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Research paper

Synthesis, pharmacological assessment, molecular modeling and *in silico* studies of fused tricyclic coumarin derivatives as a new family of multifunctional anti-Alzheimer agents

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ABSTRACT

A series of fused tricyclic coumarin derivatives bearing iminopyran ring connected to various amido moieties were developed as potential multifunctional anti-Alzheimer agents for their cholinesterase inhibitory and radical scavenging activities. In vitro studies revealed that most of these compounds exhibited high inhibitory activity on acetylcholinesterase (AChE), with IC_{50} values ranging from 0.003 to 0.357 μM which is 2–220 folds more potent than the positive control, galantamine. Their inhibition selectivity against AChE over butyrylcholinesterase (BuChE) has increased about 194 fold compared with galantamine. The developed compounds also showed potent ABTS radical scavenging activity (IC_{50} 7.98 −15.99 μM). Specifically, the most potent AChE inhibitor **6n** (IC_{50} 0.003 ± 0.0007 μM) has an excellent antioxidant profile as determined by the ABTS method (IC_{50} 7.98 ± 0.77 μM). Moreover, cell viability studies in SK N SH cells showed that the compounds **6m**–**q** have significant neuroprotective effects against H_2O_2-induced cell death, and are not neurotoxic at all concentrations except **6n** and **6q**. The kinetic analysis of compound **6n** proved that it is a mixed-type inhibitor for *Ee*AChE (K_{i1} 0.0103 μM and K_{i2} 0.0193 μM). Accordingly, the molecular modeling study demonstrated that **6m**–**q** with substituted benzyl amido moiety possessed an optimal docking pose with interactions at catalytic active site (CAS) and peripheral anionic site (PAS) of AChE simultaneously and thereby they might prevent aggregation of Aβ induced by AChE. Furthermore, *in silico* ADMET prediction studies indicated that these compounds satisfied all the characteristics of CNS acting drugs. Most active inhibitor **6n** is permeable to BBB as determined in the *in vivo* brain AChE activity. To sum up, the multipotent therapeutic profile of these novel tricyclic coumarins makes them promising leads for developing anti-Alzheimer agents.

1. Introduction

Alzheimer's disease (AD) is a deadly neurodegenerative disorder that attacks the central nervous system through progressive degeneration of its neurons [1]. It is the most common form of dementia affecting over 35 million people worldwide. AD is characterized by the loss of memory, progressive and irreversible cognitive impairments, language deterioration, severe behavioral abnormalities, and ultimately causing death [2]. Despite AD has been found for more than a century, it is still incurable as its etiology has not yet been fully explored. However, low levels of acetylcholine (ACh), oxidative stress, the inflammation of neurons and β-amyloid (Aβ) deposits are thought to play definitive roles in AD pathogenesis. Based on these factors several hypotheses which include cholinergic and noncholinergic interventions have been emerged over the past decades [3]. However, the cholinergic

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hypothesis which enhances the central cholinergic function by maintaining ACh levels via inhibition of acetylcholinesterase (AChE) is the only approved therapeutic strategy for the treatment of AD.

Recently it has been shown that AChE involves in the extraneous non-cholinergic function in the early phases of AD by binding to Aβ, thereby accelerating its polymerization into oligomers and fibrils and increasing the neurotoxicity of Aβ aggregates [4,5]. The peripheral anionic site (PAS) has been shown to play a crucial role in Aβ pro-aggregating action of AChE. It is located at the mouth of a 20 Å deep gorge and leads to the catalytic anionic site (CAS) of the enzyme. Considering these aspects, AChE inhibitors that bind to either PAS or to both the CAS and PAS may simultaneously alleviate the cognitive deficit and delay the neurodegenerative process by preventing the assembly of Aβ-peptide [6–10].

Furthermore, inhibition of butyrylcholinesterase (BuChE), an enzyme closely related to AChE has been found to be a desirable activity in design of anti-Alzheimer agents [11]. However, serious inhibition of BuChE causes peripheral side effects including gastrointestinal events, nausea, vomiting, diarrhea and dizziness as it is mainly localized in the peripheral tissues including plasma and several regions of the brain. For example, the dual AChE and BuChE inhibitor, tacrine showed severe hepatotoxicity as well as other adverse effects and so withdrawn from the use. Therefore, the development of selective AChE inhibitors to reduce side effects may be a more suitable therapeutic strategy for the treatment of AD [12].

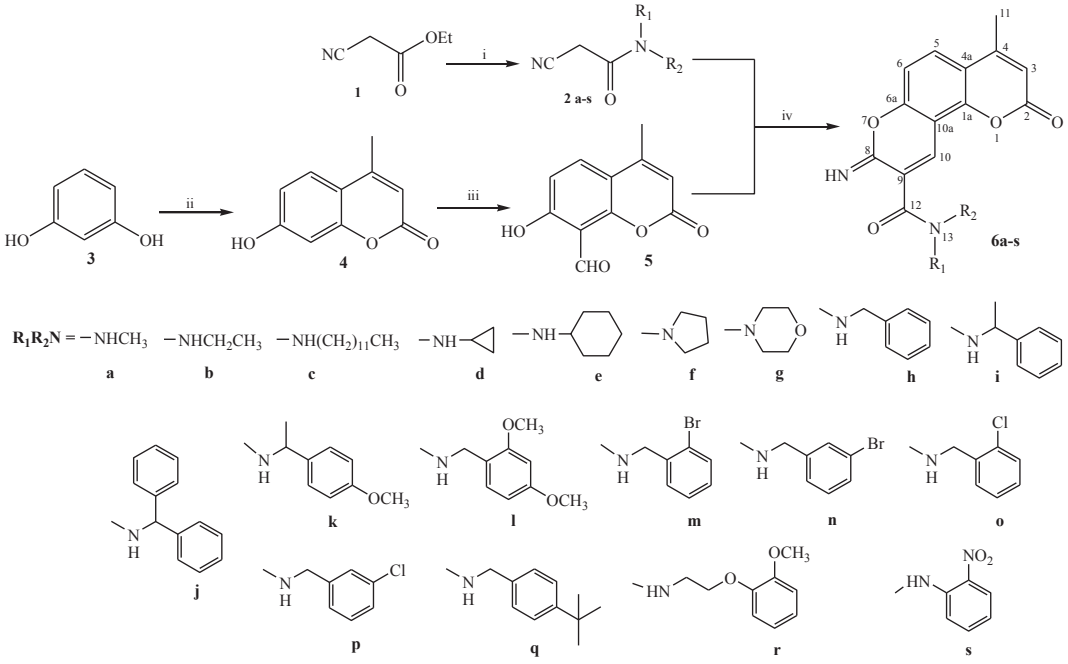
Increasing evidence supports the significant impact of oxidative stress in initiating the aggregation of Aβ and tau protein hyperphosphorylation, involved in the early stage of the pathologic cascade [13]. Oxidative cell damage marked by lipid peroxidation, nitration, reactive carbonyls, and nucleic acid oxidation is at higher level in vulnerable neurons in AD [14]. Thus, neuroprotection against oxidative stress induced cell damage in neuronal cells has become the important target for AD treatment. Therefore, the combination of selective AChEIs, antioxidants and neuroprotectives represents an additional rational approach to develop new multifunctional agents for AD treatment [15,16].

Naturally occurring and chemically synthesized coumarins have received intense attention in recent years because of their wide ranging biological activities [17]. Researchers have attempted to explore the coumarin template for developing novel AChE inhibitors with additional pharmacological activities such as decrease in Aβ deposition and β-secretase inhibition that are also important for AD management [18–21]. Coumarin derivatives have also been protecting neurons against Aβ-induced oxidative stress and free radicals [22,23]. Since coumarins primarily interact with PAS of AChE, several coumarin based dual inhibitors of AChE were designed by incorporating a catalytic site interacting moiety through an appropriate spacer [24–31]. As a result, the 3rd or 4th position of coumarin moiety has been described as the most favorable for linking CAS interacting moiety but not 6th or 7th to obtain potent dual site AChE inhibitors [18]. However, we focused our attention for the first time on angularly fused iminopyran at 7 and 8 positions of 7-hydroxy-4-methylcoumarin bearing various N-substituted amides on 9th position. Thus we report herein synthesis, biological evaluation, molecular modeling and *in silico* studies of a series of fused tricyclic coumarin amide derivatives as novel multifunctional anti-AD agents with potent and selective AChE over BuChE inhibitory and antioxidant activities.

2. Results and discussion

2.1. Chemistry

The synthetic pathway of fused tricyclic coumarins (**6a**–**s**) was outlined in Scheme 1. The target molecules consist of three parts, therefore the synthetic strategy involves the preparation of cyano acetamide derivative by reacting different amines with ethylcyanoacetate and then fused it to the formyl coumarin. At first, a series of N-substituted cyano acetamide derivatives (**2a**–**s**) were prepared by simple reaction in readily available screw cap bottle by treating amines with equivalent amount of ethylcyanoacetate [32,33]. Resorcinol (**3**) and ethylacetoacetate were treated under Pechmann conditions to give 7-hydroxy-4-methylcoumarin (**4**)



Scheme 1. Synthetic pathway of target compounds **6a-s**. *Reagents and conditions*: (i) corresponding amine, EtOH; (ii) ethylacetoacetate, H_2SO_4, 18 h; (iii) HMTA, glacial acetic acid, 90 °C, reflux, 6 h; (iv) Et_3N, EtOH.