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Research paper

Ameliorative effects of amide derivatives of 1,3,4-thiadiazoles on scopolamine induced cognitive dysfunction

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ABSTRACT

The present study reports the effect of amide derivatives of 1,3,4-thiadizoles on scopolamine induced deficit cholinergic neurotransmission and oxidative stress serving as promising leads for the therapeutics of cognitive dysfunction. Fourteen compounds **(2c–8d)** have been synthesised and evaluated against behavioural alterations using step down passive avoidance protocol and morris water maze and at a dose of 0.5 mg/kg with reference to the standard, Rivastigmine. All the synthesised compounds were evaluated for their *in vitro* acetylcholinesterase (AChE) inhibition at five different concentrations using mice brain homogenate as the source of the enzyme. Biochemical estimation of markers of oxidative stress (lipid peroxidation, superoxide dismutase, glutathione, plasma nitrite, catalase) has also been carried out to assess the role of synthesised molecules on the oxidative damage induced by scopolamine. The compounds **5c, 6c** and **8c** displayed appreciable activity with an IC_{50} value of 3 μM, 3.033 μM and 2.743 μM, respectively towards acetylcholinesterase inhibition. These compounds also decreased scopolamine induced oxidative stress, thus serving as promising leads for the amelioration of oxidative stress induced cognitive decline. The molecular docking study performed to predict the binding mode of the compounds also suggested that these compounds bind appreciably with the amino acids present in the active site of recombinant human acetylcholinesterase (rhAChE). The results indicated that these compounds could be further traversed as inhibitors of AChE and oxidative stress for the treatment of cognitive dysfunction.

1. Introduction

Cognition refers to a combination of capabilities of a person to discern, observe, reminisce and rationalize any particular situation or event [1]. Cognitive debility is manifested in various neurodegenerative disorders mainly Alzheimer's Disease (AD), Parkinson's Disease (PD), traumatic brain injury, schizophrenia, depression *etc.* [2,3]. AD is the commonest form of dementia associated with the loss of cognitive capabilities of an individual resulting in the decline of memory and intellect of a person [4]. Research undertaken during the past two decades has led to a better understanding of the underlying pathophysiology, suggesting the role of formation of amyloid-β plaques, deficit cholinergic neurotransmission and oxidative stress in cognitive decline [5,6]. The molecular basis of pathophysiology of AD has been appreciably illustrated by the formation of amyloid β (Aβ) plaques and neurofibrillary tangles which are hyperphosphorylated form of microtubule associated protein, tau [7,8]. These two pathophysiological hallmarks result in neurodegeneration, leading to deficits in the cholinergic neurotransmission by the loss of cholinergic neurons in the basal forebrain, allegedly responsible for cognitive decline and loss of short term memory [9,10]. Hence, increasing the cholinergic neurotransmission by the use of acetylcholinesterase inhibitors (AChEIs) forms a viable approach for designing and optimising a possible therapeutics for cognitive dysfunction. The past several decades have witnessed the development of several cognition enhancers, but the discovery and development of potential AChEIs has paved the way for a better therapeutic and treatment approach towards cholinergic deficit associated with cognitive decline [11]. Apart from cholinergic hypothesis, the role of oxidative stress and reactive oxygen species (ROS) in the process of aging and as the causative factor in loss of mental tenacity and cognitive disorders has been very well cited in the literature. Damage caused by the oxidative stress is attributed to disproportion between the generation of ROS and anti-oxidant enzyme activity. Hence, oxidative

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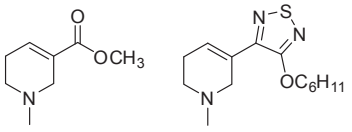
stress is also a forerunner in the pathophysiology of cognitive deterioration [12,13].

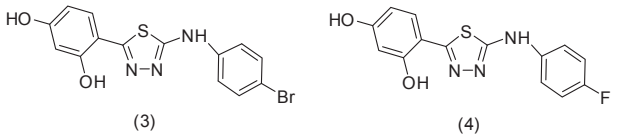
Scopolamine induced animal model of cognitive decline has been widely studied to carry out research on molecules with a propensity to be developed for the therapy for dementia [14,15]. Scopolamine is a muscarinic antagonist which causes interference in the learning and memory paradigm of both humans and animals. It is used as an inducer of dementia in AD animal models and has a prominent effect on the elevation of the enzyme acetylcholinesterase (AChE) [16] and is also responsible for the oxidative stress that has an implication in the pathophysiology of cognitive dementia associated with neurodegenerative disorders.

Five-membered heterocycles are fundamental fragments of many diversified biologically active molecules of different therapeutic categories. 1,3,4-Thiadiazole is one such five-membered heterocycle exhibiting a plethora of biological activities which is attributed to the presence of –N=C–S group. The ability of this scaffold to effectively traverse the biological membranes has been accredited to its mesoionic nature and hydrophobic character [17]. 1,3,4-Thiadiazole has been successfully implicated as a propitious scaffold for the treatment of cognitive debility [18,19]. Oral administration of acetylcholine poses challenges due to its rapid hydrolysis in the gastrointestinal tract owing to its ester and quaternary ammonium functional groups. On this line of thought, cholinomimetic ligands were designed wherein the ester group of the neurotransmitter is replaced by a metabolically stable thiadiazole scaffold. A fruitful strategy wherein the ester group of Arecoline **(1)** and its analogues has been replaced by a hydrolytically stable heterocycle was adopted. Xanomeline **(2)** is a remarkable outcome of this strategy wherein the ester function of arecoline has been replaced by (hexyloxy)-1,2,5-thiadiazole thereby conferring selective M1-receptor agonistic activity [20]. It improved both cognitive as well as behavioural symptoms of AD [21].



(1) (2)

The importance of 1,3,4-thiadiazole scaffold in the inhibition of AChE has been very well cited in literature. 1,3,4-Thiadiazole nucleus effectively creates π–π stacking interaction with the aromatic residues present at the enzymatic site of AChE [22,23]. Reports on 1,3,4-thiadiazole scaffold containing compounds, 4BrABT (2-(4-bromophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole) **(3)** [24] and 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) **(4)** [18] having neuroprotective role have surfaced suggesting the possible protective role of 1,3,4-thiadiazole containing compounds in oxidative stress induced dementia.



(3) (4)

The present study fosters the design and synthesis of novel therapeutically useful amide derivatives of 1,3,4-thiadiazole **(2c–8d)** for treating cognitive impairments and their antioxidant potential in a scopolamine induced memory deficit animal model. These newly synthesised compounds were evaluated against behavioural alterations using morris water maze and passive avoidance (laca mice) models with reference to the standard, Rivastigmine. Biochemical estimation of different markers of oxidative stress has also been carried out. The molecular docking of the synthesised compounds has been carried out on the recombinant human acetylcholinesterase (rhAChE, PDB ID: 4EY7) using Vlife MDS (version 4.3.30052013).

2. Results and discussion

2.1. Chemistry

The various starting 1,3,4-thiadiazoles (**1a-1d**) were prepared by refluxing respective aromatic acids (benzoic acid, phenyl acetic acid, 2-chloro benzoic acid and *p*-toluic acid) with thiosemicarbazide in phosphorus oxychloride at 100–120 °C. After refluxing, the solution obtained was cooled followed by basification of the solution to pH 8–9 using 50% sodium hydroxide solution. The solid obtained was filtered and recrystallised (Scheme 1) (Fig. 1). **1a** and **1b** were refluxed with various acid chlorides (3-(chloromethyl) benzoyl chloride, 3-(acetoxy) benzoyl chloride, 3,5,5-trimethyl hexanoyl chloride) in anhydrous tetrahydrofuran (THF) in the presence of triethylamine to yield **2c, 3c, 4c, 2d, 3d and 4d (2c–4d)**, respectively (Scheme 2) (Fig. 1). Similarly, **1c** and **1d** were acylated with various acid chlorides (3-(chloromethyl) benzoyl chloride, 3-(acetoxy) benzoyl chloride, 3,5,5-trimethyl hexanoyl chloride and 3,5-bis-(trifluoromethyl) benzoyl chloride) in methylene chloride in the presence of 1 M sodium bicarbonate solution followed by extraction of the organic layer to yield **5c, 6c, 7c, 8c and 5d, 6d, 7d, 8d (5c–8d)**, respectively (Scheme 3) (Fig. 1). The acylation reactions were carried out according to the principles of Schotten Baumann reaction [25]. The spectral analysis was carried out using IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The IR spectrum of all the synthesised compounds showed a characteristic peak between 1650 cm^{-1} to 1690 cm^{-1} confirming the presence of –NH of the amide function. Compounds **3c, 3d, 6c** and **6d** showed a peak at around 1740 cm^{-1}, confirming the presence of the ester functionality. In the proton NMR spectra of all the compounds, the characteristic broad singlet appears between δ 12 ppm to δ 13 ppm confirming the presence of an amide proton. Compounds **2c, 2d, 5c** and **5d** showed a characteristic singlet at δ 4.87–4.74 ppm in the ^{1}H NMR spectrum corresponding to the two protons of the 3-chloromethyl group. A singlet at a frequency of 2.03–2.40 ppm corresponds to the three protons of the acetoxy group in the proton NMR spectra of the compounds **3c, 3d, 6c** and **6d**. The corresponding amide carbon signal resonated between δ 164–169 ppm in the ^{13}C NMR spectra of the newly synthesised compounds. The C-5 of the 1,3,4-thiadiazole nucleus appears downfield in the NMR spectra in comparison to C-2 of the heterocyclic scaffold. Further structural confirmation was done using mass spectrometric data and elemental analysis. The physicochemical data of the synthesised compounds is listed in the Table 1.

2.2. Pharmacology

2.2.1. In vitro acetylcholinesterase inhibition

In vitro AChE inhibition of the target compounds was carried out on the supernatant obtained from homogenized brain tissue of fresh or untreated mice as a source of enzyme for the assay. The essay was performed based on Ellman method [26]. Five different concentrations (1, 5, 10, 25 and 50 μL) of the synthesised compounds **(2c–8d)** and the standard, Rivastigmine were taken, the concentration *vs.* percentage inhibition graph was plotted and IC_{50}