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Preliminary communication

An expedient, ionic liquid mediated multi-component synthesis of novel piperidone grafted cholinesterase enzymes inhibitors and their molecular modeling study

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ABSTRACT

Series of hitherto unreported piperidone grafted pyridopyrimidines synthesized through ionic liquid mediated multi-component reaction. These compounds were evaluated for their inhibitory activities against AChE and BChE enzymes. All the compounds displayed considerable potency against AChE with IC_{50} values ranging from 0.92 to 9.11 μM, therein compounds **6a**, **6h** and **6i** displayed superior enzyme inhibitory activities compared to standard drug with IC_{50} values of 0.92, 1.29 and 2.07 μM. Remarkably, all the compounds displayed higher BChE inhibitory activity compared to galantamine with IC_{50} values of 1.89–8.13 μM. Molecular modeling, performed for the most active compounds using three dimensional crystal structures of *Tc*AChE and *h*BChE, disclosed binding template of these inhibitors into the active site of their respective enzymes.

1. Introduction

According to World Alzheimer's Report 2012, there are 36 million people living with dementia worldwide, which is predicted to increase up to 115 million by 2050 unless there is a cure or treatment to delay the onset or progression of the disease [1]. Alzheimer's disease (AD) is the most common type of dementia. Biochemical deficits in AD patients arise from degeneration of the cholinergic neurons caused by the phosphorylation of tau proteins leading to development of neurofibrillary tangles and formation of β-amyloid senile plaques. This neurodegeneration leads to remarkable reduction of neurotransmitter acetylcholine at the synaptic clefts [2,3]. Since acetylcholine plays a major role in cognitive processes, increasing acetylcholine levels to restore the substantial impairment of memory and cognitive dysfunctions in AD patients, the so-called cholinergic hypothesis, has gained interest [4].

Currently approved pharmacological treatments for AD are limited to cholinesterase inhibitors (ChEI's), working by inhibiting cholinesterase enzymes from hydrolyzing acetylcholine to restore the cholinergic function as well as the *N*-methyl D-aspartate receptor antagonist (e.g. memantine), which acts at the glutaminergic pathway [5–7]. Despite the tremendous efforts in search of disease modifying agents working along with the β-amyloid or tau pathways, none are clinically available due to their adverse effects. Therefore, the search for new cholinesterase enzymes inhibitors is still ongoing worldwide.

Both cholinesterase enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are involved in the hydrolysis of acetylcholine; however studies showed that as the disease progresses, the activity of AChE decreases while the activity of BChE remains unaffected or even increases [8]. In the brain of advanced staged AD patients, BChE can compensate for AChE when the activity of AChE is inhibited by AChE inhibitors. Thus, BChE hydrolyses the already depleted levels of ACh in these patients [9,10]. It has been

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

Entry	Site name	Residue composition in *Tc*AChE	Residue composition in *h*BChE [34]
1	Catalytic triad	Ser200, His440 and Glu327	His438, Ser198 and Glu325
2	Choline binding site (α-anionic site)	Trp84 and Phe330	Trp82 and Phe329
3	Acyl-binding pocket	Phe288 and Phe290	Leu286 and Val288
4	Oxyanion hole	Gly118, Gly117 and Ala201	Gly116, Gly117 and Ala199
5	Peripheral anionic site (β-anionic site)	Tyr 70, Asp72, Tyr 121, Trp279 and Tyr334	Trp231, Val288, Leu286 and Phe398

also proposed that individuals with low-activity of BChE can sustain cognitive functions better comparing to individuals with normal BChE activity [11]. Furthermore, restoration of ACh levels by BChE inhibition seems to occur without apparent adverse effects [9].

Molecular modeling plays an important role in the rational drug design and is used to predict the bonding affinity, spatial orientation and total binding energy of the small molecule drug candidates to the active site of their target enzymes [12]. Active site of AChE and BChE enzymes is located deep in the center of the molecule with a narrow gorge made up of five important regions to accommodate and hydrolyze the acetylcholine substrate, namely, catalytic triad [13], oxyanion hole [14], choline binding site [15], acyl binding pocket [16] and peripheral anionic site (Table 1) [17]. In AChE, aromatic residues such as tryptophan (Trp) and phenylalanine (Phe) comprise the active site gorge, whilst in BChE the gorge is lined with hydrophobic residues such as valine (Val), which allows accommodation of bulkier substrates [17].

In the context of green chemistry, ionic liquid (IL) mediated multicomponent reactions gained much attentions as an efficient synthetic tool from the viewpoint of evasion from intermediate isolation and purification steps, effectively merged with unique properties of green ionic solvents such as strong solvating ability, catalytic behavior and recyclability [18,19]

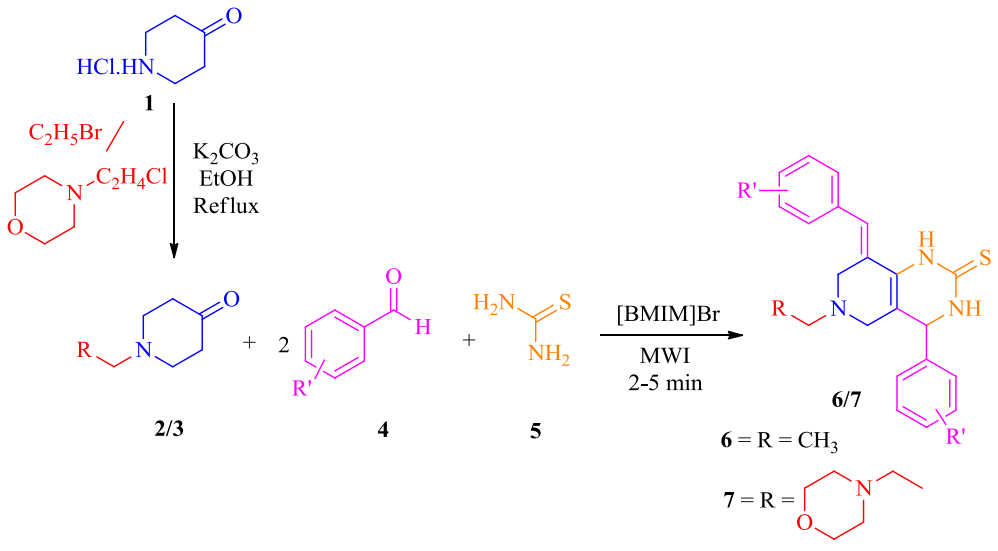
Natural and synthetic biologically active compounds with pyrimidine moiety, find wide applications in pharmaceutical field [20] as antihypertensive [21], α_1-adrenergic receptor antagonist [22], antibacterial, anti-inflammatory, antitumor [23] and anti-HIV agents [24–26]. Moreover, compounds comprising thiourea and pyrimidine entities are reported to possess potent dual cholinesterase and Aβ-aggregation inhibitory properties [27–31]. Inspired by the aforementioned inhibitory potential of pyrimidine derivatives on cholinesterase enzymes activity and in search of new potent AChE/BChE enzymes inhibitors, in the present study we report an efficient method to synthesize biologically active piperidone grafted pyridopyrimidines *via* microwave assisted, multicomponent reaction methodology in ionic liquid and their cholinesterase enzyme inhibitory activities.

2. Result and discussion

2.1. Chemistry

N-Substituted piperidine-4-ones **2**/**3** were prepared by refluxing 4-piperidone monohydrate hydrochloride (**1**) and ethyl bromide (**2**)/4-(2-chloroethyl)morpholine (**3**) in ethanol (Scheme 1). With the functionalized piperidones **2**/**3** in hand, the aim is to synthesize pyridopyrimidine-2-thiones using ionic liquid as green solvent. To optimize the reaction conditions, initially a model reaction of 1-ethylpiperidine-4-one, benzaldehyde and thiourea in 1:2:1 molar ratio in 1 M equiv of [BMIM]Br was performed under conventional heating, the reaction progress was monitored by TLC. After completion of the reaction (60 min), the product was isolated (52%) through flash column chromatography. Subsequently, the same reaction was investigated also, under microwave irradiation which as in the case of thermal reaction, the reactants in 1 M equiv of [BMIM]Br was subjected to microwave irradiation. After completion of the reaction (2 min), the pyridopyrimidine-2-thiones were isolated as the single reaction product through column chromatography in 71% yield. The above results clearly showed that microwave irradiation led to an enhancement in the yield of the product and the reaction time has also been reduced over the conventional thermal method. Consequently, all other reactions were performed under microwave irradiation.

The structure of the pyridopyrimidine-2-thiones **6**/**7** is in accordance with the combustion data, 1D and 2D NMR spectroscopic data and IR spectroscopy (*vide infra*). In the ^{1}H NMR spectrum of **7c**, the singlet at 5.58 ppm is readily assigned to H-4, which shows HMBC correlations with the doublets at 2.90 and 3.18 ppm (J = 16.75 Hz) enabling their assignment to H-5a and H-5b. A doublet and a multiplet at 3.40 (J = 13.60 Hz) and 3.52–3.54 ppm



Scheme 1. Synthesis of **6**(**a**–**j**) and **7**(**a**–**j**).