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Synthesis and anti-acetylcholinesterase activity of scopoletin derivatives

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ABSTRACT

A series of scopoletin derivatives incorporated with the pyridinium moiety was synthesized and evaluated for their acetylcholinesterase (AChE) inhibitory activity by the colorimetric Ellman's method. A 2-fluorobenzylpyridinium derivative was the most potent among the tested compounds, with an IC_{50} value of 0.215 ± 0.015 μM, which was greatly improved from that of scopoletin. Docking studies revealed that the scopoletin portion of the mentioned compound was bound to the peripheral anionic site of the AChE, whereas the *N*-benzylpyridinium residue to the catalytic anionic site.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the brain, which most commonly leads to dementia among the elderly. Multiple cognitive impairments that are characteristics of AD include the loss of memory, judgment, and learning ability. In a recent report [1], approximately 36 million people worldwide have problems with AD; this number is estimated to reach 115 million by 2050. Major pathological features of AD are beta-amyloid (Aβ) plaque deposition and the presence of neurofibrillary tangles in the brain [2]. Most of the existing treatments can only delay the development of AD and cannot cure the disease. Among the various biochemical theories regarding the disease, the cholinergic hypothesis is the most widely accepted. In this hypothesis, the decreased cognitive and mental function is related to the loss of cortical cholinergic neurotransmission. Rationally, the inhibition of acetylcholinesterase (AChE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine (ACh), can restore and enhance cholinergic neurotransmission. To date, most of the drugs approved for treating AD are acetylcholinesterase inhibitors (AChE*i*), such as galanthamine, donepezil, rivastigmine, and tacrine (Fig. 1) [3]. However, undesirable common side effects including nausea, vomiting and weight loss were observed with these acetylcholinesterase inhibitors while tacrine was found to be hepatotoxic [4]. Therefore, the design of novel AChE*i* agents is still urgently needed for AD treatment. Based on the three-dimensional structure of the *Torpedo californica* acetylcholinesterase (TcAChE) reported in 1991 [5], two binding sites were evident, namely the active site and the peripheral anionic site (PAS). Experimental studies have shown that the PAS of AChE interacted with Aβ and thus promoted the formation of the amyloid fibrils [6]. It has been shown also that the molecules interacting either exclusively with PAS or with both the active site and PAS can prevent AChE-induced Aβ aggregation [7]. Therefore, molecules capable of binding to both PAS and the active site are potential targets for the development of anti-AD agents.

Computational studies revealed that compounds containing planar and aromatic scaffolds can interact with the PAS of AChE and inhibit the AChE-induced aggregation of the Aβ peptide [8,9]. Coumarin was one of the compounds possessing this capability [10] leading to its attractive feature to be further developed as an anti-AD agent. Thereafter, a number of studies reported the use of coumarin as a scaffold for the AChE*i* synthesis [7,11–14]. Scopoletin or 7-hydroxy-6-methoxycoumarin (**1**, Fig. 2) is a phenolic coumarin isolated from several different species, genera, and families of plants. Its medicinal properties have been recently reported [15]. Scopoletin is known to possess diverse biological properties including antimicrobial [16–18], antiviral [19], anti-inflammatory [20], and antiproliferative [21,22] effects. Over the past decade, the pharmacophore-based virtual screening approach indicated potent AChE inhibitory activity of scopoletin, although the *in vitro* tests showed a rather high IC_{50} of 168 μM against AChE [23]. In this study, we aim to improve the AChE inhibitory activity

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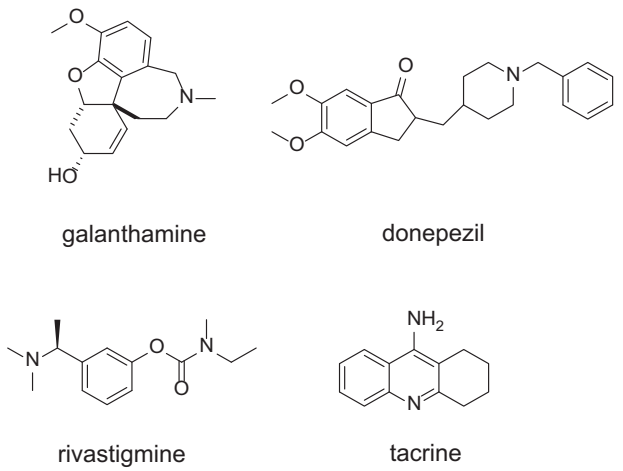


Fig. 1. Structures of acetylcholinesterase inhibitors approved as drugs for AD treatment.

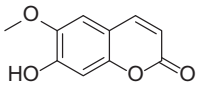


Fig. 2. Structure of scopoletin (**1**).

of scopoletin by incorporating different side chains which possess a quaternary amine or basic nitrogen. We hypothesized that, with the PAS binding capability of scopoletin and the positively charged side chain, the binding affinity of scopoletin derivatives with AChE could be improved; the resulting compound could be an effective inhibitor for this enzyme. Benzyl pyridinium-type side chains were of our interest due to their recently reported promising inhibitory activity [24–26]. Therefore, scopoletin derivatives linked with different benzyl pyridinium moiety were synthesized and their inhibitory activity against AChE was evaluated.

2. Results and discussion

2.1. Chemistry

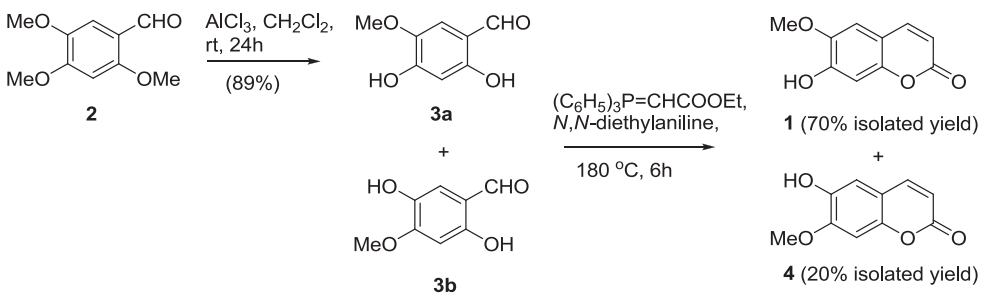
Scopoletin (**1**) was prepared from 2,4,5-trimethoxybenzaldehyde (**2**) in two steps [27] as shown in Scheme 1. Treatment of **2** with 4 equiv. of aluminum (III) chloride in dichloromethane led to the formation of an inseparable mixture of 2,4-dihydroxy-5-methoxybenzaldehyde (**3a**) [28] and 2,5-dihydroxy-4-methoxy benzaldehyde (**3b**) [29] in an approximate ratio of 5:1. The Wittig reaction of a mixture of **3a** and **3b** with ethoxycarbonylmethylene triphenylphosphorane in *N*,*N*-diethylaniline at 180 °C for 4 h provided a mixture of scopoletin (**1**) [30] and isoscopoletin (**4**) [31], which were separated by silica column chromatography in 70% and 20% yields, respectively.

The methyl pyridine moiety was attached to scopoletin by using 4-(chloromethyl)pyridine hydrochloride in the presence of cesium carbonate as a base to furnish compound **5**. Compound **5** was converted to the corresponding *N*-alkyl and *N*-benzyl pyridinium salts (**6a**–**6o**) by treating **5** with various alkyl and benzyl halides (Scheme 2).

2.2. Inhibition of AChE

The AChE inhibitory activity of all the synthesized scopoletin derivatives was evaluated according to Ellman's method [32] using *Electrophorus electricus* AChE; donepezil hydrochloride was used as the reference compound. The tested compounds with more than 50% inhibition (at concentration of 10 μM) were further evaluated for their half maximal inhibitory concentration (IC_{50}) values, and the results are shown in Table 1. Scopoletin exhibited only poor inhibitory activity against AChE (23% inhibition at 10 μM), similar to reported previously [13,23]. Introduction of the 4-pyridyl methylene moiety to the hydroxy group at C7 via ether yielded compound **5** exhibiting AChE inhibitory activity (21% inhibition at 10 μM) comparable to that of the parent scopoletin. According to the work by Nadri [24] and Alipour [25], introducing a benzyl moiety into the pyridine ring to form its pyridinium salts improved the AChE inhibitory activity. Therefore, in the present study, different alkyl and benzyl moieties were incorporated into the pyridine ring of compound **5** to form the corresponding pyridinium salts (**6a**–**6o**). The test assays showed that the pyridinium derivatives with alkyl side chains (**6a**–**6c**) exhibited two- to threefold higher activity than scopoletin. Further improved activity was obtained when an alkyl benzene was incorporated instead of the long-chain alkyl side chain. The derivatives **6d** and **6f** were approximately 7.5- to 8.5-fold more active than **6a**–**6c**; both derivatives were much more potent than the parent scopoletin. For further optimization, **6d** was selected as the hit compound.

Based on its promising IC_{50} value (1.170 ± 0.054 μM), **6d** was selected for further modifications. Docking results in literature [25,26] revealed that introducing halogen to the benzene ring at a proper position can modulate the binding properties of the corresponding compound at the active site of AChE and leads to higher activity. In the current study, chlorine and fluorine substituents were attached to the benzyl moiety of **6d** at the *ortho*-, *meta*-, and *para*-positions to yield derivatives **6h**–**6m**. For the chlorine substituent, the *ortho*- and *meta*-position substitution (**6h** and **6i**) gave comparable IC_{50} values (0.360 ± 0.024 and 0.397 ± 0.023 μM, respectively) which were approximately 15-fold more potent than that of chlorine substitution at the *para*-position. For the fluorine substituent, the activity followed the order of *ortho* > *meta* > *para*. Among these substituents (**6k**–**6m**), fluorine substitution at the *ortho*-position (**6k**) displayed the highest potency with IC_{50} value of 0.215 ± 0.015 μM.



Scheme 1. Synthesis of scopoletin (**1**).