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Cholinesterase inhibitory activity versus aromatic core multiplicity: A facile green synthesis and molecular docking study of novel piperidone embedded thiazolopyrimidines

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ABSTRACT

Novel thiazolopyrimidine derivatives have been synthesized via microwave assisted, domino cascade methodology in ionic liquid and evaluated in vitro for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities. Among the newly synthesized compounds **6d**, **6a**, **6e** and **6b** displayed higher AChE inhibitory activity than standard drug, galanthamine, with IC_{50} values of 0.53, 1.47, 1.62 and 2.05 μM, respectively. Interestingly, all the compounds except for **6m–r** and **6x** displayed higher BChE inhibitory potentials than galanthamine with IC_{50} values ranging from 1.09 to 18.56 μM. Molecular docking simulations for **6d** possessing the most potent AChE and BChE inhibitory activities, disclosed its binding interactions at the active site gorge of AChE and BChE enzymes.

1. Introduction

In the context of green chemistry, microwave assisted organic synthesis (MAOS) has gained widespread acceptance in drug discovery laboratories. Those reactions, which are assisted by unrivaled features of ionic liquids such as remarkable catalytic behavior, excellent chemical/thermal stability and good solvating ability, displayed significant merits of reduced reaction time and improved yields.[1,2] Compounds comprising pyrimidine entity were found to exhibit a wide range of biological activities suchlike antitumor,[3–5] anti-inflammatory,[6] adenosine A_{2A} receptor antagonists,[7] antiviral[8,9] as well as potent cholinesterase/Aβ-aggregation inhibitory properties.[10–16] In our pervious study, we reported synthesis and cholinesterase inhibitory activities of novel pyrimidine and thiopyrimidine derivatives, comprising two aromatic rings. These new dual core compounds displayed reasonable cholinesterase inhibitory activities with more selectivity toward BChE.[16]

Alzheimer's disease (AD) is a neurodegenerative disorder that based on the World Health Organization (WHO) report, has affected more than 37 million people worldwide.[17] The etiology of AD is not completely known, however the deposition of extracellular β-amyloid plaques and formation of intracellular neurofibrillary tangles are thought to play principal roles in the pathophysiology of this disease, causing loss of cholinergic neurons in the forebrain, cortex and hippocampus of AD patients.[18–22] The decline in acetylcholine (ACh) neurotransmitter level causes the cognitive impairments seen in AD patients. Thus, increasing ACh levels to restore the cholinergic neurotransmission and improve the cognitive functions in AD patients are of prime importance.[7,23]

Current clinically approved treatments for AD are limited to cholinesterase inhibitors (ChEI's), working by inhibiting cholinesterases from hydrolyzing ACh and *N*-methyl D-aspartate receptor antagonists (e.g. memantine), which act at the glutaminergic pathway.[24–26] Despite the tremendous efforts in search of disease modifying agents working via β-amyloid or tau pathways, none are clinically available due to their adverse effects. Therefore, the search for new cholinesterase enzymes inhibitors is still a promising approach and ongoing worldwide. Two cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for degradation and regulation of ACh in human body, however they differ in kinetics and substrate selectivity.[27] Cholinergic therapy for AD initially focused on AChE inhibition because

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Table 1
Residue composition of active sites in *h*AChE and *h*BChE

Entry	Site name	Residue composition in *h*AChE	Residue composition in *h*BChE[38]
1	Catalytic triad	Ser203, His447 and Glu201	His438, Ser198 and Glu325
2	Choline binding site (α-anionic site)	Trp86 and Phe338	Trp82 and Phe329
3	Acyl-binding pocket	Phe295 and Phe297	Leu286 and Val288
4	Oxyanion hole	Gly121, Gly122 and Ala204	Gly116, Gly117 and Ala199
5	Peripheral anionic site (β-anionic site)	Tyr72, Asp74, Tyr124, Trp286, Tyr337 and Tyr341	Trp231, Val288, Leu286 and Phe398

this is the main enzyme involved in the breakdown of ACh in the normal brain.[28] Studies have shown that as the disease progresses, the activity of AChE decreases while the activity of BChE remains unaffected or even increases.[29] Moreover, in the brain of advanced-staged AD patients, BChE can compensate for AChE where the activity of AChE is inhibited and hydrolyze the already depleted levels of ACh.[30,31] Although overall AChE level is reduced in the brain of AD patients, it is increased within and around the amyloid plaques. Since both AChE and BChE hydrolyze ACh and involved in amyloid plaques maturation, inhibition of both enzymes using a dual inhibitor should results in the higher levels of ACh in the brain that provides more significant clinical efficacy.[28] Hence, dual inhibitors are valuable therapeutic compounds in AD therapy.

The active site of human AChE enzyme is located at the bottom of a 20 Å long, narrow gorge comprising five important regions to accommodate and hydrolyze the acetylcholine, namely; catalytic triad,[32] oxyanion hole,[33] choline binding site,[34] acyl binding pocket[35] and peripheral anionic site,[36] (Table 1). Acetylcholine or inhibitors guidance inside the gorge is facilitated by hydrophobic interactions with aromatic amino acid residues lining the gorge wall viz. phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr).[37] While the overall structure of human BChE is similar to that of human AChE, the active site of *h*BChE has many of the channel-lining aromatic residues replaced by residues with aliphatic side chains, such as leucine (Leu) and valine (Val), making BChE better able to accommodate bulkier substrates and inhibitors.[38]

Inspired by aforementioned biological significance of dual AChE and BChE inhibitors and in search for new potent Alzheimer's disease modifying agents, in the present study we wish to report ionic liquid mediated synthesis and cholinesterase inhibitory activity study of novel thiazolopyrimidine derivatives comprised of three aromatic rings. Subsequently, the effect of additional aromatic ring on AChE and BChE inhibitory activities and selectivity is compared to dual core inhibitors from our previous report. By the aid of molecular docking studies, the plausible binding interactions mechanisms of the most active derivatives at the active site of AChE and BChE are also explored.

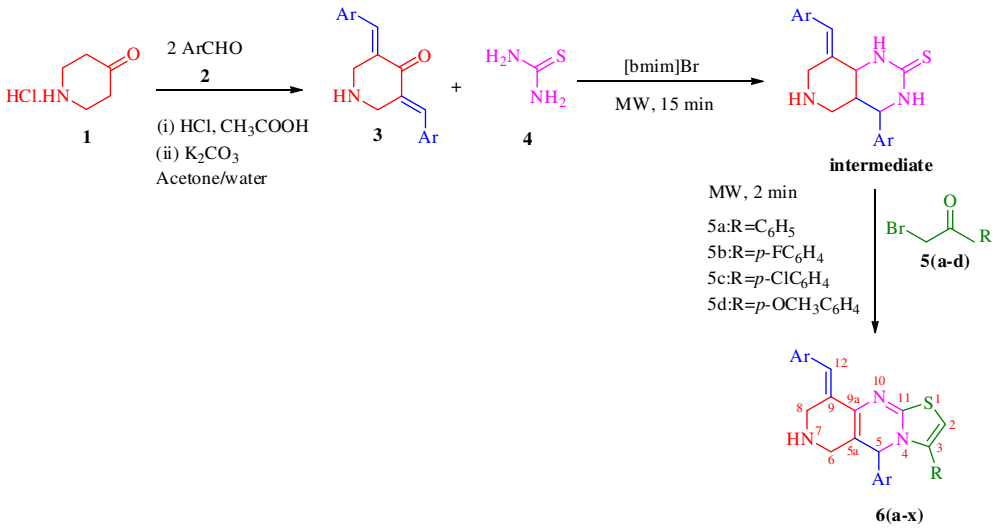
2. Results and discussion

2.1. Chemistry

The highly functionalized α,β-unsaturated ketones (**3**) required for the synthesis of (**6**) were prepared by the Claisen–Schmidt condensation of 4-piperidone hydrochloride (**1**) with a series of aromatic aldehydes in the presence of HCl in acetic acid.[39] The domino cascade reaction of 3,5-diarylidenepiperidin-4-ones (**3**), thiourea (**4**) and α-Bromoacetophenones (**5a–d**) in the ionic liquid, 1-butyl-3-methylimidazolium bromide ([bmim]Br) furnished (*E*)-9-arylidene-3,5-diaryl-6,7,8,9-tetrahydro-5*H*-pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidines (**6a–x**) in good yield. (Scheme 1)

In order to find an optimal and efficient protocol for the synthesis of **6**(**a–x**), we initiated our study by performing the model reaction of an equimolar ratio of *para*-methyl derivative of 3,5-bis-(aryledene)piperidin-4-one (**3**), thiourea (**4**) and 2-bromo-4′-chloroacetophenone (**5b**), employing two different synthetic approaches. In the first approach, the synthetic potential of a one pot, three component reaction was investigated using one molar equivalent of ([bmim]Br). Owing to the slow reaction rate of 3,5-bis-(aryledene)piperidin-4-one (**3**) with thiourea (**4**), in contrast to the relatively fast reaction of (**4**) and (**5**), this methodology failed to furnish the proposed thiazolopyrimidines (**6**).

Thus, we carried out a domino cascade two-step reaction by refluxing 3,5-bis-(aryledene)piperidin-4-one (**3**) and thiourea (**4**) in 1 molar equivalent of [bmim]Br for 2 h, as the first step. In the second step, 2-bromo-4′-chloroacetophenone (**5b**) were added to



Scheme 1. Synthesis of **6**(**a–x**).