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Juliflorine: A potent natural peripheral anionic-site-binding inhibitor of acetylcholinesterase with calcium-channel blocking potential, a leading candidate for Alzheimer's disease therapy ☆

M. Iqbal Choudhary [a,*], Sarfraz Ahmad Nawaz [a], Zaheer-ul-Haq [a], M. Kamran Azim [a], M. Nabeel Ghayur [b], M. Arif Lodhi [a], Saima Jalil [a], Asaad Khalid [a], Amir Ahmed [a], Bernd M. Rode [c], Atta-ur-Rahman [a], Anwar-ul-Hassan Gilani [b], Viqar Uddin Ahmad [a]

[a] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan*
[b] *Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi-74800, Pakistan*
[c] *Department of Theoretical Chemistry, Institute of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*

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Dedicated in the memory of Dr. Mohammad Hussain Panjwani (1940–1992), a renowned philanthropist and scholar

Abstract

The alkaloid juliflorine (**1**) from *Prosopis juliflora* inhibited acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) enzymes in a concentration-dependent fashion with IC_{50} values 0.42 and 0.12 μM, respectively. Lineweaver–Burk as well as Dixon plots and their secondary replots indicated that the nature of inhibition was purely of non-competitive type with K_i values 0.4 and 0.1 μM, against AChE and BChE, respectively. By molecular docking studies compound **1** was found to be ideally spaced inside the aromatic gorge of AChE with rings A/B remaining at the top and rings C/D penetrating deep into the gorge, that might be due to the greater hydrophobicity of rings C/D as compared to rings A/B, allowing their simultaneous interaction with the peripheral anionic and quaternary ammonium-binding sites. The **1**–AChE complex was found to be stabilized by hydrophobic contacts, hydrogen bonding, and π–π stacking between the compound **1** and amino acid residues of the aromatic gorge of AChE. Amino acid residues Tyr70, Asp72, Tyr121, Trp279, and Tyr334 of the peripheral anionic site (PAS) of AChE were found to be exclusively involved in the hydrophobic contacts with compound **1** that might be responsible for the competitive mode of inhibition. Compound **1** also showed dose-dependent (30–500 μg/mL) spasmolytic and Ca^{2+}-channel blocking activities in isolated rabbit jejunum preparations. The cholinesterase inhibitory potential along with calcium-channel blocking activity of compound **1** and safe profile in human neutrophils viable assay could make it a possible drug candidate for Alzheimer's disease.

Keywords: Juliflorine; Acetylcholinesterase; Butyrylcholinesterase; Enzyme inhibition studies; Molecular docking; Calcium-channel blocker

AChE (EC 3.1.1.7) is a key component of cholinergic brain synapses and neuromuscular junctions. The major biological role of the enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine [1]. According to the

☆ *Abbreviations:* PAS, peripheral anionic site; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; AD, Alzheimer's disease.

* Corresponding author. Fax: +92 21 9243190 91.
E-mail address: hej@cyber.net.pk (M.I. Choudhary).

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cholinergic hypothesis, memory impairments in patients with the senile dementia diseases are due to a selective and irreversible deficiency in the cholinergic functions in the brain [2]. This serves as the rationale for the use of AChE inhibitors for the symptomatic treatment of AD in its early stages. Certain classes of intestinal spasmolytics are also known to have a combination of Ca^{2+}-channel blocking and AChE inhibitory activities. Histamine H_1 blockers like promethazine, mepyramine, and H_2 receptor antagonists such as cimetidine, oxmetidine, and ranitidine have been shown to possess AChE inhibitory activities [3,4]. The role of BChE (EC 3.1.1.8) in normal aging and brain diseases is still elusive. It has been found that BChE is present in significantly higher quantities in Alzheimer's plaques than in plaques of normal age related non-demented brains [5].

The comprehensive study of the AChE/inhibitor complexes by X-ray crystallography has indicated a nearly identical three-dimensional structure of the active site in which the active center is located 20 Å from the protein surface at the bottom of a deep and narrow gorge [6]. The different positions of the known inhibitors in the binding pocket suggest that more than one clearly defined binding site exists which are called esteratic and anionic subsites. Esteratic subsite contains catalytic triad (Ser200, His440, and Glu327) [7] and oxyanion hole forming residues (Gly118, Gly119, and Ala201) [8]. The quaternary ammonium-binding locus (Trp84, Phe330, and Glu199) is responsible for binding of the quaternary trimethyl ammonium tail group of ACh by cation–π interaction [9]. The peripheral site, which is also called peripheral anionic site (PAS), includes Tyr70, Asp72, Tyr121, Tyr334, and Trp279 residues [10]. Ligand occupation of the peripheral anionic site may allosterically change the conformation of the active center [11]. Aromatic residues lining the gorge and residues located at the outer rim of the gorge have been postulated to be involved in the initial binding and guiding of the substrate towards the active site [12].

The discovery of new cholinesterase inhibitors has been a challenging area of drug development due to the involvement of cholinesterases in Alzheimer's disease and other related dementias. We have previously reported a number of novel natural inhibitors of cholinesterases (AChE and BChE) isolated from different medicinally important plants [13,14]. The inhibition kinetics, 3D-QSAR, CoMFA, and CoMSIA [15–17] studies have also been conducted for a good number of compounds. Continuing our efforts to discover new natural inhibitors of clinically valuable enzymes through high-throughput screening assays, we identified juliflorine (**1**) as having appreciable inhibitory potential against cholinesterase enzymes.

The objectives of the current investigation were threefold: first to determine the in vitro inhibitory potential of juliflorine (**1**) against AChE and BChE, second to predict the interactions of compound **1** in the active site of AChE (*Torpedo californica*) through molecular docking techniques and third to investigate correlation between the AChE inhibition and Ca^{2+}-channel blocking potential of compound **1** for its therapeutic relevance in AD and finally to evaluate the cytotoxicity of compound **1**.

Results and discussion

Juliflorine (**1**), a piperidinium alkaloid, was isolated from the leaves of *Prosopis juliflora* by a collaborative group, Ahmad et al. [18]. The structure was elucidated by using spectroscopic techniques. Compound **1** has two piperidine rings A and B that are connected with a dihydroindilizine moiety through two aliphatic chains A and B, respectively (Fig. 1). The piperidine rings contain secondary hydroxyl and methyl substituents. The inhibitory potential of compound **1** against cholinesterases was determined by using the electric-eel (*T. californica*) AChE because of two reasons: first, the oligomeric forms of AChE in the electric eel are structurally similar to those of vertebrate's nerves and muscles [7]. Second, the results obtained with this enzyme allow molecular modeling studies to be conducted using the coordinates of the published eel AChE X-ray structure [7]. Moreover, horse-serum BChE has similarities with synaptic AChE in primary amino acid sequence, deduced secondary structure, and active-site chemistry; the two enzymes also have overlapping specificities for substrates and inhibitors [19]. Compound **1** inhibited AChE and BChE enzymes in a concentration-dependent manner with K_i values 0.4 and 0.1 μM and IC_{50} values 0.42 and 0.12 μM against AChE and BChE, respectively. K_i values were calculated in three ways: first, the slopes of each line in the Lineweaver–Burk plot were plotted against different concentrations of compound **1**, second, the $1/V_{maxapp}$ was calculated by plotting different fixed concentrations of (substrate) ATCh or BTCh versus ΔV in the presence of different fixed concentrations of compound **1** in the respective assays of AChE or BChE,

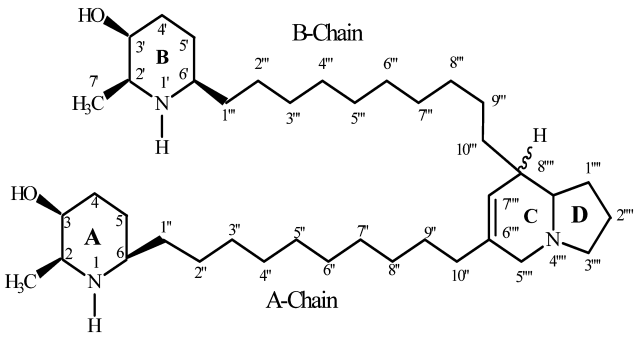

Fig. 1. Structure of juliflorine (**1**).