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Novel tacrine–ebselen hybrids with improved cholinesterase inhibitory, hydrogen peroxide and peroxynitrite scavenging activity

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ABSTRACT

A series of tacrine–ebselen hybrids were synthesised and evaluated as possible multifunctional anti-Alzheimer's disease (AD) agents. Compound **6i**, which is tacrine linked with 5,6-dimethoxybenzo[*d*][1,2]selenazol-3(2*H*)-one by a six-carbon spacer, was the most potent acetylcholinesterase (AChE) and butylcholinesterase (BuChE) inhibitor, with IC_{50} values of 2.55 and 2.80 nM, respectively. Furthermore, this compound demonstrated similar hydrogen peroxide and peroxynitrite scavenging activity as ebselen by horseradish peroxidase assay and peroxynitrite scavenging activity assay, indicating that this hybrid is a good multifunctional drug candidate for the treatment of AD.

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, causing progressive dementia amongst the ageing population.[1] It is characterised by irreversible neuronal damage that causes memory loss, impaired cognitive functions, loss of speech and behavioural abnormalities. The main features of AD include low levels of acetylcholine, amyloid plaques composed of β-amyloid (Aβ), neuro-fibrillary tangles, τ-protein aggregation,[2] oxidative stress and reactive oxygen species (ROS) formation.[3]

Because the pathogenesis of AD is complicated and is related to the abnormality and dysfunction of multiple systems, no ideal drug has been found for preventing and treating this disease. Currently, the most efficacious treatment approach for AD is increasing cholinergic neurotransmission in the brain by inhibiting cholinesterases (ChEs).[4] Clinically, the acetylcholinesterase inhibitors (AChEIs), such as tacrine, donepezil, rivastigmine and galantamine, have been approved by the FDA. (In addition, memantine, an uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, also has been approved for treatment of moderately to severe AD in 2003[5,6]). However, these drugs show modest improvements in memory and cognitive function but do not appear to prevent or slow the progressive neuro-degeneration.[7] Moreover, tacrine was withdrawn from the pharmaceutical market shortly after its approval due to hepatotoxicity issues.[8] Therefore, a more appropriate approach to addressing the multifaceted nature of AD may be the development of multi-target directed ligands (MTDLs).[9,10]

The brain of a man, which accounts for 2% of the body mass yet consumes 20% of the total oxygen in a resting individual, is the most aerobically active organ in the body because of its high metabolic requirements.[11–13] As a consequence of the high oxygen demand, the brain inevitably induces the generation of large amounts of ROS, which are considered to be important causative agents in inducing oxidative damage in cellular structures, which precedes the appearance of the other pathological hallmarks of AD.[14] Moreover, β-secretase expression is increased by oxidative stress,[15,16] which could explain why hypoxia up-regulates β-secretase because hypoxia leads to local oxidative stress due to metabolic inefficiency.

ROS include superoxide ($O_2^{\cdot -}$), dihydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$).[17,18] Another notable class of reactive oxidants are the reactive nitrogen species (RNS), including nitric oxide radical ($^{\cdot}NO$) and peroxynitrite ($ONOO-$), which can impair protein functioning and stability by causing the nitration of tyrosine residues.[19] The nitration of proteins results in the inactivation of several important mammalian proteins, such as Mn superoxide dismutase (SOD) and Cu/Zn SOD, which play an important role in the clearance of ROS. Furthermore, it is believed that a deficiency of SOD-2 has been shown to accelerate plaque deposition[20] and increase tau phosphorylation in AD mice models,[21] and overexpression of Mn-SOD can protect against β-amyloid-induced neuronal death and improve mitochondrial respiratory function.[22] Thus, drugs that specifically scavenge ROS and/or RNS could be useful for either the prevention or the treatment of AD.[23,24]

Tacrine, the first inhibitor of both AChE and butylcholinesterase (BuChE) approved by the FDA for the treatment of AD, suffers from therapy-limiting side effects, such as liver toxicity.[8] This side effect

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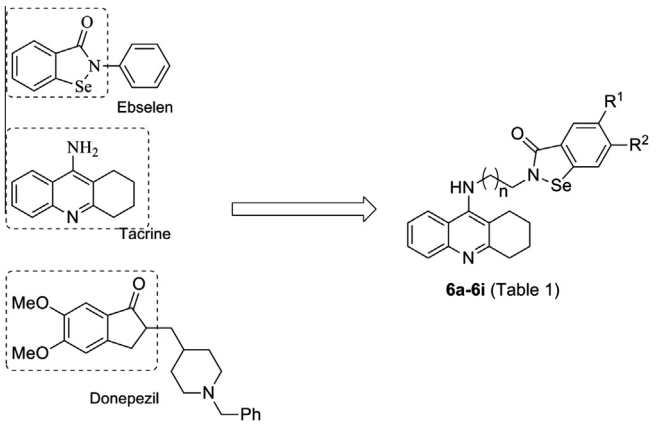


Figure 1. Synthesis strategy for compounds **6a–6i** (Table 1).

can be prevented by a free radical scavenger, such as anethole dithiolethione glutathione or vitamin E.[25,26] Thus, tacrine derivatives endowed with additional antioxidant properties might be beneficial for reducing toxicity.[27] Recently, this strategy has led to the discovery of several anti-AD drug candidates, for example, tacrine–ferulic acid hybrids,[28] tacrine–melatonin hybrids,[29] tacrine-8-hydroxyquinoline hybrids,[27] and tacrine-4-oxo-4*H*-chromene hybrids.[30]

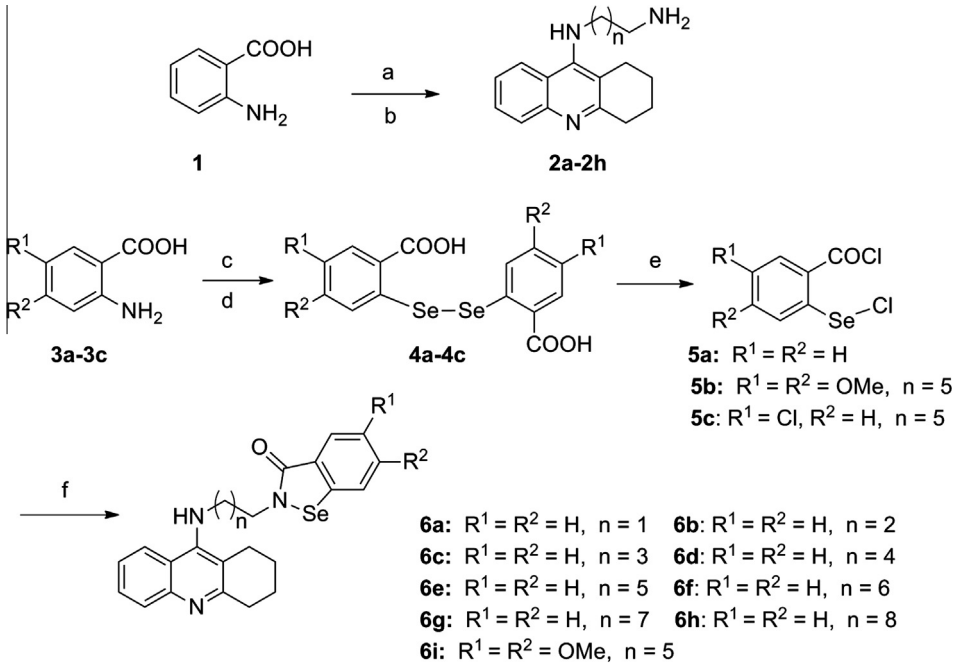
Selenium, an essential antioxidant nutrient for humans and other higher animal species, is abundant in fish and is an ingredient of glutathione peroxidase, which is an important antioxidant enzyme that converts H_2O_2 to water via GSH. Recently, a study showed that feeding a selenium-deficient diet to Tg2576 transgenic mice resulted in more than a twofold increase in the total area of the Aβ plaques compared to that of mice with a selenium-adequate diet.[31] Thus, antioxidative selenoproteins and/or selenium-containing species are one of the factors that may affect the risk of cognitive decline.

Ebselen, a organoselenium found in 1996, is known as a classic glutathione peroxidase mimic,[32,33] reacts in a rapid reaction with peroxynitrite,[34] possesses antioxidant activity and anti-inflammatory properties[35] and exhibits neuroprotective effects in several preclinical studies.[36–38]

In our previous work, we designed a series of hybrid molecules by reacting *N*-(aminoalkyl)tacrine with salicylic aldehyde or derivatives of 2-aminobenzaldehyde, which was exhibited multifunctional anti-Alzheimer's disease activities.[39] In this work, we disclose the synthesis and evaluation of a series of tacrine–ebselen hybrids (**6a**–**6i**) by connecting tacrine and ebselen (it also takes origin from the structures of donepezil–which is thought to be the most effective drug for AD at present.[40,41]) as an inhibitor of AChE and BuChE and a scavenger of hydrogen peroxide and peroxynitrite (Fig. 1).

The synthesis of tacrine–ebselen hybrids and their analogues (**6a**–**6i**) is shown in Scheme 1. Intermediate **2** was synthesised according to the reported procedure.[42,43] Simultaneously, disodium diselenide (Na_2Se_2) reacted with the benzenediazonium salt, which was prepared from 2-aminobenzoic acid by a diazotisation reaction, to give 2,2′-diselenobisbenzoic acid, that is compound **4**. The subsequent reaction of compound **4** with thionyl chloride under reflux yielded intermediate **5**, which was then reacted with amine intermediate **2** at room temperature to provide target compound **6** in moderate yields (45–68%). The purities of all synthesised compounds were determined by HPLC. It should be noted that some of the hybrids do not show the chemical shift value for the secondary amine NH as exchange with $CDCl_3$.

The biological profiles of tacrine–ebselen hybrids (**6a**–**6i**) as inhibitors of ChEs were evaluated in vitro using AChE from the electric eel and BuChE from equine serum following the methods of Ellman et al.[44] with tacrine and bis(7)-tacrine as the references compound. As shown in Table 1, all nine compounds proved to be potent inhibitors of AChE and BuChE (with IC_{50} values ranging from 2.55 to 657 nM for AChE and from 2.80 to 38.88 nM for BuChE). The results also revealed that the length of the alkyl linkage affects the inhibition of both AChE and BuChE. In addition to



Scheme 1. Synthesis of tacrine–ebselen hybrids. Reagents and conditions: (a) cyclohexanone, $POCl_3$, 0 °C → reflux; (b) diamine, KI, 1-pentanol, 160 °C; (c) HCl, H_2O, $NaNO_2$; (d) Na_2Se_2; (e) $SOCl_2$, DMF_{cat}, reflux; (f) **2a–2h** $(Et)_3N$, CH_2Cl_2, rt.