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Methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives: A new class of acetylcholinesterase inhibitors

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Abstract—A new class of inhibitors of acetylcholinesterase (methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives) is described. Compounds **4b** and **4i** were found to be more potent than galanthamine in inhibiting acetylcholinesterase.

In the past two decades, treatment for Alzheimer's disease (AD) has largely involved replacement of neurotransmitters that are known to be lacking in AD, mostly based on the "cholinergic hypothesis" of AD.[1,2] A deficit in central cholinergic transmission caused by degeneration of the basal forebrain nuclei is an important pathological and neurochemical feature of AD. One possible approach to treating this disease is to restore the acetylcholine levels by inhibiting acetylcholinesterase (AChE) with highly selective inhibitors. AChE inhibitors such as tacrine, donepezil, rivastigmine and galanthamine are able to enhance memory in AD patients (Fig. 1). Unfortunately, the potential effectiveness offered by most common inhibitors often is limited by the appearance of central and peripheral side effects.[3,4] Recent data focus on the complex nature of AD and disclose the involvement of other neurotransmitters such as serotonin, dopamine, noradrenalin, histamine, excitatory amino acids and neuropeptides among others.[5] The present tendency in the search for new compounds to treat AD is to look for inhibitors of AChE endowed with additional therapeutic effects, such as antioxidant[6] and anti-inflammatory agents,[7] activation of other neurotransmitter systems like GABA or monoaminergic systems[8] and calcium-channel blocking potential.[9]

4-Hydroxybenzaldehyde (**1**) is an active constituent of *Gastrodia elata* (Chinese name: tianma), which is a very important Chinese herbal medicine used for the medical treatment of headaches, migraine, dizziness, epilepsy, rheumatism, neuralgia, paralysis, and other neuralgic and nervous disorders.[10,11] *Gastrodia elata* was reported to contain one or more compounds that dramatically reduce amyloid β-peptide induced neuronal cell death in vitro.[12] Recent studies showed that **1** is not only a potent antioxidant agent but also a GABA transaminase inhibitor ($IC_{50} = 4.1$ μg/mL),[13] and there was a report further exactly suggesting that the aldehyde group and the hydroxyl group at C-4 are necessary for the action.[14] Based on these cases, we supposed that **1** might contribute to the treatment of AD. In our previous high throughout screening work, **1** was also found to show weak anti-AChE activity and the value of IC_{50} was 37.8 mM. Therefore, a series of methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives were designed using **1** and amino-acid methyl esters as starting materials, in order to explore new AChE inhibitors with additional antioxidant and GABA transaminase inhibitory effects.

Our strategy is based on the crystallographic structure of the active-site gorge of AChE from *Torpedo californica*.[15] The active centre of AChE contains a catalytic triad (Ser 200, His440, Glu327) which locates at the bottom of the narrow gorge. Trp84, a residue of the anionic site, is located near the active center. It is supposed that the aromatic ring of those compounds

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Keywords: AChE inhibitors; Methyl 2-(2-(4-formylphenoxy) acetamido)-2-substituted acetate; 4-Hydroxybenzaldehyde; Synthesis.

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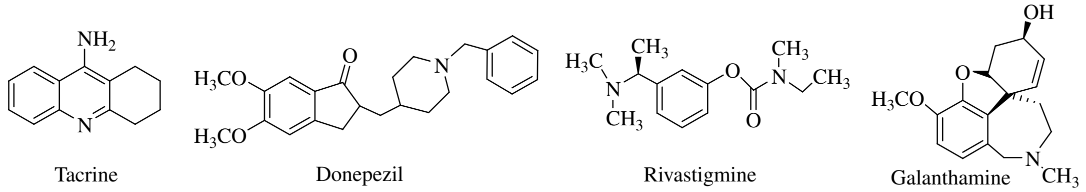
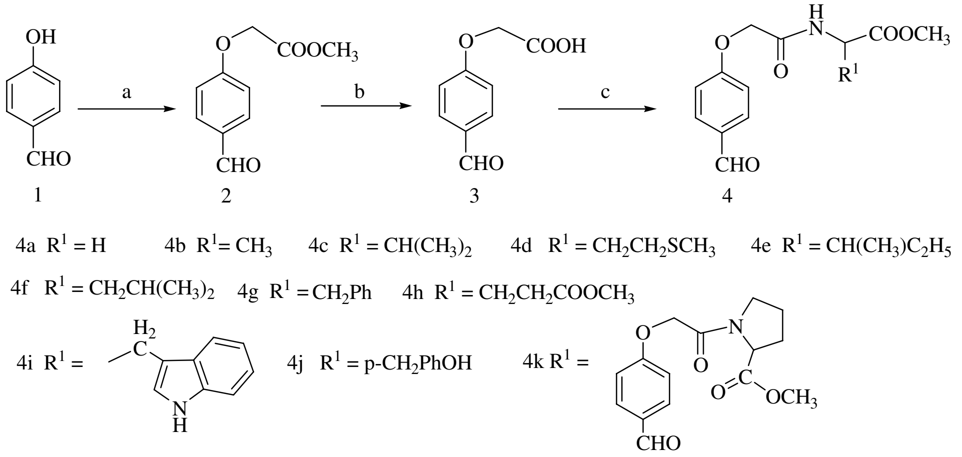


Figure 1. AChE inhibitors currently used in therapeutics.



Scheme 1. Synthesis of **4a–k**. Reagents and conditions: (a) $ClCH_2COOCH_3$, K_2CO_3, acetone, 60 °C, 5h, 100%; (b)—(i) NaOH, H_2O, (ii) H^+, H_2O, 95%; (c) amino-acid methyl ester, DCC, DMAP, $CHCl_3$, 0 °C stirred rt, overnight, 26–80%.

synthesized here may interact with Trp84 through π–π stacking,[16] and the amido fragment would be able to interact with the catalytic triad of the active site.

Methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives could be prepared by the condensation of amino-acid methyl esters and 4-formylphenoxyacetic acid, and the latter was synthesized by the reaction of **1** and methyl chloroacetate. From Scheme 1, when 1 equivalent of **1** and 1.2 equivalents of methyl chloroacetate reacted in acetone at 60 °C for 5 h in the presence of anhydrous K_2CO_3, compound **2** was afforded. Then compound **2** was hydrolysed in 1 M NaOH, acidified to pH 3–4 with 1 M HCl to produce compound **3**.[17] Compound **3** condensed with the appropriate amino-acid methyl esters in the presence of *N*,*N′*-dicyclohexyl-carbodiimide (DCC) and 4-(dimethylamino) pyridine (DMAP) to provide the desired compounds (**4a–k**). All those methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives were characterized by (^1H, ^{13}C) NMR, MS and IR spectroscopy. The anti-AChE activity of compounds **4a–k** was evaluated using a slightly modified colorimetric method of Ellman et al.[18] The obtained IC_{50} values, together with that of galanthamine for comparison, are shown in Table 1.

Table 1. AChE inhibition of methyl 2-(2-(4-formylphenoxy)-acetamido)-2-substituted acetate derivatives

Compound	AChE IC_{50}[a] (μM)	BuChE IC_{50}[b] (μM)	Selectivity for AChE[c]
1	>1000	Nd[d]	
4a	98.86	Nd	
4b	0.19	Nd	
4c	Nd	Nd	
4d	Nd	Nd	
4e	Nd	Nd	
4f	Nd	Nd	
4g	4.96	Nd	
4h	>200	Nd	
4i	0.28	33.94	121.21
4j	Nd	Nd	
4k	Nd	Nd	
Galanthamine	0.55[e]	15.24	27.70

[a] Assay performed using ChE from Electric eel. Values are means of three different experiments.

[b] Assay performed using horse serum.

[c] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).

[d] Not determined at 1000 μM concentration.

[e] Values in the literature[16,19] is 0.36–0.61 μM.

From Table 1, five results could be found about the change of AChE inhibitory activity on introduction of amino-acid moieties into the backbone of **1**, as compared with AChE inhibitory activity of **1** itself. First, **1** possessed a very weak AChE inhibitory activity, however, **4b**, **4g** and **4i** showed a higher AChE inhibitory activity than **1**, therefore, it is sure that the introduction of three amino-acid moieties, L-alanine, L-tryptophan and L-phenylalanine into the molecule of **1**, respectively, could make the inhibitory activities of **1** increase obviously. Second, the introduction of L-glycin into **1** (for **4a**) could just slightly increase the AChE inhibitory activity. Third, for **4c–f**, **4h**, **4j** and **4k**, although amino-acid moieties like L-valine, L-methionine, L-leucine, L-isoleucine, L-aspartic acid, L-tyrosine and L-proline were attached to the structure of **1**, respectively, the AChE inhibitory values were almost same as that of **1**. Fourth, both of **4b** ($IC_{50} = 0.19\ \mu M$) and **4i** ($IC_{50} = 0.28\ \mu M$) showed higher AChE inhibitory values than galanthamine ($IC_{50} = 0.55\ \mu M$). Lastly, all of the tested compounds showed poor inhibition to butyrylcholinesterase (BuChE). Especially for compounds **4b** and **4i**, they all demonstrated a high selectivity, respectively, for AChE inhibitory activity over BuChE, **4b** (selectivity, >1000) and **4i** (selectivity, 121.21), whereas the selectivity value of galanthamine is only 27.70. Therefore, the remarkable selectivity feature of compounds **4b** and **4i** might shed light on the future design and preparation of AChE inhibitors. However, the reason why **4b** and **4i** showed a better inhibitory activity and selectivity than the other synthesized compounds is not clear, which will be further studied in the future in our laboratory.

In conclusion, methyl 2-(2-(4-formylphenoxy)acetamido)-2-substituted acetate derivatives represented a new class of highly active and selective AChE inhibitors, which indicated that the modification based on the structure of **1** is a practical approach to increase its AChE inhibitory activities. Among all the tested compounds, **4b** and **4i** showed significant AChE inhibitory activities ($IC_{50} = 0.19\ \mu M$, $0.28\ \mu M$) and a high selectivity over BuChE (>1000, 121.21), and thus they may be applied for potent AChE inhibitors. Further efforts aiming at developing potent AChE inhibitors based on modification of **1** would be continued in our laboratory and the research results will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.091.

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