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Microwave assisted synthesis, cholinesterase enzymes inhibitory activities and molecular docking studies of new pyridopyrimidine derivatives

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ABSTRACT

A series of hitherto unreported pyrido-pyrimidine-2-ones/pyrimidine-2-thiones were synthesized under microwave assisted solvent free reaction conditions in excellent yields and evaluated in vitro for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes inhibitory activity. Among the pyridopyrimidine derivatives, **7e** and **7l** displayed 2.5- and 1.5-fold higher enzyme inhibitory activities against AChE as compared to standard drug, galanthamine, with IC_{50} of 0.80 and 1.37 μM, respectively. Interestingly, all the compounds except **6k**, **7j** and **7k** displayed higher inhibitory potential against BChE enzyme in comparison to standard with IC_{50} ranging from 1.18 to 18.90 μM. Molecular modeling simulations of **7e** and **7l** was performed using three-dimensional structure of *Torpedo californica* AChE (*Tc*AChE) and human butyrylcholinesterase (*h*BChE) enzymes to disclose binding interaction and orientation of these molecule into the active site gorge of respective receptors.

1. Introduction

According to the World Alzheimer report 2012, Alzheimer's disease (AD) is among the most significant social, health and economical crisis of the 21st century.[1] AD is a complex disease characterized by accumulation of β-amyloid (Aβ) plaques and neurofibrillary tangles composed of tau amyloid fibrils, associated with synapses loss and neuro-degeneration leading to impairment of memory and other cognitive dysfunctions.[2] The cognitive deficit is thought to be due to loss of cholinergic neurons in basal forebrain.[3] One of the pharmacological approaches to restore cholinergic function is by blocking the breakdown of neurotransmitter in the cholinergic neurons, acetylcholine (ACh), leading to the use of cholinesterase inhibitors to treat AD.[4]

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes are involved in the breakdown of acetylcholine in the brain and inhibition of these enzymes may increase the efficacy of treatment and broaden the indications.[5] Effects of cholinesterase inhibitors are mainly due to enhancement of cholinergic transmission at cholinergic autonomic synapses and at the neuromuscular junction.[6] Commercially available drugs such as galanthamine, donepezil, rivastigmine and tacrine showed positive results in symptomatic improvements of mild to moderate AD patients.[7] Donepezil and galanthamine being most selective for AChE while rivastigmine inhibit AChE and BChE at the same extent and tacrine shows lower selectivity for AChE than BChE.[8]

The recent development of inhibitors includes drugs with high selectivity for BChE, which also showed enhancement of ACh levels in rats brain.[9] BChE inhibitors were also found to reduce amyloid precursor protein levels in animals with a cholinergic lesion in the forebrain.[10] These agents represent an additional advantage for long-term stabilization of cognitive and behavioral symptoms in patients with advanced AD.[11] A selective BChE inhibitor, may produce significant increase in brain ACh levels without triggering severe peripheral or central cholinergic adverse effects.[8]

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] AChE's active site is located on the bottom of a long and narrow gorge, acetylcholine and substrate guidance down the gorge is facilitated by cation–π interactions with aromatic side-chains residues such as phenylalanine, tryptophan and tyrosine lining gorge wall.[13] The overall structure of human BChE is very similar to that of AChE

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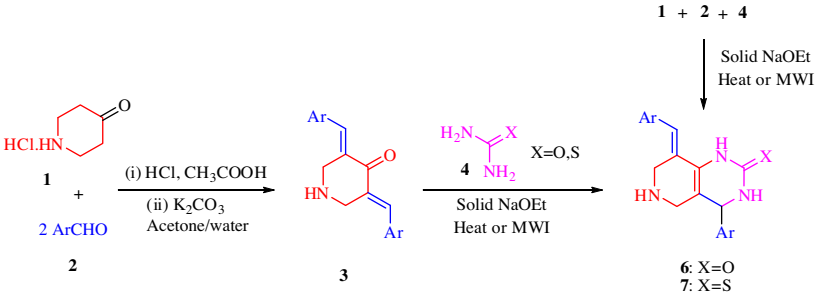


Scheme 1. Synthesis of **6a–l** and **7a–l**.

from *Torpedo California*. Most differences between BChE and *Tc*AChE are confined to the residues composing the gorge, whereby in BChE it is replaced with hydrophobic Leu286 and Val288. These changes make it possible for the binding of bulkier butyrate substrate moiety and inhibitors in BChE.[14]

Natural and synthetic biologically active compounds with pyrimidinone moiety, find applications in pharmaceutical and biochemical fields[15] as antihypertensive,[16] α_{1a}-adrenergic receptor antagonists,[17] antibacterial, anti-inflammatory and antitumor agents.[18] Compounds which constitute the core structural elements of pyrimidinone are commonly present in the polycyclic marine alkaloids which show anti cancer, anti HIV and cytotoxic properties.[19–21] Inspired by the aforementioned significance of pyrimidinone derivatives, in the present study two series of pyrimidinone derivatives were synthesized and their cholinesterase inhibitory activities were explored. Further molecular modeling was performed on the most active compounds to understand the possible reasons for their selectivity.

2. Results and discussion

2.1. Chemistry

In the present investigation, the reaction of a series of N-unsubstituted dibenzylidenepiperidine-4-ones with urea/thiourea in presence of catalytic amount of solid sodium ethoxide afforded the functionalized hitherto unreported pyridopyrimidine-2-ones (**6**)/pyridopyrimidine-2-thiones (**7**) in excellent yields (Scheme 1). The N-unsubstituted dibenzylidenepiperidine-4-ones (**3**) required for the present study was synthesized following the literature reported method by Dimmock et al.[22]

In order to find the optimal, efficient, eco-friendly and handy protocol for the synthesis of pyridopyrimidine derivatives, the reaction was examined under different conditions by choosing the model reaction between an equimolar ratio of 3,5-bis(4-methylbenzylidene)piperidin-4-one and thiourea in the presence of catalytic amount of solid sodium ethoxide. Initially, the neat reaction mixture in a semi micro boiling tube was heated in a water bath, for about 4–5 min during which period a transparent viscous liquid formed showing completion of reaction and double examined by TLC. Water (50 mL) was added to the mixture, precipitated solid was filtered and dried in vacuo to afford the pyridopyrimidine-2-thiones (**7c**) in 72% yield (Method A). The product obtained by this method doesn't require further purification as it is evident from TLC and ^{1}H NMR spectra. The same reaction was conducted under microwave irradiation condition (Method B), as this method is evolved as an alternative to conventional heating. Interestingly, the reaction was completed in just 30 seconds affording the product in excellent yield (95%) with high purity.

Alternatively, an attempt was made to synthesize the pyrido-pyrimidine derivatives through one pot pseudo four-component reaction of 4-piperidone hydrochloride monohydrate (**1**), 4-methylbenzaldehyde (**2**) and thiourea in a molar ratio 1:2:1, respectively in presence of catalytic amount of solid sodium ethoxide (Method C). The reaction mixture in a semi-micro boiling tube was heated on a water bath with constant grinding. The reaction took quite a longer time (10 min) than the earlier methods (Method A and B) for completion, resulting a yellow viscous liquid in 61% yield. ^{1}H NMR analysis of this liquid showed the presence of pyridopyrimidine along with some non-characterizable impurities. In order to reduce the impurities and to lessen the reaction time, the same pseudo four-component reaction was conducted under microwave irradiation condition (method D). As expected the reaction was completed in 3–4 min affording the product along with some impurities as method C. The above results disclose that a maximum yield of pyridopyrimidine derivative was obtained with high purity when the reaction was performed under microwave irradiation method.

Structural elucidation of the pyrido-pyrimidine-2-ones (**6**)/pyrimidine-2-thiones (**7**) was accomplished by FT-IR, 1D and 2D

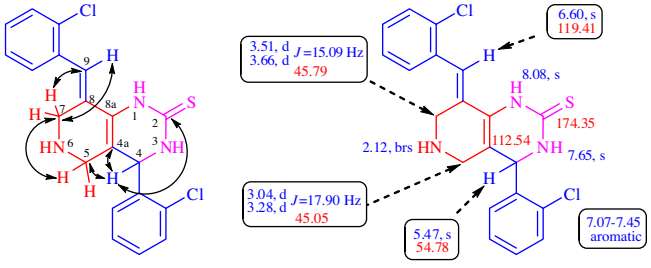
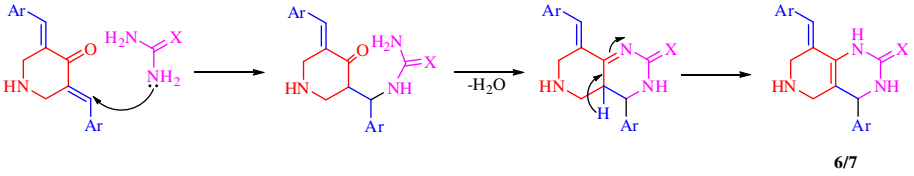


Figure 1. Selected HMBCs and ^{1}H and ^{13}C chemical shifts of **7c**.



Scheme 2. Mechanism for the formation of novel pyridopyrimidines **6**/**7**.