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Design, synthesis and evaluation of isaindigotone derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors

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ABSTRACT

A series of isaindigotone derivatives **5a–d** and **6a–d** were designed, synthesized and evaluated as acetylcholinesterase and butyrylcholinesterase inhibitors. Results showed that the novel class of isaindigotone derivatives could inhibit both cholinesterases and the selectivity of AChE over BuChE inhibition was related to the aromatic, the species and length of the alkyl amino side chain of compounds. The structure–activity relationships were discussed and their multiple binding modes were further clarified in the molecular docking studies.

Alzheimer's disease (AD) is a progressive and degenerative neurological disorder characterized by loss of cognition and memory. Observation of a deficiency in cholinergic neurotransmission in AD led to the statement of cholinergic hypothesis.[1] Based on the cholinergic hypothesis, the mainstays of current pharmacotherapy are drugs aimed at increasing the levels of acetylcholine (ACh) through inhibition of the cholinesterases (ChEs).

Two types of ChE enzyme are found in the central nervous system – acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Both enzymes are able to hydrolyse acetylcholine (ACh), but AChE has a 10^{13}-fold higher hydrolytic ACh activity than BuChE at the same temperature and pH.[2] In the normal brain, AChE predominates over BuChE activity.[3] Moreover, it is reported that AChE could play a key role in accelerating senile β-amyloid peptide (Aβ) plaque deposition which appears to be toxic to neurons.[4] Therefore, five FDA-approved drugs are used for treatment of AD, four of which are acetylcholinesterase inhibitors.

However, some evidences suggest that inhibition of brain BuChE may represent an important therapeutic target for AD. It is reported that the BuChE has a key role that can partly compensate for the action of AChE.[5] Meanwhile, it is noteworthy that AChE activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10–15% of normal values, whereas BuChE levels are unchanged or even rise with disease progression.[6] The ratio of BuChE to AChE changes dramatically in cortical regions affected by AD from 0.2 up to as much as 11.[3] Furthermore, BuChE may also have a role in the aggregation of Aβ besides the AChE.[7] Therefore, a good balance between AChE and BuChE inhibition profiles may result in higher effect, reflected by the fact that drug rivastigmine, which inhibits both enzymes, represents improvement of cognition, activities of daily living and global function in mild to moderate AD patients.[8]

The design of AChE and BuChE inhibitors is required to clarify the structural characteristics and functions of the enzymes. X-ray crystallography of the AChE/inhibitor complexes indicated that the active site of AChE, containing a catalytic triad and a binding site of the quaternary amino group at the bottom of a deep narrow gorge. Furthermore, an aromatic midgorge recognition site and a peripheral anionic site (PAS) at the lip of the gorge were also discovered. AChE and BuChE share 65% amino acid sequence homology at the molecular level. Structure of BuChE was similar to that of AChE with the active site, a midgorge interaction site and PAS.[8–10] Most differences between BuChE and AChE were confined to the acyl-binding pocket, where amino acid residues Phe288 and Phe290 in AChE were replaced by Leu286 and Val288 of BuChE.[9] These changes made it possible for the binding of the bulkier butyrate substrate moiety in BuChE. On the other hand, three aromatic residues of the AChE PAS are missing in the PAS of BuChE.[8] Resulting in the PAS of BuChE had weaker affinity than AChE for typical PAS ligands and mediates substrate activation. Based on these observations, multiple binding with the active site, midgorge recognition site and the PAS had been considered as an important rule in designing powerful and selective ChE inhibitors, such as the typical AChE inhibitor E2020 (donepezil, Fig. 1a).[11]

In the search of nature products as ChE inhibitors, we found the alkaloid isaindigotone (Fig. 1b) was a good leading compound for

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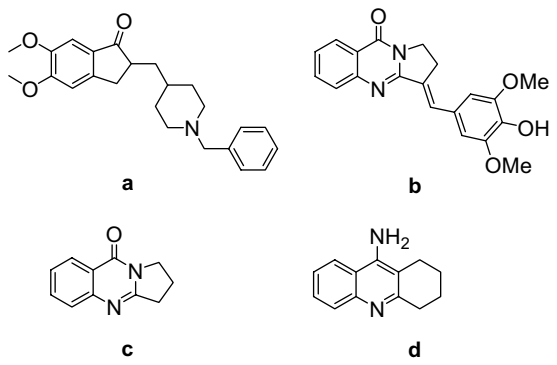

Figure 1. Chemical structure of E2020 (a), isaindigotone (b), deoxyvasicinone (c) and tacrine (d).

chemical modification. Isaindigotone was isolated from the root of *Isatis indigotica Fort*, which is a biennial herbaceous plant widely present in China.[12] Its chemical structure consisted of the deoxyvasicinone (Fig. 1c) moiety conjugated with a substituted benzylidene. Chemical structure of deoxyvasicinone showed some resemblance to the classical AD drug – tacrine (Fig. 1d) and exhibited a selective inhibitory activity towards BuChE with IC_{50} of 25.1 μM against 82.5 μM for AChE.[13] Further investigations of its analogues also showed a wide range of ChE inhibition and selectivity to the BuChE and have potential of π–π stacking with PAS.[13,14] Moreover, we speculated that the benzylidene moiety in isaindigotone was capable of π–π stacking with the aromatic residue of ChE at midgorge site.

In view of the above structural characteristics of enzymes and isaindigotone, a series of isaindigotone derivatives were designed and synthesized as AChE and BuChE inhibitors by introduction of terminal amine side chains. These side chains might be protonated at physiological pH, thus they could occupy the anionic binding site of quaternary amino group via cation–π interaction, and further promote the multiple binding interactions of deoxyvasicinone with PAS and benzylidene moieties with midgorge recognition site for both enzymes. As a result, controlling the steric and aromatic properties of the derivatives via changing the species and length of the amine side chain or reducing the ethylene in the conjugated system was important. The aim was to investigate whether these modifications would influence the AChE and BuChE inhibitions or the balance between them. Finally, several compounds were chosen for the further molecular docking studies.

The synthetic steps of the target compounds were shown in Figure 2. Deoxyvasicinone **1** was prepared by the condensation of 2-pyrrolidone and methyl anthranilate in the presence of phosphorous oxychloride. Treatment of **1** with 4-hydroxybenzaldehyde gave compound **2** by the Claisen–Schmidt condensation.[15] Compound **2** was assigned as *E* configuration on the basis of the Nuclear Overhauser Enhancement Spectroscopy (NOESY). On irradiation of the proton H-7′, only enhancement of the equivalent protons H-6′ and H-2′ was observed. Moreover, on irradiation of the equivalent protons H-6′ and H-2′, signal of protons H-7′ and H-2 was enhanced, respectively. Reaction of **2** with 1,2-dibromoethane or 1-bromo-3-chloropropane led to compounds **3a**–**b** and further reduction of **3a**–**b** in H_2/Pd gave compounds **4a**–**b**. The target compounds **5a**–**d** and **6a**–**d** were then prepared by substitution of **3a**–**b** and **4a**–**b** with appropriate secondary amines.

The IC_{50} values for AChE and BuChE inhibitions are summarized in Table 1. Due to the poor solubility, isaindigotone, compounds **2**, **3a**–b and **4a**–**b** were not measured. Generally, most of the synthetic compounds showed inhibition selectivity for AChE over BuChE. Higher inhibitory effects on AChE and BuChE were found in compounds **5a**–**d** with native chromophore than **6a**–**d** with the hydrogenation of a double bond. Compound **5c**, the most potent for AChE inhibition, presented an IC_{50} value of 0.16 μM, while **5d**, the most potent for BuChE, presented an IC_{50} value of 1.66 μM. Moreover, higher inhibitory potency was found to be associated with piperidine at the end of side chain, while diethylamine derivatives showed less potency. In addition, elongation of side chain significantly decreased AChE inhibition but increased the inhibitory potency of BuChE. On the other hand, control of the aromatic and steric properties via, respectively, reducing the ethylene in the conjugated system and changing the space and length of side chain could modulate the balance between AChE and BuChE inhibition.

First, inhibitory activity of AChE was more susceptible to the reduction of 3, 7′ carbon–carbon double bond. Compound **6d** led to a 7.8 time drop of AChE inhibition and presented an IC_{50} value of 5.58 μM compared with the IC_{50} value of 0.72 μM of **5d**. Nevertheless, its BuChE inhibition was less influenced with about a 3.4 time drop and presented an IC_{50} value of 5.72 μM compared with the IC_{50} value of 1.66 μM of **5d**. Similar correlation was also found in compounds **5c** and **6c**. These observations were in accordance with the multiple binding interactions of deoxyvasicinone and benzylidene moieties with PAS and aromatic residue at the midgorge. Reduction of the double bond in benzylidene moiety demolished the consecutive aromaticity of the chromophore, hence weakening the π–π interaction with the aromatic residues of the enzyme. As a result, weaker inhibitory effects on AChE and BuChE were found in compounds **6a**–**d**. Moreover, it was reported that

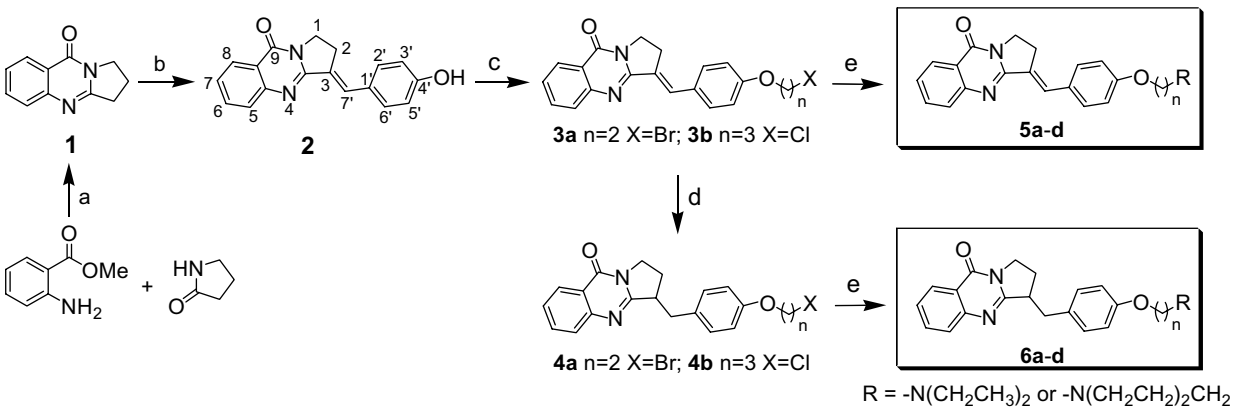

Figure 2. Synthesis of derivatives. Reagents and conditions: (a) $POCl_3$, CH_2Cl_2, 40 °C; (b) 4-hydroxybenzaldehyde, Ac_2O, reflux; (c) 1,2-dibromoethane or 1-bromo-3-chloropropane, K_2CO_3, acetone, reflux; (d) H_2, Pd/C, methanol, rt; (e) secondary amine, KI, K_2CO_3, MeCN, reflux.