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Development of cyanopyridine–triazine hybrids as lead multitarget anti-Alzheimer agents

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ABSTRACT

A series of new cyanopyridine–triazine hybrids were designed, synthesized and screened as multitargeted anti-Alzheimer's agents. These molecules were designed while using computational techniques and were synthesized via a feasible concurrent synthetic route. Inhibition potencies of synthetic compounds **4a**–**4h** against cholinesterases, $A\beta_{1-42}$ disaggregation, oxidative stress, cytotoxicity, and neuroprotection against $A\beta_{1-42}$-induced toxicity of the synthesized compounds were evaluated. Compounds **4d** and **4h** showed promising inhibitory activity on acetylcholinesterase (AChE) with IC_{50} values 0.059 and 0.080 μM, respectively, along with good inhibition selectivity against AChE over butyrylcholinesterase (BuChE). Molecular modelling studies revealed that these compounds interacted simultaneously with the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. The mixed type inhibition of compound **4d** further confirmed their dual binding nature in kinetic studies. Furthermore, the results from neuroprotection studies of most potent compounds **4d** and **4h** indicate that these derivatives can reduce neuronal death induced by H_2O_2-mediated oxidative stress and $A\beta_{1-42}$ induced cytotoxicity. In addition, in silico analysis of absorption, distribution, metabolism and excretion (ADME) profile of best compounds **4d** and **4h** revealed that they have drug like properties. Overall, these cyanopyridine–triazine hybrids can be considered as a candidate with potential impact for further pharmacological development in Alzheimer's therapy.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder, characterized by progressive cognitive impairment, dementia and neuropsychiatric symptoms.[1,2] It starts with minuscule changes of hippocampal synaptic adequacy, simultaneous neuronal degeneration, and ultimately death of the patient. It is a chronic disorder that cannot be prevented, cured or slowed hence is the third leading cause of death after cardiovascular diseases and cancer worldwide.

The complete etiology of the disease is still not clear. However, the synaptic dysfunction is believed to be caused due to extracellular amyloid-β (Aβ) aggregation,[3] produced by the proteolytic cleavage of a transmembrane protein amyloid precursor protein (APP) by the β- and γ-secretases. Intracellular tangle formation due to hyperphosphorylation of microtubule associated protein tau[4] which in turn is governed by the overexpression of glycogen synthase kinase beta (GSK-3β),[5] also accounts for the synaptic dysfunction. These two protein misfoldings are considered to be the main hallmarks of this ailment. Another popular physiological target in AD is cholinergic pathway that tides symptoms of dementia and learning difficulties to the significant decrease of acetylcholine

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; eelAChE, electric eel acetylcholinesterase; BuCh, butyrylcholine; BuChE, butyrylcholinesterase; eqBuChE, equine serum butyrylcholinesterase; ChE, cholinesterase; AD, Alzheimer's disease; Aβ, amyloid beta; AChEI, acetylcholinesterase inhibitor; BChEI, butyrylcholinesterase inhibitor; PAS, peripheral anionic site; CAS, catalytic active site; TcAChE, Torpedo Californica acetylcholinesterase; ROS, reactive oxygen species; SAR, structure activity relationship; SEM, scanning electron microscope; TEM, transmission electron microscopy; ThT assay, thioflavin T assay; equiv, equivalent.

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level. AChE, a key member of serine hydrolase family, catalyses the breakdown of acetylcholine (ACh) an important neurotransmitter. Its catalytic triad Ser-His-Glu is located at the bottom of a deep and narrow gorge mainly covered with the aromatic residues which are considered to be responsible for cation–π interaction with the substrate Ach.[6,7] Peripheral anionic site (PAS) of AChE constitutes Tyr 121, Tyr70 and Trp279 residues, present at the opening of the gorge and guides the substrate approaching the active site. In addition, Aβ binds to the peripheral anionic site (PAS) of the AChE which enhances the rate of Aβ fibril formation.[8,9] Therefore, due to their non-classical roles, cholinesterase inhibitors could also be useful as potential disease-modifying drugs.[10,11]

On the other hand, evasion of Aβ stockpile is considered to be the potentially influential step approaching the treatment of AD.[12] In addition to this, oxidative stress occurs in the early breakthrough of AD.[13] Brain aging is directly correlated to the AD development which in turn is believed to be due to a gradient between production of reactive oxygen species (ROS) and antioxidant defences.[14] Several evidences suggest that oxidative stress has a prominent role in the AD buildup. Therefore, therapeutics targeting clearance or prevention of the free radicals in the brain would be beneficial against AD.

Till date drugs like tacrine, donepezil, rivastigmine, galanthamine, caproctamine and memantine targeting cholinesterases, cholesterol esterase, and lipase are used to treat AD but these drugs only curtail the symptoms and do not produce a complete cure. These drugs are also recognized to have many side effects including hepatotoxicity, gastrointestinal disturbance, dizziness, diarrhoea, vomiting and nausea.[15,16]

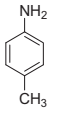
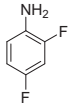
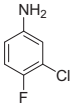
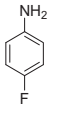
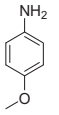
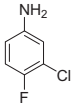
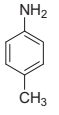
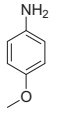
1,3,5-Triazine scaffold has been serving medicinal chemists for a long time for the development of antifungal,[17] anticancer,[18] antimalarial,[19] antiviral,[20] antibacterial agents.[21] The construction of triazine and its derivatives could be easily achieved by routine chemical reactions.

Keeping the above aspects into consideration, here we have designed and synthesized a series of eight new cyanopyridine–triazine hybrids as persuasive multifunctional agents for the treatment of AD. All the synthesized compounds were assessed for their inhibitory activity towards cholinesterases and their Aβ anti-aggregating activity. Further, the mechanism of AChE inhibition was investigated by kinetic studies. Selected cholinesterase inhibitors were subsequently examined for neuroprotective properties in SH-SY5Y neuronal cells. In order to obtain a better understanding of possible interactions with biological targets, molecular docking studies were also performed.

2. Results and discussion

2.1. Chemistry

The target molecules were synthesized via multiple steps as depicted in Schemes 1 and 2. The mono and di-substituted triazine compounds (**2a**–**2h** and **3a**–**3h**) were synthesized according to the reported literature procedure.[22] 2,4,6-Trichloro-1,3,5-triazine was first treated with 3-(trifluoromethyl)aniline (**2a**–**2e**, **2h**), 2-fluoroaniline (**2f**), 3-chloro-4-fluoroaniline (**2g**) at −10 °C in THF and K_2CO_3. The mono-substituted triazine compounds were further treated with different amines to obtain disubstituted compounds (**3a**–**3h**). On the other hand, 2-(piperazin-1-yl)nicotinonitrile (**6**) was synthesized as shown in Scheme 2.[23] Finally, the di-substituted triazine compounds (**3a**–**3h**) were treated with compound (**6**) in presence of K_2CO_3 and 1,4-dioxane at 110 °C to obtain the targeted compounds as tri-substituted triazines (**4a**–**4h**). The reactions involved in the synthesis of the target compounds were temperature dependent nucleophilic aromatic substitution reactions. The monosubstituted triazines were synthesized at below 0 °C, disubstituted at room temperature and the targeted compounds were obtained at higher temperature, i.e., 110 °C. All the newly synthesized compounds were purified by column chromatography and were characterized by different spectroscopic techniques—^{1}H NMR, ^{13}C NMR, ESI MS and elemental analysis.

Compound	R_1	R_2
4a		
4b		
4c		
4d		
4e		
4f		
4g		
4h		

2.2. Design of multifunctional ligands

In order to develop a persuasive drug candidate for multifaceted AD, we undertook structure based drug design approach. Triazine was selected as preferred scaffold, because its roughly planar structure was expected to intercalate between beta-amyloid sheets and was expected to enhance the beta-amyloid disaggregation. It was also expected to engage efficiently with the active site residues of AChE via weak non-covalent interactions like H-bonding, π–π stacking interaction, and alkyl–π-interaction. In addition, the molecules substituted with *p*-anisidine are known to have neuroprotective and radical scavenging capability.[24] Inclusion of piperazine was a natural choice to connect two electrophilic centres. Nitrogen of piperazine was expected to be in protonated state and hence may engage in π–cation interactions with the residues of aromatic gorge. Pardock module of *Sanjeevini* (*SCFBIO*, IITD)[25,26]