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Cholinesterases inhibition and molecular modeling studies of piperidyl-thienyl and 2-pyrazoline derivatives of chalcones

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ABSTRACT

Super-activation of cholinesterases (acetylcholinesterase and butyrylcholinesterase) are linked to various neurological problems most precisely Alzheimer's disease (AD), which leads to senile dementia. Therefore, cholinesterases (AChE & BChE) inhibition are considered as a promising strategy for the treatment of Alzheimer's disease. FDA approved drugs for the treatment of AD, belong to a group of cholinesterase inhibitors. However, none of them is able to combat or completely abrogate the disease progression. Herein, we report a series of newly synthesized chalcone derivatives with anti-AD potential. For this purpose, a series of piperidyl-thienyl and 2-pyrazoline derivatives of chalcones were tested for their cholinesterases (AChE & BChE) inhibitory activity. All compounds were found as selective inhibitor of AChE. In piperidyl chalcones derivatives compound **1e** having IC_{50} of 0.16 ± 0.008 μM and **2m** in 2-pyrazoline chalcones with IC_{50} of 0.13 ± 0.006 μM, were found to be the most potent inhibitors of AChE, exhibiting ≈142 and ≈ 173-fold greater inhibitory potential compared to the reference inhibitor i.e., Neostigmine (IC_{50} ± SEM = 22.2 ± 3.2 μM). Molecular docking studies of most potent inhibitors were carried out to investigate the binding interactions inside the active site. Molecular docking study revealed that potent compounds and co-crystalized ligand had same binding orientation within the active site of target enzyme. Most of these compounds are selective inhibitors of AChE with a potential use against progressive neurodegenerative disorder and age related problems.

1. Introduction

Cholinesterases (ChEs) belong to a supper family of esterase/lipase enzyme that catalyze the hydrolysis of a neurotransmitter acetylcholine (ACh) into choline and acetic acid and thus terminate the cholinergic neurotransmission [1]. This catalytic reaction plays an important role to allow a cholinergic neuron to come back into its resting state after activation. Mostly present in cholinergic and non-cholinergic tissues and other body fluids including plasma [2]. On the basis of substrate specificity and inhibitors, two types of cholinesterase co-exist simultaneously throughout the body; acetylcholinesterase and butyrylcholinesterases [3]. Both these forms are highly homologous i.e., >65% but are products of different genes on the chromosomes 7 and 3 in case of human, respectively [4]. Acetylcholinesterase (AChE; E.C. 3.1.1.7) is a membrane-bound enzyme found in many types of conducting tissues; nerve and muscle, cholinergic and non-cholinergic fibers, central and peripheral tissues, sensory and motor neuron fibers while butyrylcholinesterase (BChE; E.C.3.1.1.8), also called plasma cholinesterase or pseudo-cholinesterase is mainly distributed in the liver, intestine, heart and lungs [5,6]. The main biological role of AChE is the termination of impulse transmission by quick hydrolysis of the cationic neurotransmitter acetylcholine [7] while BChE precisely catalyze butyrylcholine (BCh), however upto some extent it can also hydrolyzes ACh as well [8,9]. On the basis of cholinergic hypothesis, memory impairment in the patients of Alzheimer's disease (AD) and dementia is due to selective and irreversible insufficiency in the cholinergic functions in the brain [10]. Dementia, rottenly also called senility, is a vast group of brain diseases that often cause long term and gradual decline in thinking ability of the person. It is great enough for affecting a person's daily life functions

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[11]. Other most common syndrome includes problem with language, emotional problem and a decrease in motivation [12]. Usually, a person's consciousness is not affected in dementia [13]. There are different types of dementia, most common is Alzheimer's disease which is responsible for 50–60% cases of dementia are noticed in the adults of USA and Europe. Other common types include vascular dementia (25%), Lewy body dementia (15%) and frontotemporal dementia. Less common types include Parkinson's disease, syphilis, normal pressure hydrocephalus and Creutzfeldt-Jakob disease. In DSM-5, dementia was reclassified on the basis of various degree of severity as a neurocognitive disorder [14].

According to WHO report 2001, the number of dementia especially, AD cases in western countries will be doubled by every twenty years and will become tripled in China and India with twenty nine million peoples in 2020, mostly owed to increased human longevity [15]. Even though the unknown morphology of AD, elevation of ACh amount through AChE inhibition has been accepted as the most potent scheme against AD treatment [16]. Therefore, AChE and BChE inhibitors have become the prime option in the treatment of AD patients. However, existent drugs (donepezil, tacrine and rivastigmine) having AChE inhibitory are only convincing against the mild type of AD while there is no drug available that shows BChE activity to present, yet [17]. Consequently, a lot of pressure develop on researchers to discover new drugs in order to conflict dementia and AD. Our group selected a series of chalcones derivatives to test the inhibitory activity of Cholinesterases because chalcones (natural and synthetic) illustrate a lot of biological activities like anti-fungal [18], anti-tuberculosis [19], analgesic [20], anti-oxidant [21], anti-leishmanial [22], anti-malarial [23], anti-viral [24,25], anti-inflammatory and molluscicidal [22], anti-amoebic [26], anti-depressant, anti-convulsant properties [27], and monoamine oxidase inhibitory (MAO) activity [28] etc. Keeping in-mind the aforementioned biological importance of chalcones derivatives, herein we explored a series of chalcones based pyrazoline derivatives as potent cholinesterase inhibitors along with their molecular docking, ADME properties and established SAR.

2. Materials and methods

2.1. Synthesis of chalcones derivatives

A systematic scheme for the synthesis of piperidyl-thienyl chalcones (**1a-1j**) and 2-pyrazoline derivatives of quinolyl thienyl chalcones (**2a-2ab**) has been already published in our previous papers [29,30].

2.2. Materials

All the chemicals and reagents including Electric eel AChE, quine serum BChE, acetylthiocholine chloride, butyrylthiocholine chloride, 5,5′-Dithiobis[2-nitrobenzoic acid]] (DTNB) were purchased from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All chemicals used were of analytical grade. Neostigmine and donepezil were used as standard drugs.

2.3. Cholinesterases (AChE and BChE) assay protocol

The cholinesterases (AChE& BChE) inhibition studies were determined by Ellmann's spectrophotometric method [31] with slight modifications using acetylthiocholine chloride and butyrylthiocholine chloride as substrates for AChE & BChE, respectively. Total reaction mixture was 100 μL that contain 60 μL phosphate buffer (50 mM, pH 7.7), 10 μL test compound (1% DMSO, final conc. of compound 0.1 mM well^{-1}) and 10 μL of AChE (0.015 U/well, E.C.3.1.1.7, from electric eel) or 10 μL of BChE (0.01 U/well, E.C.3.1.1.8, from equine serum). The contents of each well was mixed thoroughly followed by incubation at 37 °C for 10 min and their absorbance was recorded at 405 nm as optical density. Then, 10 μL substrate (0.5 mM acetylthiocholine chloride) for AChE inhibition assay or (0.5 mM butyrylthiocholine chloride) for BChE inhibition assay was added followed by addition of 10 μL DTNB (0.5 mM well^{-1}). Then the mixture was further incubated at 37 °C for 20 min. Finally, absorbance at 405 nm was recorded using 96-well plate reader (BioTek ELx800, Instruments, Inc. USA). All experiments were performed in triplicate with their respective control. Neostigmine (0.1 mM well^{-1}) was used as positive control. Percent inhibition was calculated by using the following formula

$$\%\ \text{inhibition} = 100 - (A_t/A_c) \times 100$$

where "A_t" and "A_c" are absorbance obtained for respective enzyme (AChE & BChE) in the presence and absence of the inhibitors, after subtracting the respective background (pre read absorbance).

2.4. Molecular docking studies

Molecular docking studies of most potent compounds in both series were carried out against AChE using MOE [32]. Prior to docking of potent compounds inside the target enzyme, structure were drawn and protonated in the molecules sketcher tool of MOE. The required protonated 3D structures of these compounds were obtained using the three-dimensional tool of MOE. Subsequently, the energy minimization of generated molecules was carried out using the MMFF94x force field with the adjustment of hydrogen. Finally, the created database was used as input file for docking studies in MOE. For docking purpose X-ray structure of AChE (PDB ID 1EVE) was selected as template and downloaded from RSC Protein Data Bank [33]. Prior to docking process protonation of target structure was accomplished using MOE protonate 3D tools which was followed by energy minimization up to 0.05 Gradient using Amber99 force field. Prior to molecular docking, active site of receptor was selected around the co-crystallized ligands. Then the required ligands were docked into the active site of protein using Triangular Matching docking method and 30 conformations of each Ligand protein complex were generated with docking score. Each complex was analyzed for interactions and their respective 3D pose was visualized using discovery studio visualizer v4 [34]. Binding free energies were determined and tabulated in Table 3. Those poses having lowest free binding energy values were considered as the most stable-one and selected for visualization of binding interactions with the target enzyme.

3. Results and discussion

3.1. Chemistry

4-(Piperidine-*l*-yl) benzaldehyde was prepared by *N*-arylation of piperidine with 4-flurobenzaldehyde in the presence of cetyltrimethylammonium bromide (CTAB) as catalyst. It was then condensed with wide range of different substituted acetylthiophenenes and acetylfurans for getting respective chalcones, Scheme 1 [30]. Another Chalcones based quinolone series was prepared by reacting it with substituted 2-chloro-3-formylquinolines and was further reacted with hydrazine hydrate to get 2-pyrazoline derivatives, Scheme 2 [29]. All these newly synthesized compounds were characterized by different analytical techniques. The relevant spectroscopic data and physicochemical properties of all these compounds (**1a-1j** and **2a-2ab**) have been already reported previously [29,30].