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Design and Synthesis of Dual Inhibitors of Acetylcholinesterase and Serotonin Transporter Targeting Potential Agents for Alzheimer's Disease

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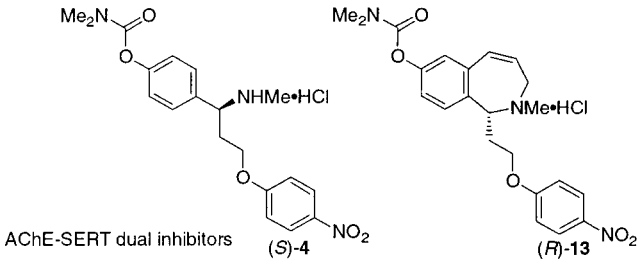
ABSTRACT



Highly efficient acetylcholinesterase (AChE) and serotonin transporter (SERT) dual inhibitors, (*S*)-4 and (*R*)-13 were designed and synthesized on the basis of the hypothetical model of AChE active site. Both compounds showed potent inhibitory activities against AChE and SERT.

Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disorder characterized by progressive deterioration of memory and cognition.[1] Enhancement of central cholinergic function by inhibition of acetylcholinesterase (AChE) is, so far, the only clinically effective approach for treatment of AD.[2] However, the clinical usefulness of marketed AChE inhibitors (AChEIs) is limited mainly by adverse effects on peripheral organs.[3] AD patients often exhibit psychiatric symptoms such as irritability, anxiety, and depression. Depression in AD patients has been successfully treated with inhibitors of serotonin transporter (SERT),[4] antidepressants that lack anticholinergic action. Thus, it is anticipated that combining SERT and AChE inhibitory activity could offer greater therapeutic benefits, because the antidepressive effect of SERT inhibitors might reduce a demand on cholinergic systems in the brain to ameliorate cognitive deficits. These dual inhibitors would be a novel class of anti-AD drugs more effective in alleviating the symptoms of AD than the known AChE inhibitors. Two groups have studied AChEIs that possess serotonin transporter inhibitory activity.[5] The reported

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(1) (a) Wurtman, R. J. *Sci. Am.* **1985**, *252*, 48. (b) *Alzheimer's Diseases Problems, Prospects and Perspectives*; Altman, H. J., Ed.; Plenum Press: New York, 1987.

(2) (a) Enz, A.; Amstutz, R.; Boddeke, H.; Gmelin, G.; Malanowski, J. *Prog. Brain Res.* **1993**, *98*, 431. (b) Millard, C. B.; Broomfield, C. A. *J. Neurochem.* **1995**, *64*, 1909.

(3) (a) Benzi, G.; Moretti, A. *Eur. J. Pharmacol.* **1998**, *346*, 1. (b) Giacobini, E. *Jpn. J. Pharmacol.* **1997**, *74*, 225.

(4) Lyketsos, C. G.; Sheooard, J. M.; Steele, C. D.; Kopunek, S.; Steinberg, M.; Baker, A. S.; Brandt, J.; Rabins, P. V. *Am. J. Psychiatry* **2000**, *157*, 1686.

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compounds showed potent AChE inhibitory activities and moderate SERT inhibitory activities in vitro. No compound to date shows simultaneous activation of both the cholinergic and serotonergic nervous systems in the brain following oral administration, probably because of inappropriate combination of activities. We describe here the design and synthesis of highly efficient dual inhibitors of AChE and SERT.

On the basis of the X-ray structure of the complex of AChE and donepezil[6] and molecular modeling studies,[7] we proposed a hypothetical model for inhibitor interaction at the active site of AChE, which involves four binding sites (Figure 1). To design the dual inhibitor, we selected one of

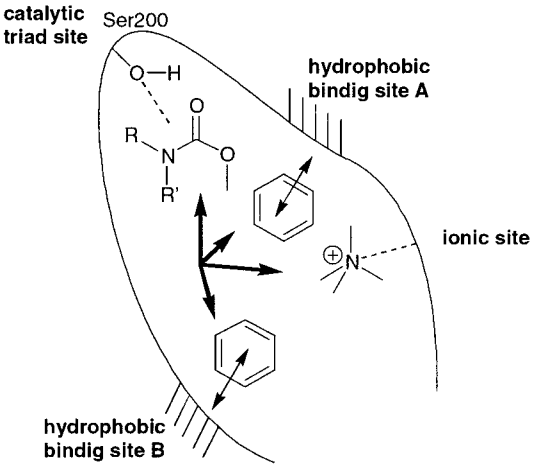
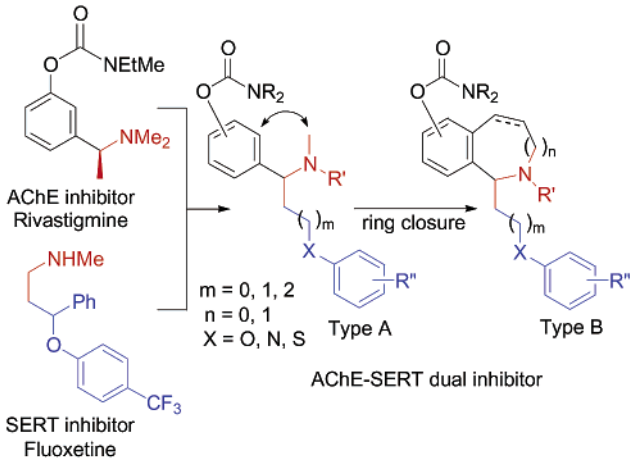


Figure 1. Hypothetical model of AChE active site with proposed pharmacophore.

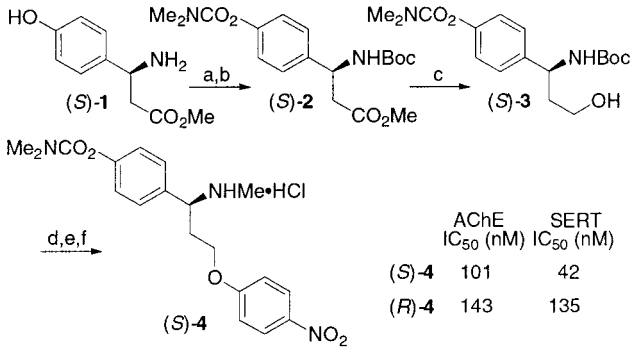
the marketed AChEIs, rivastigmine,[8] as a lead compound. Rivastigmine possesses three elements that are able to bind to the proposed binding sites of AChE: a carbamate moiety to the catalytic triad site, an amino moiety to the ionic site, and an aromatic ring to the hydrophobic binding site A. Focusing on the fourth binding site of our model, we chose fluoxetine[9] as the pharmacophoric element corresponding to the hydrophobic binding site B. Fluoxetine has potent inhibitory activity against SERT and possesses an ethylamine moiety that could be overlapped with that of rivastigmine. Therefore, the type A compound designed by hybridization of these two compounds at the common ethylamine moiety was expected to be a dual inhibitor of AChE and SERT. The type B ring-closed compound was also designed to explore the effect of conformational restriction (Scheme 1).

Scheme 1. Design of AChE−SERT Dual Inhibitors



We synthesized and evaluated various type A derivatives and found that most have inhibitory activities against both AChE and SERT. Optimization of the carbamate group, the tether-length,[10] and the R″ substituent showed 4-nitrophenoxy ether derivative **4** (m = 1, X = O) to be the best type A dual inhibitor. Both optical isomers of compound **4** were synthesized as shown in Scheme 2. The primary amine of

Scheme 2[a]



[a] (a) Boc_2O, THF, rt, 72%; (b) Me_2NCOCl, K_2CO_3, DMF, rt, 96%; (c) $LiAlH_4$, −78 to 0 °C, 97%; (d) DEAD, PPh_3, 4-nitrophenol, THF, rt; (e) NaH, MeI, DMF, 0 °C to rt, 77% (2 steps); (f) 2 N HCl−AcOEt, rt, 100%.

known (*S*)-**1**[11] was Boc-protected, followed by treatment with dimethylcarbamyl chloride to afford (*S*)-**2**. Reduction of the ester group of (*S*)-**2** with $LiAlH_4$ gave primary alcohol (*S*)-**3**. The alcohol (*S*)-**3** was reacted under Mitsunobu conditions

(5) (a) Mackenna, M. T.; Proctor, G. R.; Young, L. C.; Harvey, A. L. *J. Med. Chem.* **1997**, *40*, 3516. (b) Hirai, K.; Kato, K.; Nakayama, T.; Hayako, H.; Ishihara, Y.; Goto, G.; Miyamoto, M. *J. Pharmocol. Exp. Ther.* **1997**, *280*, 1261.

(6) (a) Kryger, G.; Silman, I.; Sussman, J. L. *J. Physiol. Paris* **1988**, *92*, 191. (b) Sugimoto, H. *Chem. Rec.* **2001**, *1*, 63.

(7) Kaur, J.; Zhang, M.-Q. *Curr. Med. Chem.* **2000**, *7*, 273.

(8) (a) Enz, A.; Amstutz, R.; Hofmann, A.; Gmelin, G.; Kelly, P. H. *Klin. Pharmakol.* **1989**, *2*, 271. (b) Sramek, J. J.; Anand, R.; Wardle, T. S.; Irwin, P.; Hartman, R. D.; Cutler, N. R. *Life Sci.* **1993**, *58*, 1201. (c) Spencer, C. M.; Noble, S. *Drugs Aging* **1998**, *13*, 391. (d) Jann, M. W. *Pharmacotherapy* **2000**, *20*, 1.

(9) Golstein, J.; Schreiber, S.; Velkeniers, B.; Vanhaelst, L. *Eur. J. Pharmacol.* **1983**, *91*, 239.

(10) There is tether-length dependence to AChEI potency enhancement. The compounds possessing a longer tether showed stronger AChEI activities; however, only the type A compounds possessing adequate tether length (m = 1) showed SERT inhibitory activities.

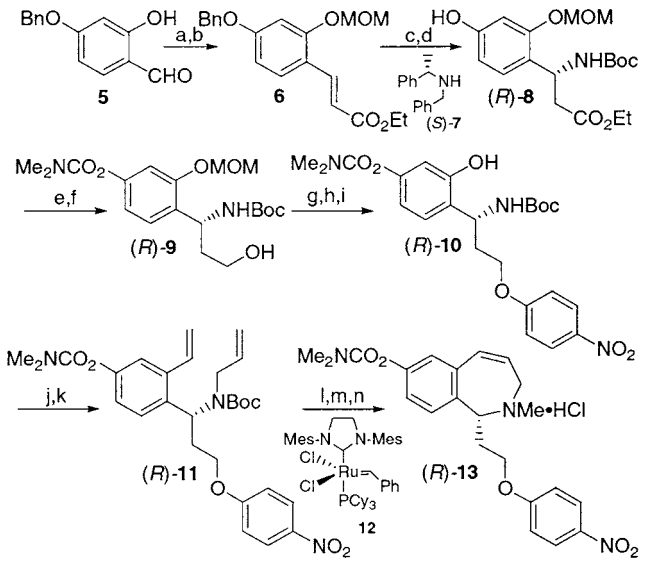
(11) Davies, S. G.; Ichihara, O. *Tetrahedron: Asymmetry* **1991**, *2*, 183.

with *p*-nitrophenol, followed by methylation of the Boc-protected amine with iodomethane. Finally, deprotection of the Boc group with 2 N HCl in ethyl acetate provided (*S*)-**4**. Enantiomer (*R*)-**4** was prepared by the same method starting from (*R*)-**1**.[11]

Compound (*S*)-**4** showed potent inhibitory activities against both AChE (IC_{50} = 101 nM) and SERT (IC_{50} = 42 nM). However, though enantiomer (*R*)-**4** showed similar potency against AChE (IC_{50} = 143 nM), SERT inhibition activity (IC_{50} = 135 nM) was slightly diminished compared to that of (*S*)-**4**.[12]

Type B compound (*R*)-**13** (*n* = 1)[13] was synthesized as shown in Scheme 3. The phenol group of **5**[14] was protected

Scheme 3[a]

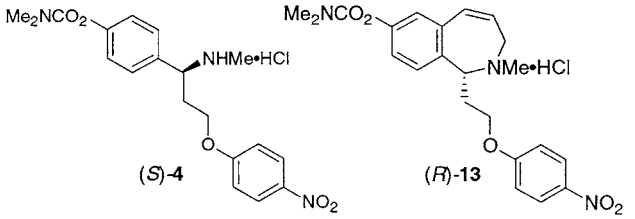


[a] (a) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 79%; (b) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 0 °C, 99%; (c) (i) (*S*)-**7**, *n*-BuLi, THF, −78 °C; (ii) H_2, 20% $Pd(OH)_2$/C, MeOH−H_2O−AcOH (20:2:1), rt, 67% (2 steps); (d) Boc_2O, Et_3N, MeOH, rt, 97%; (e) Me_2NCOCl, K_2CO_3, DMF, rt, 97%; (f) $LiAlH_4$, THF, −50 to 0 °C, 82%; (g) 4-nitrophenol, DEAD, PPh_3, THF, rt; (h) concentrated HCl, MeOH, rt, 68% (2 steps); (i) Boc_2O, Et_3N, MeOH, rt, 85%; (j) (i) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (ii) $(CH_2{=}CH)SnBu_3$, cat. $Pd(PPh_3)_4$, LiCl, dioxane, 100 °C, 86% (2 steps); (k) allyl bromide, NaH, DMF, 0 °C to rt, 86%; (l) **12** (10 mol %), CH_2Cl_2 (15mM), 45 °C, 96%; (m) 2 N HCl−AcOEt, rt, 96%; (n) (i) 37% HCHO aq., HCO_2H, 80 °C; (ii) 2 N HCl−AcOEt, rt, 78% (2 steps).

with a methoxymethyl group (MOM), and a Horner−Emmons reaction provided **6**. The ester **6** was subjected to the chiral amination method that was reported by Davies,[15] and the resulting amino group was subsequently protected with Boc to yield (*R*)-**8** (99% ee).[16] Compound (*R*)-**8** was treated with dimethylcarbamyl chloride and reduced with $LiAlH_4$ to provide alcohol (*R*)-**9**. The alcohol (*R*)-**9** was reacted with *p*-nitrophenol under Mitsunobu conditions. Treatment with concentrated HCl and Boc protection of the amino group afforded phenol (*R*)-**10**, which was converted to the aryl triflate by treatment with trifluoromethanesulfonic anhydride. The resulting aryl triflate was coupled with tributylvinylstannane by a palladium-catalyzed Stille reaction[17] to afford the corresponding styrene compound. N-Allylation of the obtained styrene compound was accomplished by treating with allyl bromide and NaH to give (*R*)-**11**. Ring-closing olefin metathesis of (*R*)-**11** with 10 mol % of Grubbs catalyst **12**[18] gave the desired seven-membered ring product in high yield. Deprotection of the Boc group in the resulting cyclic compound by 2 N HCl in ethyl acetate, followed by Eshweiler-Clarke methylation, provided (*R*)-**13**. Enantiomer (*S*)-**13** was prepared by the same method by using chiral amine (*R*)-**7**.[15]

Surprisingly, the inhibitory activities of (*R*)-**13** against both AChE and SERT (IC_{50} = 14 and 6 nM, respectively) were extremely enhanced as compared to those of **4** (Table 1).

Table 1. Inhibitory Actibities of **4** and **13** against AChE



	AChE IC_{50} (nM)	SERT IC_{50} (nM)
(*S*)-**4**	101	42
(*S*)-**13**	609	930
(*R*)-**13**	14	6

Although both enantiomers of **4** showed similar AChE inhibitory activities, (*R*)-**13** showed inhibitory activity against AChE that was extremely stronger than that of (*S*)-**13**. We supposed that in the case of the ring closure compound, the restriction of the conformational flexibility of the amine moiety induced the appropriate binding to AChE.[19]

These compounds hardly inhibited butyrylcholinesterase and choline acetyltransferase (IC_{50}'s > 100 μM) and showed weak affinities for transporters of norepinephrine and

(12) By our assay procedures, rivastigmine showed weak inhibitory activity against AChE (IC_{50} = 11 μM). Donepezil showed potent inhibitory activity only against AChE (IC_{50} = 10 nM), and fluoxetine showed inhibitory activities only against SERT (IC_{50} = 180 nM).

(13) Seven-membered ring compound (*m* = 1, *n* = 1, X = O) showed the most potent AChE and SERT inhibitory activities.

(14) Mendelson, W. L.; Holmes, M.; Dougherty, J. *Synth. Commun.* **1996**, *26*, 593.

(15) Davies, S. G.; Walters, A. S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1129.

(16) The determination of optical purity and absolute configuration of (*R*)-**8** are included in Supporting Information.

(17) For a recent review, see: Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1.

(18) For a recent review, see: (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413. (b) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3013. (c) Yet, L. *Chem. Rev.* **2000**, *100*, 2963.

(19) Since there is no information on the structure of SERT, the reason of the opposite stereochemical preference is unclear.

dopamine (IC_{50}'s > 10 μM). In addition, (*S*)-**4** and (*R*)-**13** had weak affinities for existing neurotransmitter receptors such as serotonin, norepinephrine, and dopamine (IC_{50}'s > 10 μM). Compound (*S*)-**4** shows more potent inhibitory activities than (*R*)-**13** against AChE in the brain following an oral administration (Figure 2).[20,21] The difference in efficiency between these two compounds could be attributed to the difference in absorption from the intestine and/or penetration into the brain.

In summary, we designed and synthesized AChE−SERT dual inhibitors **4** and **13** on the basis of a hypothetical model of the AChE active site. Additionally, ring-closing compound (*R*)-**13** showed extremely potent inhibitory activities against both AChE and SERT. Compound (*S*)-**4** is the first compound that can facilitate both the cholinergic and serotonergic transmission. Further pharmacological evaluation of these compounds targeting Alzheimer's disease is underway.

(20) The brain of mouse was isolated 1 h after an oral administration of compounds, and AChE and/or SERT activity were measured. Each symbol represents the mean of five mice. The doses (95% confidence interval) of (*S*)-**4** necessary for 50% inhibition of AChE and SERT in the brain were determined as 13 (11−15) and 2.4 (1.9−2.9) mg/kg, p.o., respectively. Details will be reported soon.

(21) The SERT inhibition of *R*-**13** was not determined because a low penetration of the compound into the brain was predicted from the ex vivo study of AChE inhibition.

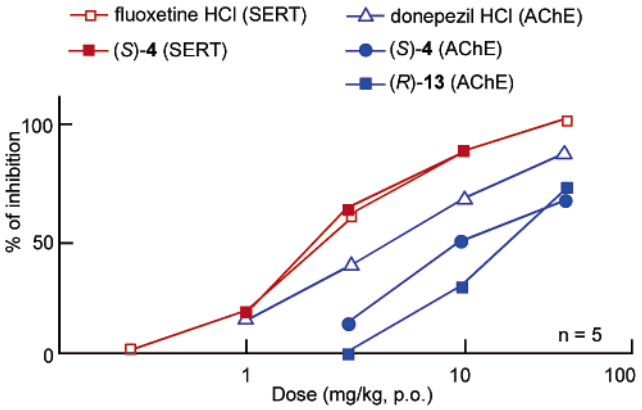


Figure 2. Inhibition of AChE and SERT in the brain following an oral administration in mice (ex vivo assay).

Supporting Information Available: Synthetic procedures and characterization of new compounds, in vitro assay of (*S*)-**4** and (*R*)-**13**, and the determination of optical purity and absolute configuration of (*R*)-**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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