Screening of Acetylcholinesterase Inhibitory Activity of Terpenoid and Coumarin Derivatives from the Genus *Ferula*

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Summary

Inhibition of acetylcholinesterase is currently regarded as the leading strategy against Alzheimer's disease. In the present study, we aimed to screen the *in vitro* inhibitory activity of 8 naturally occuring terpenoid and coumarin derivatives (auraptene, diversin, diversolide D, farnesiferol A, galbanic acid, tschimgine, umbelliferone and umbelliprenin) from *Ferula* species together with 2 other related compounds (herniarin and 7-isopentenyloxycoumarin) at 100 mM concentration against human erythrocyte acetycholinesterase using a modification of the Ellman method. The results showed that tschimgine was the most potent inhibitor of acetylcholinesterase (inhibition %: 63.5%) among the 10 tested compounds. However, the inhibitory activity of none of the tested compounds was comparable to that of galanthamine (inhibition %: 86.4%) which was used as the reference inhibitor. The esteric monoterpene, tschimgine, may be applied as a lead molecule for the design of anticholinesterase agents.

Key words: Acetylcholinesterase inhibition; *Ferula*; terpenoid derivatives; coumarin derivatives

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Introduction

Alzheimer's disease (AD) is a chronic, progressive and neurodegenerative disorder which is characterized by β-amyloid peptide deposition, neurofibrillary tangles accumulation, cognitive impairment and behavioral disturbances (1-3). AD is believed to be the most common cause of dementia among the elderly and is the fourth leading cause of death in western countries (4,5). Although several hypotheses have been put forward, the exact molecular etiology of this devastating disorder remains unknown. However, the cholinergic hypothesis is currently regarded as the most widely accepted theory for the pathophysiology of AD (6,7). This hypothesis is based on the reports about selective and profound loss of acetylcholine (Ach) synthesis in patients with AD (8). Hence, blocking the degradation of Ach by the use of acetylcholinesterase inhibitors ($AchEI_s$) is a rational approach that would lead to the enhancement of cholinergic neurotransmission and increased availability of Ach in the synaptic cleft (9,10). In addition to the functional improvement of the central cholinergic synapses, elevation of AchE (EC 3.1.1.7) levels would also lead to the protection against neuronal degeneration, amyloid precursor protein modification and increased synthesis of neurotropic molecules (11). Therefore, the application of $AchEI_s$ has become the leading and most promising strategy for the treatment of AD. However, in spite of the efficacy of $AchEI_s$ for the treatment of mild to moderate AD, these drugs possess side effects such as hepatotoxicity and several gastrointestinal disorders (12-14).

The genus *Ferula* (Apiaceae) comprises about 170 species occurring from central Asia westward throughout the Mediterranean region to northern Africa (15-17). The Iranian flora comprises of 30 species of *Ferula*, of which some are endemic (16,17). The plants of this genus are well documented as a good source of biologically active compounds such as sesquiterpene derivatives (18-22), and sulfur containing compounds (23,24). Several species of this genus have been used in traditional medicine as natural remedies (25), and have been reported by recent investigations for possessing diverse biological activities (26-32). There are also reports about the traditional application of some *Ferula* species for enhancing memory or treatment of neurological disorders and recently neuroprotective and anticholinesterasic activity have been reported for auraptene which is a prenylated coumarin found in the plants of this genus (25, 33-35).

Given the above findings and considering the previously reported anticholinesterasic activity of some terpenoid and coumarin derivatives (36), we sought to screen the inhibitory activity of 8 naturally occuring occuring terpenoid and coumarin derivatives of *Ferula* species (umbelliprenin, auraptene, 7-isopentenyloxycoumarin, diversin, diversolide D, farnesiferol A, galbanic acid and tschimgine) together with 2 related compounds (herniarin and umbelliferone) against AchE to provide a base for the development of natural drugs for AD.

Materials and Methods

Chemicals

5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), quinidine sulfate and galanthamine were purchased from Sigma; dimethyl sulfoxide and Hyamine® 1622 were purchased from Merck and Rohm and Haas Company, respectively.

Human erythrocyte AchE inhibition assay

Red blood cell (RBC) AchE was used as the source of AchE. Blood samples (2 mL) were mixed with normal saline (8 mL) and then centrifuged for 5 minutes at 3000 g. After three times of centrifugation and washing, distilled water (6 mL) was added to 0.1 mL of the packed RBC to obtain the hemolysate.

Inhibition of AChE was assessed by a modification of the colorimetric method of Ellman et al. (1961). Phosphate buffer (PH = 7.6, 3 mL) including DTNB (0.27 mM) and quinidine sulfate (0.02 mM), together with 100 μL of the test solution were added to 100 μL of the hemolysate. After incubation (5 minutes at 37°C), 100 μL of the ATCI solution (0.1 M) was added and then incubated again for 10 minutes at 37°C. The reaction was terminated by adding 1 mL of Hyamine® 1622 solution. The absorbance at 440 nm was measured spectrophotometrically (UV-1650PC, SHIMADZU). The inhibitory effect of test compound was calculated according to the formula: Inhibition (%) = $[OD_{DMSO} - (OD_{compound} - OD_{DMSO})]/OD_{DMSO} \times 100$.

Test compounds

Chemical structures of the test compounds are illustrated in Fig. 1. Diversin and diversolide A were isolated from the roots of *Ferula diversivittata* as previously described (37). Galbanic acid and farnesiferol A were isolated from the roots of *F. szowitsiana* and *F. persica*, respectively, as previously described (21, 38). Auraptene, umbelliprenin, 7-isopentenyloxycoumarin and herniarin were synthesized as described in our previous report (39). Umbelliferone was purchased from Merck. For the isolation of tschimgine, 500 g of the powdered roots of *F. ovina* were extracted by dichloromethane (3 L) using maceration method (36 h), yielding a residue (93 g). Part of the extract (21 g) was subjected to column chromatography on silica gel (5 × 60 cm) using petroleum ether:ethyl acetate (20:1) as an initial solvent with gradual increasing of solvent polarity up to 100% ethyl acetate. Tschimgine (1691 mg) was obtained as pure solid crystals from the column and its structure was confirmed by comparison of ^{1}H and ^{13}C NMR spectra as well as melting point value with those of a previous report (40).

Statistical analysis

Statistical analyses were performed using SPSS software (release 11.5, SPSS Inc., 2002). Normality of data was assessed by the Kolmogorov-Smirnov test. The results were expressed as mean ± SD. Group comparisons were made using Kruskal-Wallis test with Mann-Whitney U test for multiple comparisons.

Results and Discussion

The inhibitory activities of 10 naturally occuring terpenoid and coumarin derivatives against human erythrocyte AChE were studied for determining the rate of hydrolysis of acetylthiochoine in comparison with reference compound galanthamine using the modified method of Ellman et al. (41). The results are shown in Table 1 as percentages of inhibition. According to the results, tschimgine was found to be the most potent inhibitor of AchE among all tested compounds, though its inhibitory activity was not comparable to that of the reference inhibitor compound (galanthamine). On the other hand, herniarin, 7-hydroxy coumarin (umbelliferone) and auraptene showed lower inhibitory activities.

Regarding the side effects of traditional AD drugs, discovery of novel $AchEI_s$ of natural origin and with less adverse effects and larger therapeutic index is of great clinical importance for treatment of AD. Currently, a well-known drug for AD (galanthamine) and another promising compound (huperzine A) have herbal origin. Besides, new anticholinesterases are continously being discovered in various plant species.

In previous studies anticholinesterasic activity have been reported from some terpenoid and coumarin derivatives (36). For instance, ensaculin, a coumarin derivative, has been shown to exert a modest improvement in memory and cognitive function as well as positive effects against progressive neurodegeneration in patients with AD (42). In a later study (5), three series of coumarin analogues with substituted phenylpiperazine functions were synthesized using ensaculin as parent compound and tested for their anti-cholinesterase activities. The authors stated that coumarins with substitution on positions 3 and/or 4 had parallel anti-AchE activities compared with the reference compound (Donepezil). In another study by Fallarero et al. (43), a coumarin derivative named coumarin 106, was shown to possess promising inhibitory activity against AchE. Therefore, it was suggested that this compound may serve as a suitable lead molecule. In addition to the aforementioned studies, there are several other reports on the AchE inhibitory activity of coumarin derivatives which make them promising candidates for the development of new drugs against AD (4, 44-49).

In regard to the terpenoid derivatives, the most well-known compound is huperzine A which is a naturally occuring sesquiterpene alkalloid isolated from *Huperzia serrata* (50,51). This compound has been reported to be a potent and highly specific inhibitor of AchE and its efficacy has been confirmed in clinical trials (50, 52-55). While the potency of huperzine A has been found to be similar or superior to that of other AchEIs in use, its peripheral cholinergic side effects are minimal (55).

There are also reports on the inhibitory activity of other terpenoid compounds. In a previous report, main terpenoid components of the tea tree oil were found to possess AchE inhibitory activity both individually and in the mixed form. However the inhibitory activity was more prominent in the mixed form which is possibly due to their synergistic activity (56). There have been also reports on the promising AchE inhibitory activity of some bicyclic monoterpenoides including (+)- and (−)-α-pinene, (+)-3-carene and (+)-3-sabinene (57,58). Besides, in two other studies, some terpenoid constituents of *Salvia lavandulaefolia* essential oil were reported to have inhibitory activity against human and bovine AchE (59,60), but their potencies were not comparable to those of standard anti-cholinesterases such as physostigmine and tacrine (59).

In a previous report, *in vitro* and *in vivo* findings indicated that two natural components of some *Ferula* species namely Umbelliferone and ferulic acid act as competetive inhibitors of AchE. In addition, eugenol and limonene which are among volatile constituents of some *Ferula* species, were shown to inhibit AchE in a competitive–non-competitive and uncompetetive manner, respectively (61). Moreover, auraptene, which is a prenylated coumarin with diverse biological activities (synthesis) found in the Citrus and some *Ferula* species (21,22,62,63), has been shown to possess neuroprotective effect and also a mild inhibitory activity against AchE (34-36). However, in the present study we did not observe remarkable AchE inhibitory activity from auraptene or umbelliprenin.

In conclusion, we indicated that the tested terpenoid and coumarin derivatives do not possess considerable AchE inhibitory activity except tschimgine, for which a relatively potent inhibitory activity was observed. Therefore, this compound could be regarded as a base for the design of strong acetylcholinesterase inhibitors. However, phytoestrogenic properties of this compound should be considered (64).

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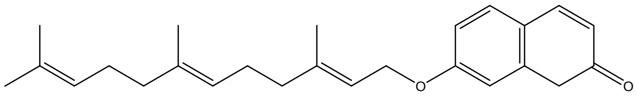
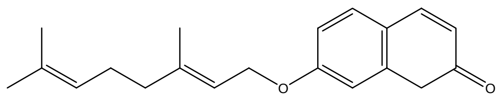
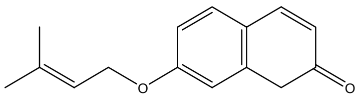


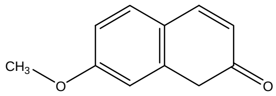
Umbelliprenin



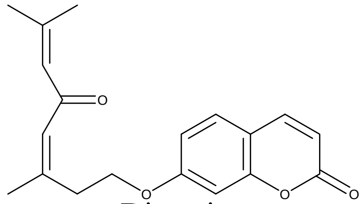
Auraptene



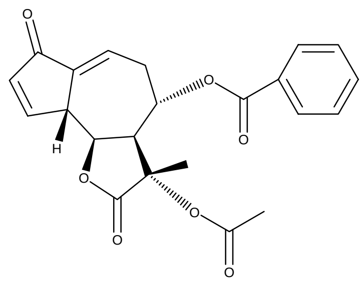
7-Isopentenyloxycoumarin



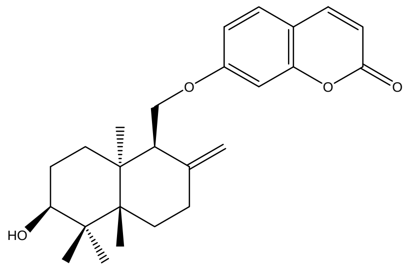
Herniarin



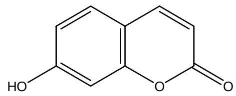
Diversin



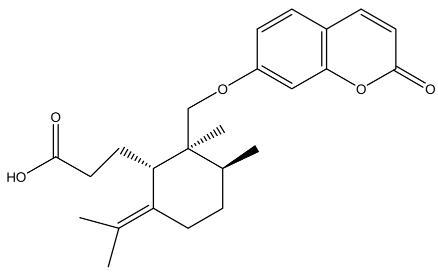
Diversolide D



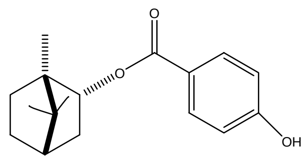
Farnesiferol A



Umbelliferone



Galbanic acid



Tschimgine

Fig. 1. Chemical structures of test compounds.

Table 1. Inhibitiory activities of tested compounds (100 μM) against human RBC AchE.

Compound	Inhibition (%)
Tschimgine	63.5 ± 8.7*†
Farnesiferol A	20.6 ± 8.2*†
Galbanic acid	19.1 ± 6.0*†
Diversolide D	19.0 ± 4.5*†
Diversin	18.4 ± 6.2*†
Umbelliprenin	17.5 ± 4.6*†
7-isopentenyloxycoumarin	11.7 ± 3.4*†
Herniarin	3.6 ± 4.5*†
Umbelliferone	3.0 ± 3.5*†
Auraptene	2.7 ± 3.6*†
Galanthamine	86.4 ± 6.1

* $p < 0.001$: comparison with galanthamine (as positive control); † $p < 0.001$: comparison with tschimgine.

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