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Ionic liquid mediated synthesis of mono- and bis-spirooxindole-hexahydropyrrolidines as cholinesterase inhibitors and their molecular docking studies

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ABSTRACT

One pot, three-component reaction of 1-acryloyl-3,5-bisarylmethylidenepiperidin-4-ones with isatin and sarcosine in molar ratios of 1:1:1 and 1:2:2 furnished to mono- and bis-spiropyrrolidine heterocyclic hybrids comprising functionalized piperidine, pyrrolidine and oxindole structural motifs. Both mono and bis-spiropyrrolidines displayed good inhibitory activity against acetylcholinesterase (AChE) with IC_{50} values of 2.36–9.43 μM. For butyrylcholinesterase (BChE), mono-cycloadducts in series **8** with IC_{50} values of lower than 10 μM displayed better inhibitory activities than their bis-cycloadduct analogs in series **9** with IC_{50} values of 7.44–19.12 μM. The cycloadducts **9j** and **8e** were found to be the most potent AChE and BChE inhibitors with IC_{50} values of 2.35 and 3.21 μM, respectively. Compound **9j** was found to be competitive inhibitor of AChE while compound **8e** was a mixed-mode inhibitor of BChE with calculated K_i values of 2.01 and 6.76 μM, respectively. Molecular docking on *Torpedo californica* AChE and human BChE showed good correlation between IC_{50} values and free binding energy values of the synthesized compounds docked into the active site of the enzymes.

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia among the elderly individuals is an irreversible neurodegenerative disorder characterized by diverse cognitive impairments, neuropsychiatric symptoms and inability to perform routine activities.[1] AD is associated with the degeneration of cholinergic neurons in the basal forebrain, which leads to substantial decrease in generation of acetylcholine (ACh) neurotransmitter. ACh deficiency severely affects the cognitive abilities, memory function and emotional responses in AD patients. Pathogenesis of AD is largely characterized by the presence of extracellular amyloid plaques (Aβ plaques) and intracellular neurofibrillary tangles (NFT) composed of phosphorylated tau proteins.[2–4] Based on so-called cholinergic hypothesis, one of the most promising therapeutic approach to enhance cholinergic function is by the use of cholinesterase inhibitors.[5,6] In spite of tremendous efforts to develop novel disease modifying agents working via β-amyloid or tau pathways, none is clinically available due to their side effects. Thus, the search for new cholinesterase inhibitor is still ongoing worldwide.

Two major cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), involved in hydrolysis and regulation of ACh in vertebrates.[7] These two cholinesterases share similar structure topology of possessing a 20 Å long, narrow active site channel located in the center of the enzyme, ending to a catalytic triad where the hydrolysis of acetylcholine takes place.[8] This channel is composed of five major binding regions, namely (i) peripheral anionic site,[9] (ii) oxyanion hole,[10] (iii) choline binding pocket,[10] (iv) acyl binding site[11] and (v) catalytic triad. In AChE, amino acid residues having aromatic side chains such as tryptophan (Trp) and tyrosine (Tyr) facilitate the insertion and transition of substrate or inhibitor inside the active site cavity. In BChE these aromatic residues are mostly replaced with hydrophobic ones such as valine (Val) and leucine (Leu) resulting in more spacious channel to accommodate bulkier substrates.[12]

Ionic liquid mediated, multi-component reactions owing to their unrivaled features such as high solvating abilities, interesting catalytic behavior and recyclability have gained great importance among the chemists for the synthesis of biologically active heterocycles.[13–15] Under this context and as a part of our ongoing

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research in the construction and biological evaluation of novel heterocyclic hybrids,[16–19] we report ionic liquid mediated synthesis of a library of novel mono- and bis-spiropyrrolidines and their *in vitro* cholinesterases inhibitory activities. Mode of inhibition, enzyme kinetics and molecular docking studies were also conducted for the most active derivatives to investigate their nature of inhibition and binding interaction template to the active site of the enzymes.

2. Results and discussion

2.1. Chemistry

The highly functionalized dipolarophiles *viz.* 1-acryloyl-3,5-diarylmethylidenepiperidin-4-ones (**5**) required for the synthesis of spiro-heterocycles were prepared by Claisen–Schmidt condensation of 4-piperidone hydrochloride (**1**) with a series of aromatic aldehydes (**2**) to furnish *N*-unsubstituted 3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**3**), which were subsequently acylated with acryloyl chloride, following the previously reported procedure.[20] The dipolarophiles (**5**) are versatile synthons for the construction of more complex spiro-heterocycles owing to presence of diverse dipolarophile functions such as three C=C and two C=O groups.

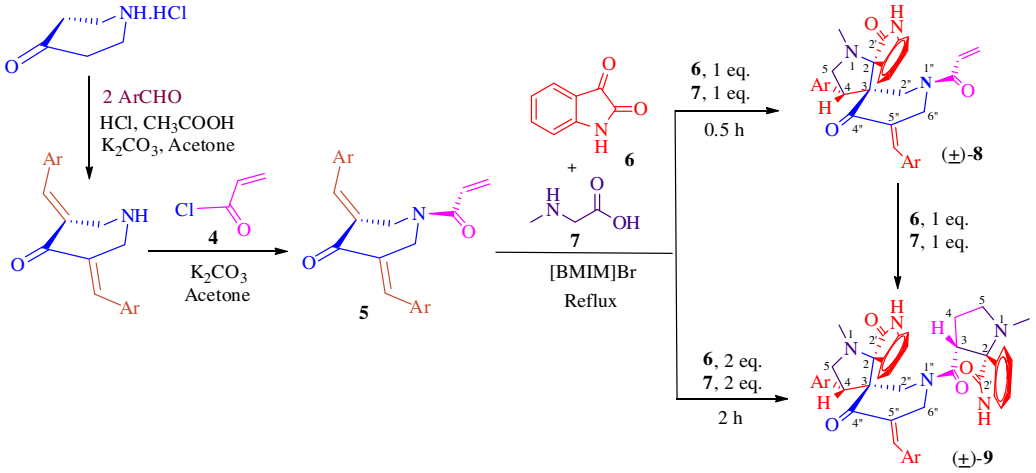
As depicted in Scheme 1, three-component [3+2]-cycloaddition reaction of dipolarophiles (**5**) with isatin (**6**) and sarcosine (**7**) in [bmim]Br as ionic solvent afforded a library of spiro-cycloadducts in good yields (Table 1). Initially, the reaction of an equimolar mixture of 1-acryloyl-3,5-diphenylidenepiperidin-4-ones (**5**), isatin (**6**) and sarcosine (**7**) was investigated under refluxing methanol for 5 h, furnishing to mono-spiropyrrolidine (**8a**) as the sole product in 62% yield. The reaction of **5**, **6** and **7** in a molar ratio 1:2:2 for a longer period of time (15 h) afforded more complex bis-spiropyrrolidine (**9a**) in moderate yield of 48%.

The above reactions were also performed in [bmim]Br, as a green and eco-friendly reaction medium. By refluxing an equimolar mixture of **5**, **6**, **7** and 1 molar equiv of [bmim]Br, the mono-spiropyrrolidine (**8a**) was obtained in 30 min and 83% yield. The bis-spiropyrrolidine (**9a**) was also afforded in a shorter reaction time of 2 h and 71% yield by refluxing 1:2:2 mixture of the reactants and 2 molar equiv of ionic solvent. In both reactions, the spiropyrrolidines [(**8a**) and (**9a**)] were purified through flash column chromatography. Further attempt to obtain the more complex tri-spiropyrrolidine cycloadduct were unsuccessful, plausibly due to the steric hindrance exerted by the cycloadduct **9** for subsequent cycloaddition. Performing aforementioned reactions in ionic solvent had noticeable advantages over methanol in terms of reaction time and yield. The high product yields were probably due to the catalytic abilities of [bmim]Br that also improved the reaction rates.

Structures of the mono and bis-spiropyrrolidines **8** and **9** are in agreement with the combustion data, IR, 1D and 2D NMR spectroscopic data. The elemental analysis results are within ±0.4% of the theoretical values. In the ^{1}H NMR spectrum of **8a**, a doublet of doublets at 5.00 ppm with J = 10.5 and 8.7 Hz is due to H-4 of the pyrrolidine ring. The HMQC correlation of H-4 assigns the carbon signal at 44.0 ppm to C-4. Further, H-4 shows HMBC correlation with the C=O of the piperidone ring at 196.5 ppm and the spiro carbon C-3″ at 63.2 ppm, besides showing correlation with the adjacent carbon C-5 at 57.1 ppm. The HMQC correlation of C-5 assigns the triplets at 3.47 ppm (J = 8.7 Hz) and 4.10 ppm (J = 10.5 Hz) to 5-CH_2. The two doublets at 2.54 and 4.33 ppm with J = 14.3 Hz are assigned to 2″-CH_2 while the doublet of doublets at 3.31 ppm with J = 18.8, 2.0 Hz and a doublet at 4.27 ppm with J = 18.8 Hz are due to 6″-CH_2 of the piperidone ring. From HMQC correlation, the carbon signals at 46.1 and 45.5 ppm are assigned to C-2″ and C-6″, respectively (Fig. 1).

Further, the piperidone ring protons, 2″-CH_2 and 6″-CH_2 show HMBC correlation with the carbonyl carbon of acryloyl moiety at 176.7 ppm. The doublet of doublets at 6.62 ppm is due to β-hydrogen of the acryloyl moiety whereas the doublets at 5.68 and 6.25 ppm are due to two α-hydrogens. The singlets at 7.82 and 8.36 ppm are due to the arylmethylidene hydrogen and NH hydrogen of the oxindole, respectively. The aromatic hydrogens appear as doublets and multiplets in region between 6.87 and 7.56 ppm. The ^{1}H and ^{13}C NMR chemical shifts of the bis-spiropyrrolidines (**9**) were also assigned by similar considerations. As the cycloadduct **9** possess two oxindolo-pyrrolidine rings, to distinguish the ^{1}H and ^{13}C chemical shifts of these two rings, the ring attached to C-3″ of 4-piperidone moiety is considered as ring A and the other ring attached to 1″-N—C=O is considered as ring B. Further, the structure and stereochemistry of mono and bis-spiropyrrolidines were confirmed by the single crystal X-ray crystallographic analysis of **8a** and **9a** (Figs. 2 and 3).

The plausible mechanism for the formation of spiropyrrolidines **8** and **9** in ionic solvent is depicted in Scheme 2. The electron-deficient hydrogen atom of [bmim]Br, forms hydrogen bonding interaction with the carbonyl function of isatin, whereby facilitates



Scheme 1. Synthesis of mono- and bis-spiropyrrolidines **8**(**a**–**k**) and **9**(**a**–**k**).