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Design, synthesis and evaluation of rivastigmine and curcumin hybrids as site-activated multitarget-directed ligands for Alzheimer's disease therapy

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ABSTRACT

A series of novel 2-methoxy-phenyl dimethyl-carbamate derivatives were designed, synthesized and evaluated as site-activated MTDLs based on rivastigmine and curcumin. Most of them exhibited good to excellent AChE and BuChE inhibitory activities with sub-micromolar IC_{50} values. Among all the compounds, **6a** demonstrated the most potent AChE inhibition with IC_{50} value of 0.097 μM, which is about 20-fold than that of rivastigmine. In addition, the three selected compounds **5a**, **6a** and **6e** demonstrated inhibitory activity against Aβ self-aggregation similar to cucurmin in TEM assay, which is obviously different from the weak activity of rivastigmine. Moreover, the hydrolysate of **6a** (compound **7**) also showed potent $ABTS^{\cdot+}$ scavenging and moderate copper ion chelating activity in vitro.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder characterized by progressive cognitive decline and memory loss. The current symptomatic treatment of AD is the four acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, galantamine and huperzine A) and one *N*-methyl-D-aspartate receptor antagonist memantine, which have resulted in a modest improvement in memory and cognitive function for AD patients, while unable to prevent progressive neurodegeneration.[1–7]

Although the exact etiology of AD is not fully known yet, it is now widely accepted that AD is a multifactorial neurodegenerative disorder.[8] The amyloid hypothesis states that the aggregation of β-amyloid (Aβ) in the brain is the leading factor in the pathogenesis of Alzheimer's disease, which has been supported by abundant genetic and pathological evidence. Compounds that are intended to reduce Aβ production, inhibit Aβ aggregation, and promote Aβ clearance are currently in clinical trials.[9] In addition, there is compelling evidence demonstrated that oxidative stress played an important role in the processes of AD pathogenesis, cellular changes show that it precedes the appearance of two hallmark pathologies of the disease, neurofibrillary tangles and senile plaques.[10] Therefore, compounds with radical scavenging activities (such as melatonin and vitamin E) were supposed to be useful for either the prevention or the treatment of AD.[11,12] Recently, elevated concentrations of copper, zinc and iron have been detected in amyloid plaques, and in vitro experiments have proved that copper and zinc metals are able to bind to Aβ and promoting its aggregation.[13,14] Moreover, evidence showed that redox-active Cu and Fe contribute to the production of reactive oxygen species (ROS) and oxidative stress.[15] Therefore, modulation of these biometals in the brain may exert potential therapeutic effect on AD, and several metal chelators such as desferrioxamine,[16] D-penicillamine,[17] clioquinol (PBT1) and PBT2[18,19] have been used for the treatment of AD in preclinical or clinical trials, while the poor target specificity and brain barrier permeability limited their widespread use in clinic.

To combat the complex profile of Alzheimer's disease, more attention has been focus on the development of novel multitarget-directed ligands (MTDLs), which interacts with multiple targets in the complex neurotoxic cascades would achieve better efficacy by a complementary manner.[20–22] Many MTDLs with a variety of scaffolds have been developed in the past few years, including AChE/BuChE and Aβ aggregation inhibitor memoquin,[23] AChE inhibitor and VDCC antagonist ITH4012,[24] iron chelating agent and MAO-B inhibitor M30.[25] Especially, an AChE and MAO-B dual inhibitor ladostigil[26] designed by Youdim had been advanced into phase II clinical trial in 2011, which confirmed the rationality and feasibility of MTDLs strategy in the treatment of AD. In continuation of our research on the design and synthesis of indole and quinoxaline

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derivatives as MTDLs for AD therapy,[27–29] we explored a new series of 2-methoxy-phenyl dimethyl-carbamate derivatives as site-activated multitarget-directed compounds.

We start our novel MTDLs from AChE/BuChE inhibitor rivastigmine and natural neuro-protective agent curcumin. Rivastigmine was the only one carbamate AChE inhibitor approved by FDA for the treatment of mild to moderately severe AD patients. It demonstrated unique central selective AChE and BuChE inhibitory activity and free of hepatic metabolism. Moreover, recent clinical trials also demonstrated that patients treated with rivastigmine showed minor cortical atrophic changes and attenuated loss of brain volume, which confirmed the hypothesis that inhibition of both enzymes may have neuroprotective and disease-modifying effects.[30,31] The crystal structure of AChE–rivastigmine complex reveals that the carbamyl moiety is covalently linked to the active-site serine and the ethylmethylamino moiety stays at the 'anionic' site.[32]

Curcumin is a famous natural compound with a variety of neuro-protective functions, including anti-inflammatory, antioxidant, metal chelating and anti-Aβ-aggregation activities,[33] and it has been advanced into clinical trial for the treatment of AD.[34] The potent antioxidant activity of curcumin is mainly due to its' *ortho*-methoxy phenol functionality, which can form an intramolecular hydrogen bond and making the H-atom of phenol abstraction surprisingly easy. Moreover, the ortho-methoxy phenol moiety also contributes to Aβ-aggregation inhibitory and metal chelating activity.[35,36]

Therefore, we incorporated the *ortho*-methoxy phenol moiety from curcumin into the structure of rivastigmine to get novel MDTLs. With the change of the position (*ortho*, *para* or *meta*) and the sort of aminoalkyl group on benzene ring, a new series of 2-methoxy-phenyl dimethyl-carbamate derivatives (**4**, **5a**–**h** and **6a**–**h**) were designed and synthesized. These novel compounds are supposed to exhibit selective AChE/BuChE inhibitory activities in the brain and site-activated by the enzymes to produce ortho-methoxy phenol, which will exert radical scavenging and metal chelating activities in vivo (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthetic route to compounds **4**, **5a**–**5h**, and **6a**–**6h** is outlined in Scheme 1. *o*-Vanillin, isovanillin and vanillin were carbamoylated by *N*,*N*-dimethyl-carbamoyl chloride in the presence of sodium hydride to give **2a**–**2c**, followed by reduction with $NaBH_4$ to get **3a**–**3c** or reacting with methyl-magnesium iodide to yield **3d**–**3e**. Then, compounds **3a**–**3e** were chlorinated by $SOCl_2$ and followed by condensation with different secondary amine in refluxing acetonitrile to yield target compounds. The hydrolysate of **6a** (compound **7**) was synthesized by reductive amination of vanillin with piperidine and $NaBH_4$ in methanol.

2.2. Biological activities and SAR

2.2.1. AChE and BuChE inhibitory activity

All synthesized compounds were evaluated for their AChE and BuChE inhibitory activities according to modified Ellman method using rat cortex homogenate (AChE) and rat serum (BuChE). Donepezil and rivastigmine were used as the reference standard and results are summarized in Table 1.

As shown in Table 1, most of these compounds demonstrated good to excellent AChE inhibitory activities and seven of them exhibited submicromolar IC_{50} values. Compound **6a** was the most potent AChE inhibitor with IC_{50} value of 0.097 μM, which is about 20-fold more potent than that of rivastigmine (IC_{50} = 2.07 μM).

The variation of aminoalkyl group position in the phenyl ring affected the AChE inhibitory activities obviously, ortho-aminoalkyl substituted compound **4** only showed weak AChE inhibitory activity with IC_{50} value of 22.7 μM. Meta-position substituted compounds (**5a**–**5g**) demonstrated moderate AChE inhibition with micromolar IC_{50} values, which is similar to that of rivastigmine. Para-position substituted compounds demonstrated the most potent AChE inhibitory activities, for example, **6a**, **6d and 6e** exhibited submicromolar IC_{50} values of 0.13, 0.11 and 0.11 μM, respectively. The sort of aminoalkyl group also affected the activity of AChE inhibition, compounds containing piperidine, pyrrolidine and diethylamine group (i.e., **5a**, **5g**, **6d**, **6g**) showed much higher activity than those with morpholine (i.e., **5b**, **5f**, **6b**, **6f**), which indicated that a lipophilic fragment was not conducive in this position. The change of R_3 from H (i.e., **5a**, **5b**, **6c**, **6d**) to CH_3 (i.e., **5e**, **5f**, **6g**, **6h**) did not affect their AChE inhibitory activities obviously.

In addition, except for the moderate inhibitory activity of compound **4** on BuChE (IC_{50} = 3.13 μM), all the other 16 compounds exert good to excellent activities with IC_{50} values ranging from 0.008 μM to 0.43 μM. Especially, compound **5a** and **6e** were the two most potent BuChE inhibitors with IC_{50} values of 0.010 and 0.008 μM, respectively, which are about 40–50 folds more potent than rivastigmine (IC_{50} = 0.37 μM). All these results suggest that the newly synthesized compounds are both AChE and BuChE inhibitors, which may be valuable to prevent hydrolysis of acetylcholine by BuChE as AD progresses.

2.2.2. Molecular docking

To gain insight into the molecular determinants that modulate the AChE inhibitory activities of these compounds, molecular docking study of **6a** with TcAChE (PDB code: 1GQR) were performed using the FlexiDock program in Sybyl 6.9 software.[32]

The Figure 2 demonstrated that **6a** has a nice fit in the active-site gorge of AChE. The *N*,*N*-dimethyl carbamate motif is directed to the catalytic active site and formed a hydrogen bond with Ser200 in a distance of 2.85 Å. The charged nitrogen of piperidine makes a cation–π interaction with Trp84 in a distance of 3.52 Å.

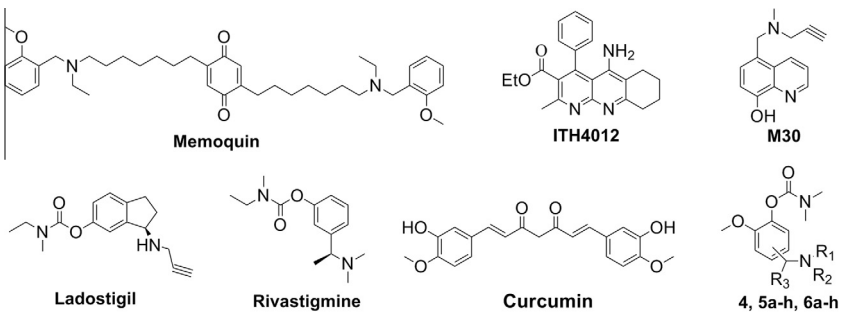

Figure 1. Structures of selected MDTLs, lead compounds and designed compounds.