Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3279–3284

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF HUPERZINE A—E2020 COMBINED COMPOUND

Fanxing Zeng, Hualiang Jiang,* Yufeng Zhai, Haiyan Zhang, Kaixian Chen,* and Ruyun Ji

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China

Received 12 July 1999; accepted 8 October 1999

Abstract: The synthesis of huperzine-E2020 combined compound (**3**) has been accomplished and the activities of **3** and the intermediates **12** and **13** to inhibit the activity of acetylcholinesterase have been measured. Conformation analyses and molecular docking studies of E2020 and the eight isomers of **12** were carried out. The results indicated that binding energies of all isomers of **12** with AChE was much lower than E2020 except for isomer RRZ, which might be the reason that the activity of **12** was lower than that of E2020. Interaction pattern of RRZ in AChE was also studied. Both binding energy and interaction pattern shows that the biological activity of RRZ might be higher than that of E2020.

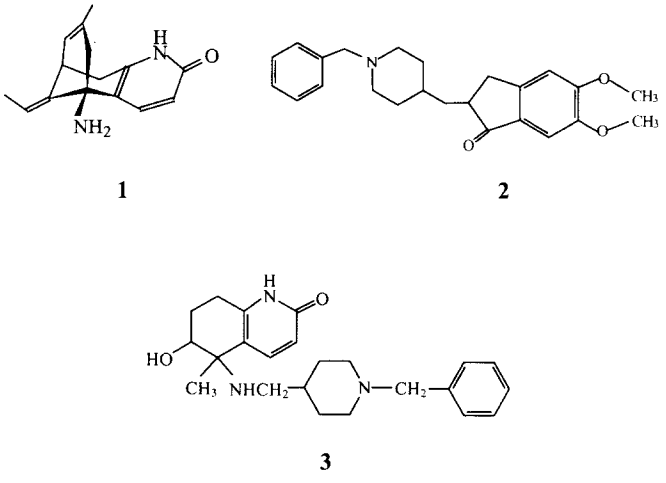
Introduction

(-)-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 nervous system renders it a particularly promising candidate for the treatment of Alzheimer's disease (AD)[3]. **1** and its derivatives have been extensively studied by the groups of Kozikowski[4] and Bai[5], some of them being tested in clinical trials. E2020(**2**), marketed as Aricept®, is a member of a large family of N-benzylpiperidine based AChE inhibitors which were developed, synthesized and evaluated by the Eisai Company in Japan, and has been approved for use in the US for the palliative treatment of AD patients [6,7].

In our program to develop new AChE inhibitors, by automated molecular docking, we have found that the dimethoxyindanone moiety of E2020 interacts with the active site of AChE and the benzyl piperidine moiety interacts with the peripheral binding site of AChE. The X-ray crystal structure of (-)-huperzine A—AChE complex[8] indicates that huperzine A only makes interaction with the active site of AChE. To combine the different binding sites of huperzine A and E2020 with AChE, compound **3** with structural feature of huperzine A and E2020 was designed which was expected to have the interaction between the 5,6,7,8-tetrahydroquinolinone of huperzine A and the active site of AChE, and the interaction between the benzyl piperidine of E2020 and the peripheral binding site of AChE. This strategy is on the assumption that a

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00597-1

compound capable of simultaneous binding to the two binding sites of AChE should have increased biological activity. Therefore, we performed the synthesis and pharmacological assay of huperzine-E2020 combined compound.



1 2

3

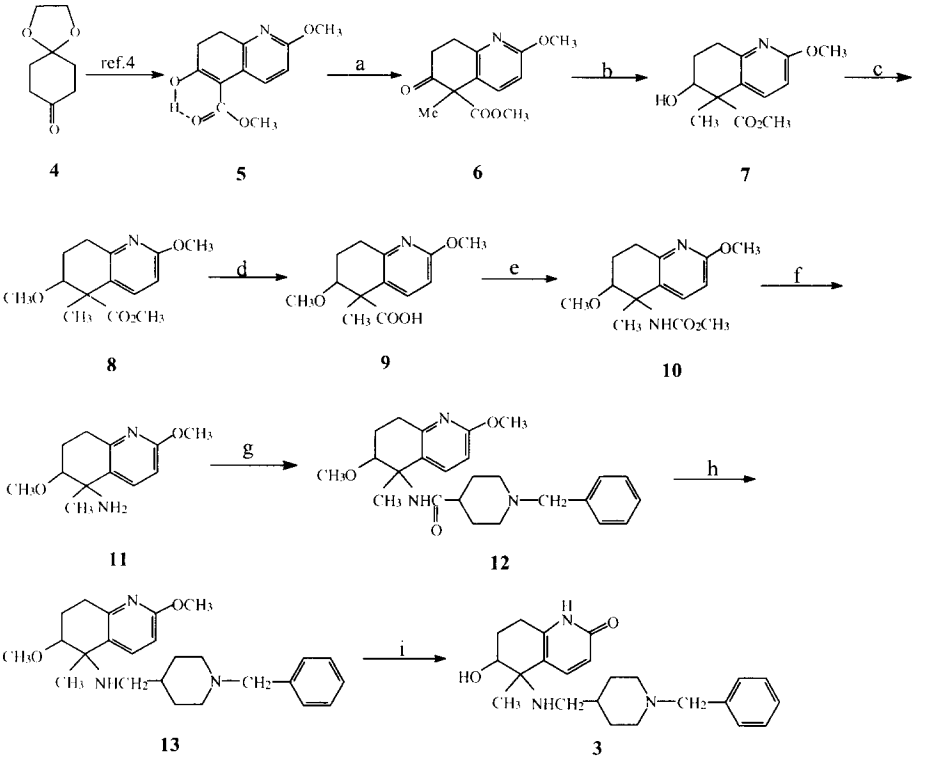
Figure 1. Structures of huperzine A (1), E2020(2) and their combined compound(3)

We report herein the synthesis and AChE inhibitory activity of this huperzine-E2020 combined compound **3**. The explored synthetic pathway is based upon the modified Kozikowski' s method developed for the synthesis of **1** and its analogues[4].

Chemistry

The synthesis of the compound **3** was accomplished as shown in **Scheme 1**. The known β-keto ester **5** prepared from commercially available 1,4-cyclohexanedione monoethylene ketal **4** according to the Kozikowski' s procedure[4] was subjected to a C-methylation reaction to provide methylated β-keto ester **6** in 75.5% yield. Treatment of **6** with $NaBH_4$ provided alcohol, whose hydroxy group was then protected to give O-methlation ester **8**(79.6%, two steps). **8** was then converted to the corresponding methyl carbamate **10** *via* a sequence of reactions involving saponification and Curtius rearrangement of the liberated acid **9**. Reaction of **10** with KOH and 18-crown-6 gave rise to the amine **11** in 81.2% yield. Acetylation of amine **11** with N-benzyl-4-piperidine carboxylic acid[9] furnished **12** in 65.7% yield. Reduction of **12** with $LiAlH_4$ in ether solution produced **13** in 72% yield. Finally, deprotection of **13** with iodotrimethylsilane(TMSI) resulted in the target compound **3**[10] in 58% yield.

Scheme 1. Synthesis of huperzine-E2020 combined compound (3)



Reagents and Conditions: a) NaH, MeI, THF, rt, 75.5%; b) $NaBH_4$, EtOH, 0°C, 95.8%; c) NaH, MeI, THF, 0°C, 83.1%; d) NaOH, THF, MeOH, reflux, 93%; e) DPPA, Et_3N, toluene, reflux, MeOH, reflux, 72%; f) KOH, 18-crown-6, toluene, 81.2%; g) N-benzyl-4-piperidylcarboxylic acid, CH_2Cl_2, DCC, rt, 65.7%; h) $LiAlH_4$, ether, reflux, 72%; i) TMSI, $CHCl_3$, reflux, MeOH, reflux, 58%

Biological Results and Discussion

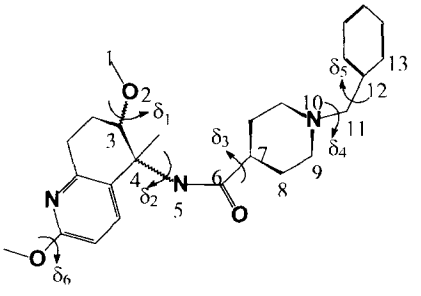
With completion of the synthesis, *in vitro* AChE inhibitory activity of **3** and **12**,**13** as compared with E2020 was measured according to the method of Ellman *et al.*[11] using rat brain hippocampal crude homogenates. The results shown in **Table 1** indicated that **12** was 850-fold less active than E2020, while neither **13** nor **3** was an effective cholinesterase inhibitor.

Table 1. Inhibitory activity against acetylcholinesterase (AChE)

Compound	IC_{50} value (μM)
E2020 (2)	0.02
12	17.2
13	>177
3	>190

To better understand the pharmaceutical results, conformation analyses of E2020 and the eight isomers of **12**[12] which was most potent among the newly synthesized three compounds were carried out to obtain their bioactive conformations, lowest-energy conformations and conformational energy. On the basis of conformation analyses, docking studies were performed from which interaction energy of E2020 and the isomers of **12** to AChE was obtained. The results showed in **Table 2** released that binding energy of all isomers of **12** was much lower than that of E2020 except for RRZ, which might be the reason that the activity of **12** was lower than E2020. In view of the higher binding energy of RRZ, interaction pattern of RRZ with AChE was also studied. The major interactions between RRZ and AChE showed in **Figure 1** indicated that RRZ could bind both the active site and the peripheral binding site of AChE, similar to the interaction between E2020 and AChE[13]. Besides keeping the major interactions of E2020 with AChE, C-8 methylene of RRZ makes an aromatic hydrogen bond with the phenyl ring of Phe 331 and C-1 methyl makes a H-bond with the backbone carbonyl of Arg 289. This interaction pattern further indicates that the activity of RRZ might be higher than E2020. The related exploring work is now in progress.

Table 2. Interaction energy of E2020 and isomers of 12 with AChE (kJ·mol^{-1})



12

Molecule and Configurations	E_{setric} [a]	E_{ele} [b]	E_{bind} [c]	ΔE_{cf} [d]	ΔE_{bind} [e]
E2020	-227.074	-135.616	-362.690	29.034	-333.656
RRE	-157.699	-94.782	-252.480	83.266	-169.215
RRZ	-213.414	-157.991	-371.401	14.454	-356.947
RSE	-168.960	-84.194	-253.153	18.032	-235.121
RSZ	-167.810	-112.087	-279.897	69.748	-210.150
SRE	-168.981	-114.695	-283.676	49.763	-233.913
SRZ	-95.124	250.441	155.312	264.895	420.207
SSE	-129.413	-127.256	-256.669	92.879	-163.789
SSZ	-100.500	-114.507	-215.002	242.929	27.927

[a] E_{steric}: steric interaction energy. [b] E_{ele}: electrostatic interaction energy. [c] E_{bind}: total binding energy; the binding energy was calculated by the following formula: $E_{bind}=E_{complex}-E_{AChE}-E_{ligand}$, where $E_{complex}$ is the total energy of complex formed between AChE and E2020 or each isomer of **12**, E_{AChE} is the total energy of AChE and E_{ligand} is the energy of E2020 or the conformational energy of each isomer of **12**; The complex models of E2020-AChE and **12**-AChE were constructed by Sybyl/FlexiDock program, and then structural optimization was performed with Tripos force field to a root-mean-square(rms) energy gradient of 0.42kJ/mol·Å. [d] ΔE_{cf}: the energy difference between active conformation and lowest-energy conformation. [e] $\Delta E_{bind} = E_{bind} + \Delta E_{cf}$.

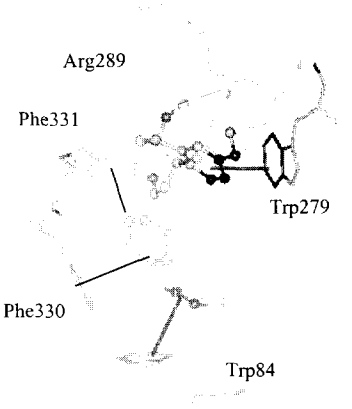


Figure 1. Majoar interactions between RRZ and AChE. H-bond is showed as dashed line, aromatic stacking are showed as pink lines, aromatic H-bond is showed as blue line and cation-π interaction is showed as black line.

In summary, we have succeeded in the synthesis of huperzine A-E2020 combined compound. The results obtained for *in vitro* AChE inhibitory activity assays on the basis of the conformation analyses and molecular docking studies should be useful for the design of novel AChE inhibitors.

Acknowledgement: The authors gratefully acknowledge the National Natural Science Foundation of China (29725203) and the Committee of 863 High Technology Program of China (863-103-04-01) for financial support.

References and Notes

1. Liu, J.-S.; Zhu, Y.-L.; Yu, C.-M.; Zhou, Y.-Z.; Han, Y.-Y.; Wu, F.-W.; Qi, B.-F. *Can. J. Chem.* **1986**, 64, 837.
2. Kozikowski, A. P.; Thiels, E.; Tang, X.-C.; Hanin, I. *Acta. Med. Chem.* **1992**, 1, 175.
3. Tang, X.-C.; De Sarno, P.; Sugaya. K.; Giacobini, E. *J. Neurosci. Res.* **1989**, 24, 276.
4. Kozikowski, A. P.; Campiani, G.; Sun, L.-Q.; Aagaard, P.; Mckinney, M. *J. Org. Chem.* **1993**, 58, 7660. b) Kozikowski, A. P.; Miller, C. P.; Yamada, F.; Pang, Y.-P.; Miller, J. H.; McKinney, M.; Ball, R. G. *J. Med. Chem.* **1991**, 34, 3399.
5. He, X.-C.; Wang, Z.-Y.; Li, Y.-L.; Xu, Z.-R.; Bai, D.-L. *Chin. Chem. Lett.* **1993**, 4, 597.
6. Kawakami, Y.; Inoue, A.; Kawai, T.; Wakita, M.; Sugimoto, H.; Hopfinger, A. J. *J. Bioorg. Med. Chem.* **1996**, 4, 1429.
7. Cardozo, M. G.; Imura, Y.; Sugimoto, H.; Yamanishi, Y.; Hopfinger, A. J. *J. Med. Chem.* **1992**, 35, 584.
8. Raves, M. L.; Harel, M.; Pang, Y.-P.; Silman, I.; Kozikowski, A. P.; Sussman, J. L. *Nat. Struct. Biol.* **1997**, 4, 57.
9. Carroll, F. I.; Ferguson, A. M.; Lewis, J. B. *J. Org. Chem.* **1966**, 31, 2957.
10. Satisfactory physical and spectroscopic data were obtained. mp: 112-114°C; IR (KBr) 3380, 3275, 2950, 1652, 1609, 1460, 1144, 743cm^{-1}; ^{1}H-NMR(300MHz, $CDCl_3$): 7.68(d, 1H, J=9.4Hz), 7.26(m, 5H), 6.39(d, 1H, J= 9.3 Hz), 3.80(m, 1H), 3.46(s, 2H), 2.95(m, 2H), 2.66(m, 1H), 2.45(m, 2H), 2.18(m, 2H), 1.90(m, 9H), 1.43(s, 3H); MS m/z 381(M^+), 363, 272, 193, 189, 174, 91. Anal. Calcd. for $C_{23}H_{31}N_3O_2$: C, 72.41; H, 8.19; N, 11.01. Found: C, 72.02; H, 8.33; N, 10.69.
11. Ellman, G. L.; Courtney, D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, 7, 88.
12. Compound **12** possesses two stereogenic centres (C*-3 and C*-4) and one cis-trans isomeric "centre", where the peptide link N5-C6 is considered as the double bond, so there are eight isomers in **12** which are named RRE, RRZ, RSE, RSZ, SRE, SRZ, SSE and SSZ.
13. Kryger, G.; Silman, I.; Sussman, J. L. *Structure* **1999**, 7, 297.