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SHORT COMMUNICATION

Discovery of potent and selective acetylcholinesterase (AChE) inhibitors: acacetin 7-*O*-methyl ether Mannich base derivatives synthesised from easy access natural product naringin

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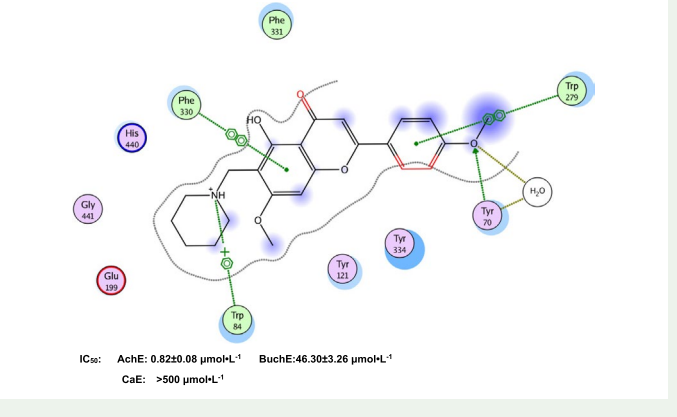
ABSTRACT

Naringin, as a component universal existing in the peel of some fruits or medicinal plants, was usually selected as the material to synthesise bioactive derivates since it was easy to gain with low cost. In present investigation, eight new acacetin-7-*O*-methyl ether Mannich base derivatives (**1**–**8**) were synthesised from naringin. The bioactivity evaluation revealed that most of them exhibited moderate or potent acetylcholinesterase (AChE) inhibitory activity. Among them, compound **7** (IC_{50} for AChE = 0.82 ± 0.08 μmol•L^{-1}, IC_{50} for BuChE = 46.30 ± 3.26 μmol•L^{-1}) showed a potent activity and high selectivity compared with the positive control Rivastigmine (IC_{50} for AChE = 10.54 ± 0.86 μmol•L^{-1}, IC_{50} for BuChE = 0.26 ± 0.08 μmol•L^{-1}). The kinetic study suggested that compound **7** bind to AChE with mix-type inhibitory profile. Molecular docking study revealed that compound **7** could combine both catalytic active site (CAS) and peripheral active site (PAS) of AChE with four points (Trp84, Trp279, Tyr70 and Phe330), while it could bind with BuChE via only His 20.



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1. Introduction

In recent years, a lot of tertiary amine derivatives originated from natural products were synthesised and evaluated as acetylcholinesterase (AChE) inhibitors for potential application in the treatment of Alzheimer's diseases (Li et al. 2013; Silva et al. 2014; Sang et al. 2015). According to the experiences in medicinal chemistry, amino moiety in drugs seemed to improve physico-chemical properties or enhance bioactivity. The Mannich reaction is one of important ways to introduction amino moiety for the natural compounds (Roman 2015). In the present study, a series of acacetin-7-*O*-methyl ether Mannich-base derivatives were synthesised from naringin, which is a low-cost and easy access natural product abundant existing in China and South-east Asia. Then the effects of them in inhibiting AChE, BuChE or carboxylesterase (CaE) were evaluated, followed by molecular docking study in further.

2. Results and discussion

2.1. Chemistry

Acacetin 7-*O*-methyl ether Mannich-base derivatives **1**–**8** were synthesised via a four steps procedure as described in Scheme 1.

According to our previous procedure (Wu et al. 2012), the acacetin and acacetin-7-*O*-methyl ether could be obtained by dehydrogenation, regioselective methylation, hydrolysis of glycosidic bond from naringin. Then the synthesis of acacetin or acacetin-7-*O*-methyl ether Mannich-base derivatives was conducted in the presence of formaldehyde, aliphatic or alicyclic amines. The structures of the new synthesised compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-MS(+) analyses. The purity of new synthesised compounds was more than 98.0% with the assay by high-pressure liquid chromatography.

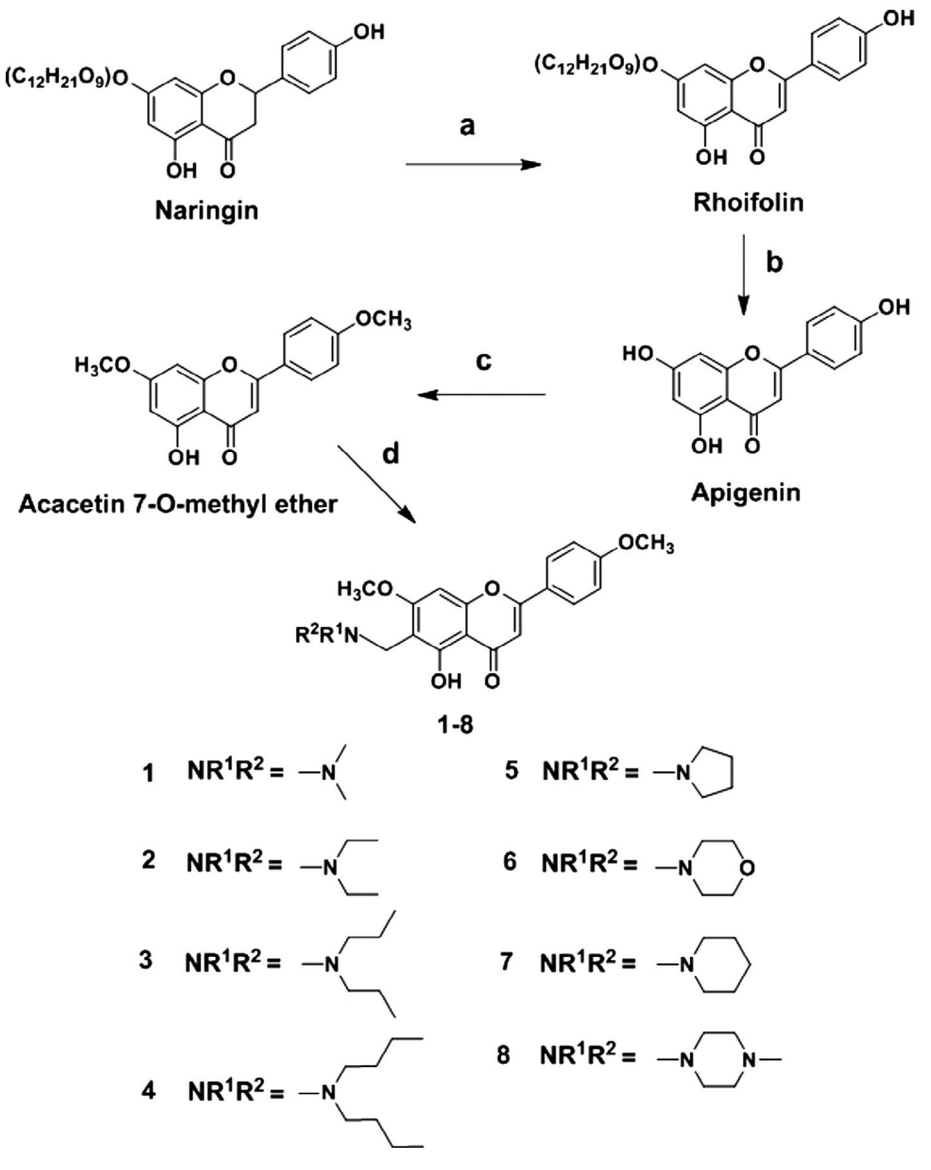
2.2. Bioactivity evaluation in inhibiting AChE and BuChE

The results for the inhibitory effects against AChE are shown in Table 1. The variation of amine groups markedly influenced the activity in inhibiting AChE. According to the data of compounds **1**–**4**, the order of inhibiting potency of them against AChE is: dimethylamino group > diethylamino group > dipropylamino group > dibutylamino group. But there is a significant difference among compounds **5**–**8**. Compound **7** ($IC_{50} = 0.82 \pm 0.08$ μmol•L^{-1}) possessed the highest activity, but compound **6** had poor activity ($IC_{50} > 500$ μmol•L^{-1}). Compound **8** had a comparable activity with compound **5**. It seemed that the introduction of morpholine group markedly decreased the inhibitory activity for AChE. Among them compound **7** had a highest selectivity in inhibiting AChE over BuChE (ratio: 56.5).

All the compounds exhibited high-specific inhibition against cholinesterase over CaE ($IC_{50} > 500$ μmol/L) compared with the control drug Rivastigmine (for CaE $IC_{50} = 31.10 \pm 1.20$ μmol/L).

2.3. Kinetic assay

The linear Lineweaver–Burk equation was applied and the graphical analysis of the steady-state inhibition data of compound **7** was shown in Figure S1. According to the results, the kinetic profile of compound **7** exhibited a mixed-type inhibition. It suggested that compound



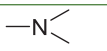
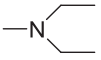
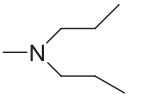
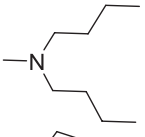
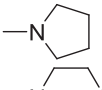
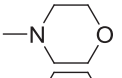
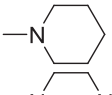
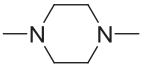
Scheme 1. Reagents and conditions: (a) I_2/Py, reflux; (b) concentrated H_2SO_4, EtOH, reflux; (c) $(CH_3)_2SO_4$, K_2CO_3, acetone, 65°C; (d) 37% HCHO, HNR^1R^2, CH_3OH, HCl, 65–70°C.

7 could bind with the catalytic site and non-catalytic site of the AChE with different equilibrium constants, and the competitive constant (K_i) and non-competitive constant (K_i') are 0.71 and 2.17 μmol/L, respectively (Table S2).

2.4. Molecular docking study

A molecular docking study was performed for compound **7** using Molecular Operating Environment (MOE). According to the results in Figure S2, A, compound **7** exhibited multiple point binding modes with AChE. The nitrogen of piperidine ring binds to the catalytic anionic

Table 1. Effect of compounds **1**–**8** on AChE and BuChE. IC_{50} (μmol•L^{-1}).

Compound	$-NR^1R^2$	CaE	AChE (IC_{50})	BuChE (IC_{50})	Selectivity[a]
1		>500	3.54 ± 0.12	25.90 ± 0.71	7.32
2		>500	7.94 ± 0.09	12.4 ± 0.13	1.56
3		>500	15.02 ± 1.31	38.9 ± 1.85	2.59
4		>500	31.50 ± 0.81	45.2 ± 2.34	1.43
5		>500	3.94 ± 0.78	76.8 ± 4.63	19.5
6		>500	>500	58.6 ± 2.21	<0.118
7		>500	0.82 ± 0.08	46.3 ± 3.26	56.5
8		>500	3.50 ± 0.17	32.4 ± 1.42	9.26
Rivastigmine*	–	31.10 ± 1.20	10.54 ± 0.86	0.26 ± 0.08	0.0247

[a]Defined as the ratio of IC_{50} for BuChE/AChE.
*Used for a positive control.

site (CAS) via the π-cation interaction with Trp84 (4.21 Å), and the aromatic ring binds to peripheral anionic site (PAS) via a π–π stacking interaction with Phe330 (4.26 Å) and Trp279 (4.07 Å).In addition, methoxyl group in benzene ring binds with Tyr70 and H_2O (Figure S2, B). However, compound **7** only binds to BuchE by His20 (4.15 Å). These result from molecular docking may be partially explained the high selectivity of compound **7** for inhibiting BuChE and AChE.

3. Conclusion

In the present study, eight Mannich-base derivatives were synthesised from naringin and bioactivity evaluation showed that most of them had better activity than that of control drug Rivastigamine in inhibiting AChE. Among them, compound **7** which possesses a piperdine side chain had more potent bioactivity and higher selectivity than the other compounds. It is a promising compound for the treatment of AD as a novel AChE inhibitor from natural product.

Supplementary material

Experimental details related to this article are available online in supplementary material.

Disclosure statement

No potential conflict of interest was reported by the authors.

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