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SYNTHESIS AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF (±)-14-FLUOROHUPERZINE A

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Abstract: The synthesis of (±)-14-Fluorohuperzine A has been accomplished and the ability of this agent to inhibit acetylcholinesterase has been measured. Taking into account its racemic form, this compound exhibits 62 times less potent activity than natural (-)-huperzine A.

Introduction

(-)-Huperzine A (**1**), an alkaloid isolated from Chinese herb *Huperzia Serrata* (*Thumb*), is a potent reversible inhibitor of acetylcholinesterase(AChE) [1,2]. The use of **1** to increase the level of neurotransmitter acetylcholine in the central system renders **1** a particularly promising candidate for the treatment of Alzheimer's disease [3].

Terashima *et al.* have reported the syntheses and AChE inhibitory activities of four fluorinated analogues **2-5** [4]. Among these fluorinated derivatives, (±)-14,14,14-trifluorohuperzine A (**3**) was found to exhibit 200 times less potent activity than **1**. The X-ray crystal structure of (-)-huperzine A—AChE complex[5] indicated that an unusual hydrogen bond (H-bond) forms between the C-14 methyl of huperzine A and the backbone carbonyl of His440 in AChE. These facts promoted us to modify the C-14 methyl with a fluoromethyl (CH_2F) for the sake of keeping the above hydrogen bond and to see whether this modification can enhance the H-bond between the C-14 methyl and AChE because of the induction effect of F. Therefore, we performed the synthesis and pharmacological assay of the target compound (±)-14-fluorohuperzine A (**6**).

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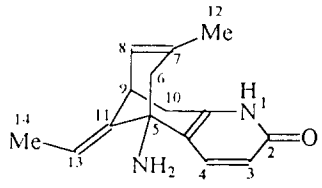

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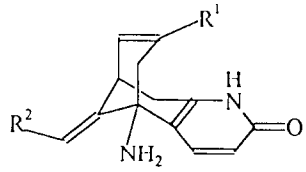


2: R^1=CF_3, R^2=Me

3: R^1=Me, R^2=CF_3

4: R^1=R^2=CF_3

5: R^1=CH_2F, R^2=Me

6: R^1=Me, R^2=CH_2F

Figure 1. Structures of huperzine A (1) and its fluorinated analogues.

We wish to report here the synthesis and AChE inhibitory activity of this novel fluorinated analogue **6**. The explored synthetic pathway to **6** is based upon Kozikowski's method developed for the synthesis of **1** and its analogues [6,7].

Chemistry

As shown in **Scheme 1**, we prepared the target compound **6** by the two methods. In **Method 1**, the (±)-14-hydroxyhuperzine A **(8)** prepared from commercially available 1,4-cyclohexanedione monoethylene ketal **7** according to the Kozikowski's procedure [7] was treated with diethylaminosulfur trifluoride (DAST) [8] to produce the fluorinated compound **6** only in 25% yield.

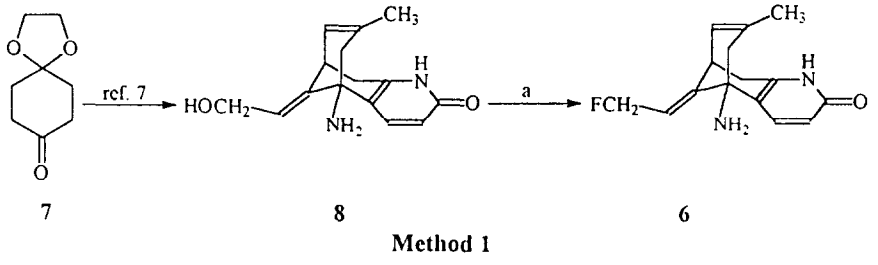
In **Method 2**, the known compound **9** [7] also prepared from **7** was reduced with diisobutylaluminium hydride (DIBAL) to give the allyl alcohol **10** in 78% yield. Treatment of **10** with DAST furnished the allyl fluoride **11** in 89% yield. Finally, deprotection of **11** with iodotrimethylsilane (TMSI) gave rise to requisite (±)-14-fluorohuperzine A **(6)** [9] in 56% yield.

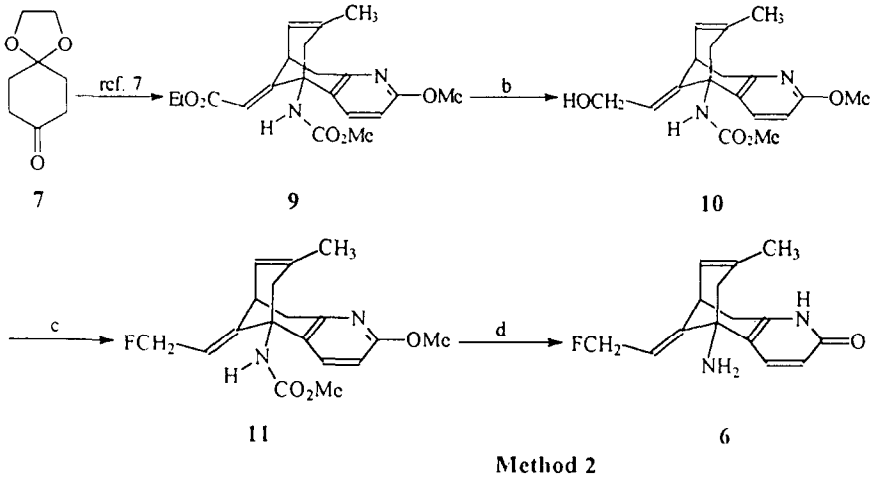
Biological Results and Discussion

With completion of the synthesis, *in vitro* AChE inhibitory activity of this compound was measured according to the method of Ellman *et al.*[10] using rat brain hippocampal crude homogenates. The results shown in **Table 1** indicated that the inhibitory activity of **6** was three times as much as that of **3**, corresponding to 62 times less potent than that of **1** taking into account its racemic form. This finding suggests that the C-14 methyl plays an important role for the activity of huperzine analogs[4,5]. Being different from **3**, the compound **6** keeps

two hydrogens in C-14, which can still form H-bond with AChE. This is one of the reasons why the anti-AChE activity of compound **6** is 3 times more potent than that of **3**. On the other hand, according to our *ab initio* quantum chemical calculation result with model molecules, the anticholinesterase activity of **6** should be higher than that of **1**, if simply considering the H-bond between the C-14 methyl and the backbone carbonyl of His440 in AChE. However, the pharmacological result did not fit with the prediction, indicating the interaction between the C-14 position of huperzine analogues and AChE is very sensitive and complicated. Besides H-bonding interaction, other interactions such as electrostatics and hydrophobicity might be essential for the binding of the C-14 group with AChE. The related exploring work is now in progress.

Scheme 1. Synthesis of (±)-14-fluorohuperzine A (6)





Reagents and Conditions: a) DAST,CH_2Cl_2,-78°C,25%; b) DIBALH, THF, -78°C, 78%; C) DAST, CH_2Cl_2,0°C, 89%; d) TMSI, $CHCl_3$, reflux, MeOH, reflux, 56%

Table 1. Inhibitory activity against acetylcholinesterase (AChE)

Compound	IC_{50} value (μ M)
(-)-huperzine A (1)	0.08
(±)-14-fluorohuperzine A (6)	10

In summary, we have succeeded in the first synthesis of novel fluorinated analogue of huperzine A, (±)-14-fluorohuperzine A (6). The result obtained for *in vitro* AChE inhibitory activity assay should be useful for the further modification of Huperzine A and design of new AChE inhibitor .

References and Notes

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9 Satisfactory IR, ^{1}H-NMR and mass spectral data were obtained. IR(KBr) 3419, 3277, 2930, 1655, 1614, 1558, 1460, 1410, 1310, 1118, 980, 825, 731 cm^{-1} ; ^{1}H-NMR (300MHz, $CDCl_3$): 7.90 (d, 1H, J= 9.4 Hz) , 6.41 (d, 1H, J=9.5 Hz), 5.62 (tt, 1H, J=10.2 Hz), 5.46 (m, 1H), 5.10 (dd, 2H, J=47 Hz,6.3Hz), 3.58 (m, 1H), 2.90 (dd, 1H, J=16.8, 5.0 Hz), 2.75 (d, 1H, J=15.7 Hz), 2.15 (br, s,2H), 1.55 (s, 3H); ^{19}F-NMR (300MHz, CF_3COOH): -132.5 (t, J= 47 Hz); EIMS m/z 260 (M^+), 241, 227, 217, 197,185, 167, 149, 83; HRMS calcd for $C_{15}H_{17}FN_2O(M^+)$: 260.1324, found: 260.1330.

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