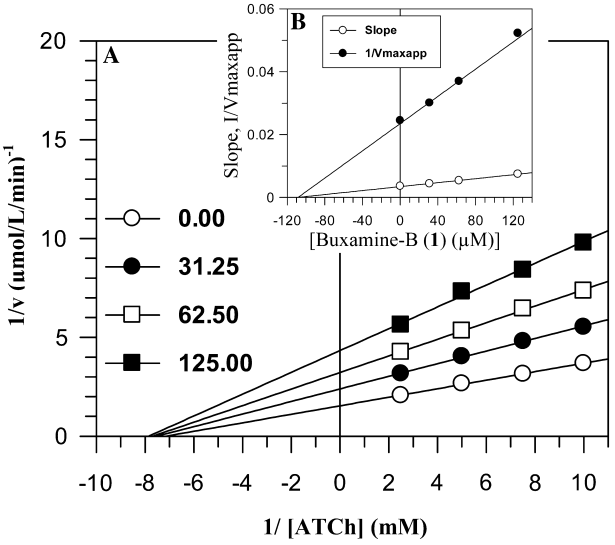

Fig. 2. Steady-state inhibition of AChE by buxamine-B (**1**), (A) Lineweaver–Burk plot of reciprocal of the initial velocities versus reciprocal of ATCh in the presence of various concentrations of the inhibitor. (B) Secondary replots of the Lineweaver–Burk plot: slope and $1/V_{maxapp}$ versus various concentrations of buxamine-B (**1**).