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Synthesis, evaluation and molecular dynamics study of some new 4-aminopyridine semicarbazones as an antiamnesic and cognition enhancing agents

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ABSTRACT

Some new semicarbazones of 4-aminopyridine were synthesized and evaluated for antiamnesic, cognition enhancing and anticholinesterase activities. The results illustrated a significant cognition enhancing effect on elevated plus maze model with a significant reversal of scopolamine-induced amnesia. A significant inhibition in acetycholinesterase (AChE) activity by all the synthesized compounds in specific brain regions that is, prefrontal cortex, hippocampus and hypothalamus was observed. Compound **4APi** exhibited significant antiamnesic and cognition enhancing activity which was comparable with standard drug donepezil. Its enzyme kinetic study revealed a non-competitive inhibition of AChE and a competitive inhibition of butyrylcholinesterase (BChE). Docking studies predicted the binding modes of these compounds in AChE active site, which were further processed for molecular dynamics simulation for calculating binding free energies using Molecular Mechanics–Generalized Born Surface Area (MM/GBSA). All the computational study confirmed their consensual interaction with AChE justifying the experimental outcome.

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder, characterised by selective loss of cholinergic neurons and accumulation of β-amyloid protein in the selective brain areas such as cortex and hippocampus.[1,2] Loss of memory, cognitive decline, impaired performance of activities of daily life and behavioural changes are hallmark of Alzheimer's disease.[3–5] Augmentation of cholinergic neurotransmission in the brain plays a pivotal role in the treatment strategy of Alzheimer's disease. Various drugs, clinically used to treat Alzheimer's disease elevates the level of acetylcholine at the synapse by inhibiting the metabolising enzyme AChE.[6,7] Through various molecular docking and dynamics study of different AChE inhibitors, it has been concluded that, the active centre of human acetylcholinesterase (hAChE) consists of several major domains such as, an esteratic site[8] containing the catalytic triad Ser-203, His-447 and Glu-334, an anionic site (Trp-86) that binds through cation–π interactions with the quaternary ammonium of choline and hydrophobic sites which binds aryl substrates and other uncharged ligands.[9–11] The allosteric modulation of hAChE catalytic activity is possible through binding of some ligands at the peripheral anionic site constituted by amino acid residues Tyr-72, Tyr-124, Glu-285, Trp-286, and Tyr-341.[12,13] Currently, derivatives of 4-aminopyridine (**4AP**) are under intensive investigation owing to their antiacetylcholinesterase activity which have shown promising effects in alleviating memory related dysfunctions.[14,15] Several carbamate derivatives of **4AP** and Schiff bases of styrylpyridine have been synthesized and evaluated for their anticholinesterase activity.[16,17] Some 4-aminobutyric acid (GABA) and 2-indolinone derivatives of **4AP** have been also reported to possess antiamnesic activity.[18] Also, the hydrazone derivatives of dihydropyridine and indolinones have been reported as potent anticholinesterase, antibutyrylcholinesterase and β-amyloid aggregation properties.[19,20] 3-Methylpyridinium and 2-thionaphthol derivatives of berberine have evaluated for AChE and BChE inhibitory activity, respectively.[21] Some quinazolinimines have been shown selective butyrylcholinesterase inhibitory activity.[22,23] A number of *N*-benzylpiperidine–purine derivatives have been reported as dual inhibitors of AChE and BChE.[24]

In our earlier studies, we have reported some new schiff bases, anilide and imide derivatives of **4AP** for their cognition enhancing, antiamnesic and anticholinesterase activities among which benzophenone and imide derivatives were found to be highly significant. In continuation of our previous research work[25,26] we have designed and synthesized some new semicarbazones of **4AP** having potential antiamnesic and cognition enhancing activities.

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2. Results and discussion

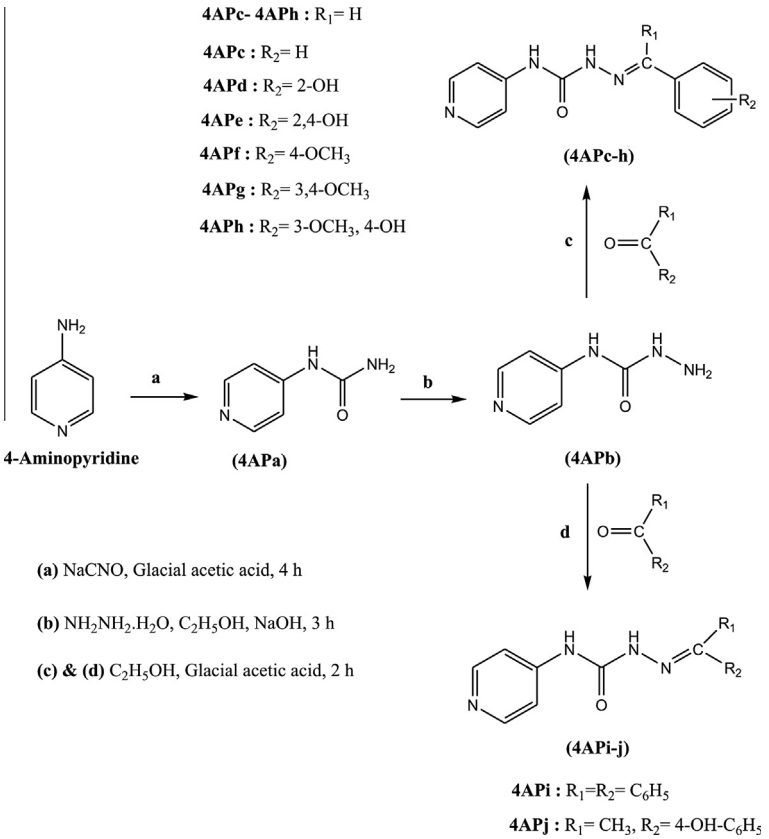
2.1. Chemistry

4-Aminopyridine urea (**4APa**) was synthesized by treating 4-aminopyridine with sodium cyanate in presence of glacial acetic acid. The urea derivative on condensation with hydrazine hydrate with ethanol in the presence of sodium hydroxide yielded the 4-aminopyridine semicarbazide (**4APb**). The semicarbazones (**4APc–j**) were prepared by reaction of the appropriate aldehyde or ketone with 4-aminopyridine semicarbazide as represented in Scheme 1. The purity of compounds was checked by TLC and all synthesized derivatives were characterised by FT-IR, ^{1}H, ^{13}C NMR and elemental analysis. In IR, peak of C=N and NH streching vibrations was observed at 1590 cm^{-1} and 3433–3326 cm^{-1}, respectively. In ^{1}H NMR spectra, compounds **4APa** and **4APb** showed peak at δ 6.51 ppm due to the presence of $-NH_2$ proton. In subsequent steps of synthesis, both the protons of $-NH_2$ were substituted by different groups which resulted into the formation of N=CH (**4APc–h**) and N=C (**4APi–j**) bond that caused the disappearance of peak at δ 6.51 ppm, confirming the substitution. The ^{13}C NMR value of δ 155–158 ppm also confirmed the formation of N=CH and N=C bond.

2.2. Biological activity

The IC_{50} values of all the derivatives on AChE and BChE was determined as per Ellman method.[27] For AChE inhibitory activity, **4APi** was found to elicit comparable activity (0.052 ± 0.01 μM) and selectivity (355) with respect to standard donepezil (0.04 ± 0.012 μM), (381) among all the derivatives (Table 1). Further, enzyme kinetics study[28] was also performed for all derivatives to gain an insight on their nature of inhibition (Table 2). The most active compound **4APi** demonstrated a non-competitive inhibition for AChE (K_i = 0.082 ± 0.60) and competitive inhibition for BChE (K_i = 16.82 ± 0.76) enzymes. The non-competitive inhibition[29] is attributed to a possible interaction of compound with the peripheral anionic site (PAS) of AChE and was also confirmed by docking studies. The synthesized derivatives were then evaluated for antiamnesic and cognition enhancing activities in rat elevated plus maze (EPM) model[30] which is a simple method for assessment of learning and memory that depends upon measuring the transfer latency of rats. Rats are known for their natural dislike for open and high spaces. In these types of studies, animals usually spend more time in enclosed arms rather than open arms in plus maze test.[31] This behaviour suggest that the transfer latency reduces day by day, if the animal have previous experience to move from the open arms to the enclosed arms, which attributed to an enhanced memory. Pre-treatment with tested compounds resulted in reduced transfer latency as compared to control group on first and second day of EPM exposure in significant and dose dependant manner, indicating facilitated learning process (Fig. 1). Scopolamine (1.5 mg/kg), significantly increased the transfer latency as compared to control group (p <0.001), resulting in amnesia which was reversed significantly by tested compounds and donepezil[32] (p <0.001) (Table 3).

Prefrontal cortex, hippocampus and hypothalamus are three specific brain regions involved in processing of memory with high innervations of cholinergic neurons. It is reported that 50–90% reduction in ChAT, an enzyme which synthesizes acetylcholine is observed in hippocampus, cortex and hypothalamus in patients with dementia of Alzheimer's Type (DAT).[33,34] Hippocampus is permanently involved in memories that have to be acquired and retrieved. It is also temporarily involved in the process of memory



Scheme 1. The synthetic pathway of the compounds here presented.