Coumarin, Anthroquinone and Stilbene Derivatives with Anticholinesterase Activity

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are the key enzymes in pathogenesis of Alzheimer's disease (AD), which is characterized by a deficit in central cholinergic transmission. In the current study, AChE and BChE inhibitory activities of seven coumarin derivatives [umbelliferone (**1**), 4-methylumbelliferone (**2**), 4-hydroxycoumarin (**3**), scopoletin (**4**), 8-methoxypsoralen (**5**), bergapten (**6**), and *iso*-bergapten (**7**)], a furanocoumarin mixture obtained from *Heracleum crenatifolium* Boiss. (Umbelliferae), as well as of two anthroquinone derivatives [rhein (**8**) and aloe-emodine (**9**)] and one stilbene, rhapontin (**10**), were tested by the spectrophotometric method of Ellman using an ELISA microplate-reader at 1 mg mL^{-1}. Among them, the furanocoumarin mixture [(68.8 ± 0.76)%], bergapten [(62.4 ± 0.74)%], aloe-emodine [(57.2 ± 1.32)%], scopoletin [(53.1 ± 0.83)%], and 4-methylumbelliferone [(62.3 ± 1.03)%] showed over 50% inhibition against AchE, while umbelliferone [(54.3 ± 0.23)%], 4-methylumbelliferone [(80.9 ± 1.17)%], scopoletin [(73.5 ± 1.01)%], 8-methoxypsoralen [(67.1 ± 0.98)%], as well as the furanocoumarin mixture [(76.7 ± 0.95)%] had a notable anti-BChE effect.

Key words: Coumarin, Acetylcholinesterase, Butyrylcholinesterase, Alzheimer's Disease

Introduction

Alzheimer's disease (AD), the most common form of dementia and clinically characterized by progressive cognitive decline, is a major threat to the ageing population in developed countries (Scarpini *et al.*, 2003). Although the etiology of the disease is not quite clear yet, several therapeutic agents and strategies have emerged up to date including acetylcholinesterase (AChE) inhibitors, amyloid-β-peptide vaccination, secretase inhibitors, cholesterol-lowering drugs, metal chelators, and anti-inflammatory agents (Cummings, 2004; Mattson, 2004; Francis *et al.*, 2005).

Nowadays coumarins are an important group of natural compounds that are used as additives in food and cosmetics (O'Kennedy and Thornes, 1997; Borges *et al.*, 2005). These compounds, having a benzo-2-pyrone nucleus, are unsaturated aromatic lactones which derive from the phenylacrylic skeleton of cinnamic acids. Furanocoumarins are also an important class of coumarins that have an additional furan ring attached to the phenyl ring of coumarins in linear fashion. In certain plant families such as Leguminosae, Rutaceae, and Umbelliferae, coumarins exist in larger quantities. On the other hand, some *Heracleum* species, containing a bulky sum of coumarins, have been reported to be used as antiseptic, carminative, digestive and also as a flavouring agent and spice for foods (Souri *et al.*, 2004; Sonboli *et al.*, 2007).

In this study, we have screened seven coumarin derivatives, namely umbelliferone (**1**), 4-methylumbelliferone (**2**), 4-hydroxycoumarin (**3**), scopoletin (**4**), 8-methoxypsoralen (**5**), bergapten (**6**), and *iso*-bergapten (**7**), along with two anthroquinone derivatives, rhein (**8**) and aloe-emodine (**9**), and one stilbene, rhapontin (**10**) as well as a furanocoumarin mixture obtained from *Heracleum crenatifolium* Boiss. (Umbelliferae) by the spectrophotometric method of Ellman using an ELISA microplate-reader at 1 mg mL^{-1} concentration.

Materials and Methods

Test compounds

Umbelliferone (U7626), 4-methylumbelliferone (M1381), 4-hydroxycoumarin (H2253), scopoletin (S2500), 8-methoxypsoralen (M3501), rhein (R7269), rhapontin (R7753), and aloe-emodine (A7687) were purchased from Sigma Chemicals (Co., St. Louis, MO, USA). The furanocoumarin mixture was obtained from *Heracleum crenatifolium* Boiss. as described elsewhere from which bergapten and *iso*-bergapten were also isolated in pure form (Tosun and Akyüz, 2007; Tosun *et al.*, 2008).

Determination of AChE and BChE inhibitory activities

AChE and BChE (butyrylcholinesterase) inhibitory activities of the test compounds were determined by slightly modifying the spectrophotometric method developed by Ellman *et al.* (1961). Electric eel AChE (Type-VI-S, EC 3. 1.1.7, Sigma) and horse serum BChE (EC 3. 1.1.8, Sigma) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were employed as substrates of the reaction. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma) was used for the measurement of the anticholinesterase activity. All other reagents and conditions were same as described in our previous publications (Orhan *et al.*, 2004, 2007). The measurements and calculations were evaluated by using Softmax PRO 4. 3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to a blank sample (ethanol in phosphate buffer, pH 8) using the formula $[(E-S)/E] \cdot 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. All experiments were done in triplicate. Galanthamine, the anticholinesterase alkaloid-type of drug firstly isolated from the bulbs of snowdrop (*Galanthus* sp.), was purchased from Sigma (St. Louis) and was used as reference.

Statistical analysis of data

Data obtained from *in vitro* experiments were expressed as mean standard error (± SEM). Statistical differences between the treatments and the control were evaluated by ANOVA test. $P < 0.05$ was considered to be significant ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Results and Discussion

At 1 mg mL^{-1}, seven coumarin derivatives, a furanocoumarin mixture, two anthroquinones, and one stilbene derivative were screened for their *in vitro* AChE and BChE inhibitory capacities (Fig. 1). Among them, the furanocoumarin mixture [(68.8 ± 0.76)%], bergapten (**6**) [(62.4 ± 0.74)%], aloe-emodine (**9**) [(57.2 ± 1.32)%], scopoletin (**4**) [(53.1 ± 0.83)%], and 4-methylumbelliferone (**2**) [(62.3 ± 1.03)%] showed over 50% inhibition against AchE, while umbelliferone (**1**) [(54.3 ± 0.23)%], 4-methylumbelliferone (**2**) [(80.9 ± 1.17)%], scopoletin (**4**) [(73.5 ± 1.01)%], 8-methoxypsoralen (**5**) [(67.1 ± 0.98)%], as well as the furanocoumarin mixture [(76.7 ± 0.95)%] had an anti-BChE effect (Table I). While the anthroquinones had some degree of inhibition only against AChE, rhapontin (**10**), the stilbene, was completely inactive against both of the enzymes.

Various active compounds and extracts obtained from medicinal plants such as *Gingko biloba*, *Huperzia serrata*, *Galanthus nivalis*, and *Salvia officinalis* were assessed for their efficacy against AD (Mantle *et al.*, 2000). However, synergistic interactions can usually occur in a single herb due to the presence of dozens of bioactive compounds and therefore, it is very important to elucidate the active component(s). As a matter of fact, single compounds are more preferred for any disease treatment in order to explain the mechanism of the action. For this purpose, we have herein screened pure coumarin, anthroquinone, and stilbene derivatives along with a furanocoumarin mixture for their anticholinesterase effect *in vitro*.

Up to date, there have been a few reports on the anticholinesterase capacity of coumarins. Kang *et al.* (2003) previously described that the methanolic extract of *Angelica gigas* roots (Umbelliferae) displayed a significant anti-AChE activity, which further led to the isolation of twelve coumarin derivatives. Among them, decursin, a major coumarin-type compound present in the extract, was evaluated for its antiamnesic activity in mice by passive avoidance and water maze tests. Moreover, decursin inhibited the AChE activity in hippocampus of the mouse brain at 1 mg mL^{-1} (34%, $P < 0.05$). Relevantly, in a very recent study, another furanocoumarin derivative called "nodakenin", isolated also from *A. gigas* of Korean origin, was examined for its effect on learning and memory impairment induced by scopolamine (Kim *et al.*, 2007). Nodakenin was observed to reverse significantly scopolamine-induced cognitive impairment in passive avoidance and Y-maze tests. Besides, this compound was found to inhibit the AChE activity in a dose-dependent manner and to show this inhibition for 6 h in an *ex vivo* study. In the same study, decursinol, a coumarin-type compound from *A. gigas* roots, was also shown to have a higher anti-AChE effect than decursin and nodakenin. However, decursinol did not exert antiamnesic activity *in vivo*.

In another study using a structure-based pharmacophore model, scopoletin and scopolin, two coumarins isolated from *Scopolia carniolica*, were investigated for their AChE inhibitory activity, at

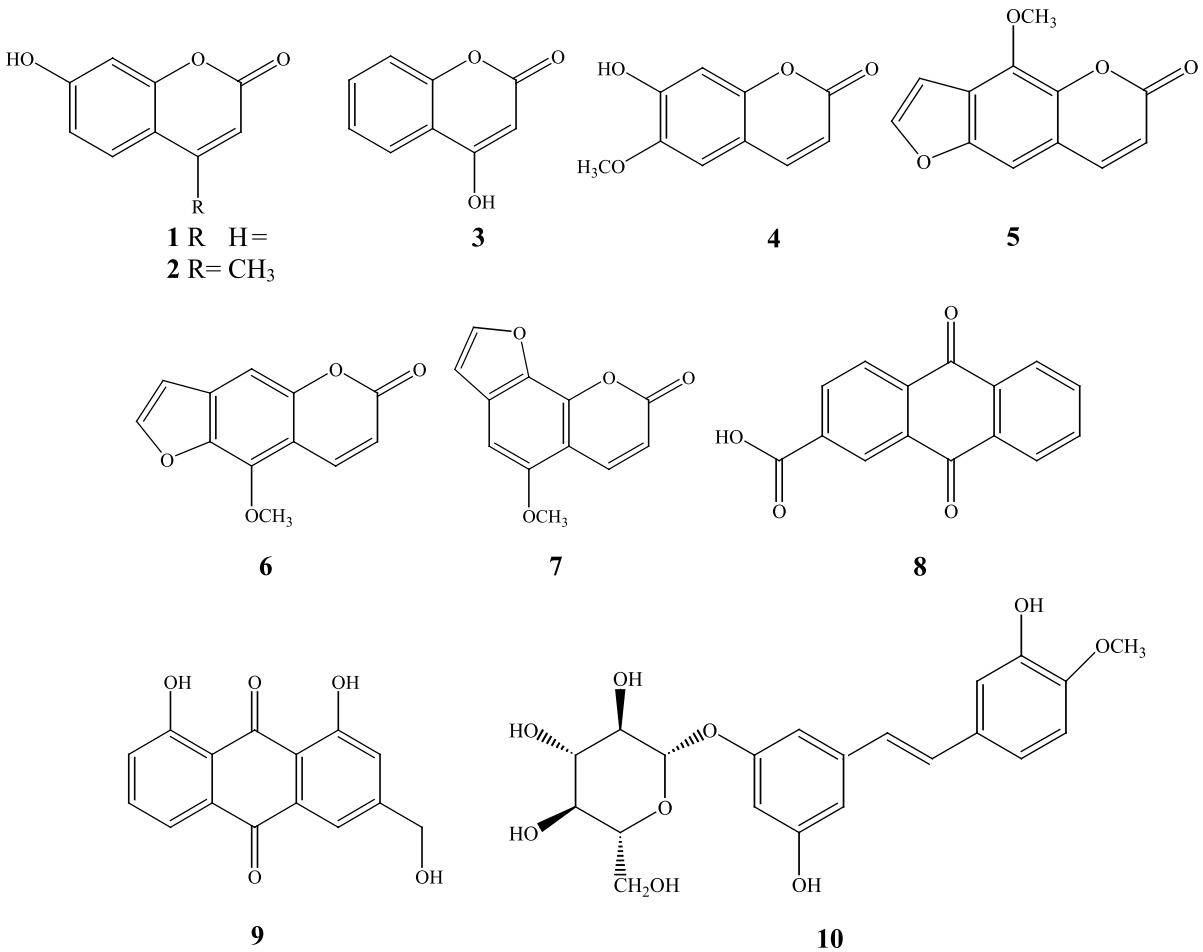

Fig. 1. Chemical structures of the coumarin, anthroquinone, and stilbene derivatives.

Table I. AChE and BChE inhibitory activities of the coumarin, anthroquinone, and stilbene derivatives (at 1 mg ml^{-1}).

Compound	Inhibition (%)	
	AChE	BChE
Umbelliferone (**1**)	11.3 ± 0.55	54.3 ± 0.023***
4-Methylumbelliferone (**2**)	62.3 ± 1.03***	80.9 ± 1.17***
4-Hydroxycoumarin (**3**)	–[a]	–
Scopoletin (**4**)	53.1 ± 0.83***	73.5 ± 1.01***
8-Methoxypsoralen (**5**)	33.5 ± 0.61	67.1 ± 0.98***
Bergapten (**6**)	62.4 ± 0.74***	29.1 ± 0.97
iso-Bergapten (**7**)	28.4 ± 0.48	12.7 ± 0.38
Furanocoumarin mixture	68.8 ± 0.76***	76.7 ± 0.95***
Rhein (**8**)	18.1 ± 0.24	–
Aloe-emodine (**9**)	57.2 ± 1.32***	–
Rhapontin (**10**)	–	–
Galanthamine	99.8 ± 0.31	80.3 ± 1.14

[a] No inhibition.
Values are expressed as mean ± SEM ($n = 3$), $P > 0.05$; *$P < 0.05$; ** $P < 0.01$; ***$P < 0001$.

first evaluated by a bioautographic thin-layer chromatography (TLC) test, which was consistent with our data (Rollinger *et al.*, 2004). These compounds were revealed to have moderate, but remarkable, dose-dependent and long-lasting inhibitory activities. Besides, they were shown in *in vivo* experiments to increase the extracellular acetylcholine concentration in rat brain to approx. 170% and 300% compared to basal release, respectively. The authors also suggested after their *in vitro* and *in vivo* results that at least one path of their ability to alleviate the extracellular concentration of acetylcholine in the nucleus accumbens may occur *via* AChE inhibition as shown in their pharmacophore model. Moreover, scopoletin was previously shown to cause a monoamine oxidase (MAO) inhibition, which is suggested as another strategy of AD treatment (Yun *et al.*, 2001). Additionally, dual inhibition of MAO and AChE enzymes by 3,4,7-substituted coumarin derivatives was formerly reported (Bruehlmann *et al.*, 2001).

On the other hand, the dichloromethane extract of *Peucedanum ostruthium* roots exhibited a significant inhibition of AChE (Urbain *et al.*, 2005). Through bioactivity-guided fractionation, four coumarin derivatives, ostruthin, imperatorin, ostruthol, and oxypeucedanin hydrate, were identified as the active components.

There has been one report on the AChE inhibitory effect of stilbene derivatives in which (+)-α-viniferin (IC_{50} = 2.0 μM) and kobophenol (IC_{50} = 115.8 μM) were investigated and found to be active (Sung *et al.*, 2002). (+)-α-Viniferin, a stilbene trimer isolated from the methanolic extract of the underground parts of *Caragana chamlague*, was found to be specific, reversible, and noncompetitive. Moreover, the authors also examined the anti-AChE activity of resveratrol and rhapontin, two stilbene monomers, whose AChE inhibition activity was not significant. Consistently, rhapontin showed no inhibition of AChE and BChE enzymes in our experiment as well.

The furanocoumarin mixture consisting of four major furanocoumarin derivatives, pimpinellin, *iso*-pimpinellin, bergapten, and *iso*-bergapten, possessed the highest anticholinesterase effect herein. Due to the scarce amount of pimpinellin and *iso*-pimpinellin, we were not able to test these compounds for their anticholinesterase activity. However, it seems that bergapten could be principally responsible for the notable anti-AChE activity of the furanocoumarin mixture, whereas *iso*-bergapten was not active against both of the enzymes. Doubtlessly, pimpinellin and *iso*-pimpinellin should also be tested in the same manner to reach the final conclusion about elucidation of the active components of this mixture from *H. crenatifolium*. To the best of our knowledge, this is the first report on BChE inhibitory activities of coumarins, stilbenes and anticholinesterase activity of anthroquinones.

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