Novel Piperidine Derivatives. Synthesis and Anti-Acetylcholinesterase Activity of 1-Benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine Derivatives

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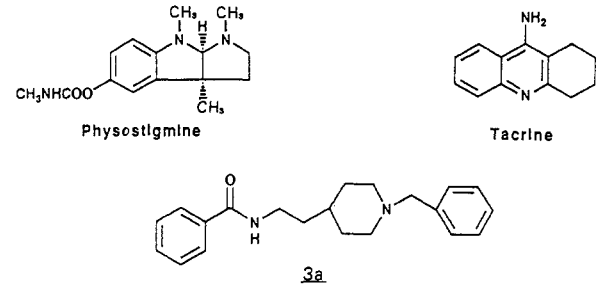
A series of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine derivatives was synthesized and evaluated for anti-acetylcholinesterase (anti-AChE) activity. Substituting the benzamide with a bulky moiety in the para position led to a substantial increase in activity. Introduction of an alkyl or phenyl group at the nitrogen atom of benzamide dramatically enhanced the activity. The basic quality of the nitrogen atom of piperidine appears to play an important role in the increased activity, since the *N*-benzoylpiperidine derivative was almost inactive. We found that 1-benzyl-4-[2-[*N*-[4′-(benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine hydrochloride (**21**) (IC_{50} = 0.56 nM) is one of the most potent inhibitors of acetylcholinesterase. Compound **21** showed an affinity 18 000 times greater for AChE than for BuChE. At a dose of 3 mg/kg, **21** produced a marked and significant increase in acetylcholine (ACh) content in the cerebral vortex and hippocampus of rats. Compound **21** was chosen for advanced development as an antidementia agent.

In senile dementia of the Alzheimer's type (SDAT), there is a selective loss of choline acetyltransferase (ChAT) from the cerebral cortex. This enzyme catalyzes the synthesis of ACh from choline.[1,2] The degree of dementia and memory impairment that occurs in this condition is well correlated with the decrement in cortical cholinergic transmission.[3] Moreover, scopolamine, a cholinergic antagonist, can cause memory impairment in normal individuals similar to that in aging.[4] These findings suggest that impaired cortical cholinergic transmission may be at least partly responsible for the symptoms of Alzheimer's disease. Therefore, enhancement of the activity of cholinergic neurons has been regarded as one of the most promising methods for treating these patients. In support of this suggestion, it has been reported that physostigmine and 1,2,3,4-tetrahydro-9-aminoacridine (tacrine), which potentiate the actions of ACh by inhibiting the degrading enzyme AChE, can bring about memory improvement in Alzheimer's patients.[5,6]

The purpose of the present study was to find a new type of acetylcholinesterase (AChE) inhibitor. Using random screening, we found that 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine (**3a**) had anti-AChE activity. We report here the results of our study on the synthesis and the structure–activity relationships of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine derivatives (Chart I).[7]

Chart I



Chemistry

Compound **3m** was synthesized as shown in Scheme I. Compound **1** was reduced by $LiAlH_4$ to yield amine **2**, which underwent acylation to form compounds **3a–r**. Reaction of *p*-bromothiophenol (**4**) with benzyl bromide yielded *p*-bromophenyl benzyl thioether, which was subjected to the Grignard reaction to yield *p*-(benzylthio)-benzoic acid. The acid derivative was subjected to oxidation with H_2O_2 and treated with $SOCl_2$ followed by a condensation with 4-(2-aminoethyl)-1-benzylpiperidine (**2**) to give 1-benzyl-4-[2-[*N*-[4′-(benzylsulfonyl)benzoyl]-amino]ethyl]piperidine hydrochloride (**3m**).

Compound **1** was converted to the 1-benzyl-4-piperidineacetaldehyde (**6**) as shown in Scheme II. Phenacyl bromide (**7**) was converted to diethyl (2-oxo-2-phenylethyl)phosphonate (**8**) by reaction with triethyl phosphite. Condensation of compound **6** with compound **8**, followed by catalytic hydrogenation, gave the 4-(1-benzylpiperidin-4-yl)butylophenone (**9**). Condensation of benzaldehyde (**10**) with compound **2** gave a Schiff base (**11**), which was subjected to reduction with sodium borohydride to yield compound **12**.

N-Substituted benzamide derivatives **13–15** and **20** were synthesized as shown in Scheme III. Compound **3s** was allowed to react with alkyl iodides in the presence of sodium hydride to give *N*-alkylbenzamide derivatives **13** and **14**. Reaction of compound **12** with benzoyl chloride led to the formation of *N*-benzylbenzamide derivative **15**. 1-Benzoyl-4-piperidine (**16**) was converted to *N*-benzoyl-4-[phenylcarbamoyl)methyl]piperidine (**19**) in five steps. Compound **19** was reduced with $LiAlH_4$ followed by a condensation with benzoyl chloride to give compound **20**.

Compound **13** underwent catalytic reduction (H_2/Pd–C) followed by reaction with the appropriate alkyl, aroyl, and aralkyl chloride to afford 4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]-1-substituted-piperidine derivatives **25–36** (Scheme IV).

Structure–Activity Relationships

A new series of piperidine derivatives were tested for biological activity in vitro. A mouse brain homogenate was used as the AChE source and the esterase activity was determined according to the method of Ellman et al.[8]

(1) Davies, P.; Maloney, A. J. *Lancet* **1976**, *2*, 1403.
(2) Richter, J. A.; Perry, K. E.; Tomlinson, B. E. *Life Sci.* **1980**, *26*, 1683.
(3) Perry, K. E.; Tomlinson, B. E.; Blessed, G.; Bergman, K.; Gibson, P. H.; Perry, R. H. *Br. Med. J.* **1979**, *2*, 1457.
(4) Drachman, D. A.; Leavitt, J. B. *Arch. Neurol.* **1974**, *30*, 113.
(5) Davis, K. L.; Mohs, R. C.; Timklenberg, J. R. *N. Engl. J. Med.* **1979**, *301*, 946.
(6) Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Aachiki, K.; Kling, A. *N. Engl. J. Med.* **1986**, *315*, 1241.
(7) Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Kawakami, Y.; Araki, S.; Nakamura, T. *J Pharm. Sci.* **1987**, *76*, S173.
(8) Ellman, G. L.; Courtney, D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

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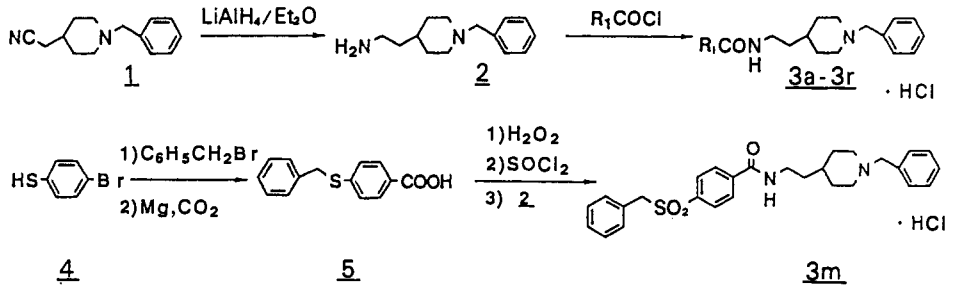
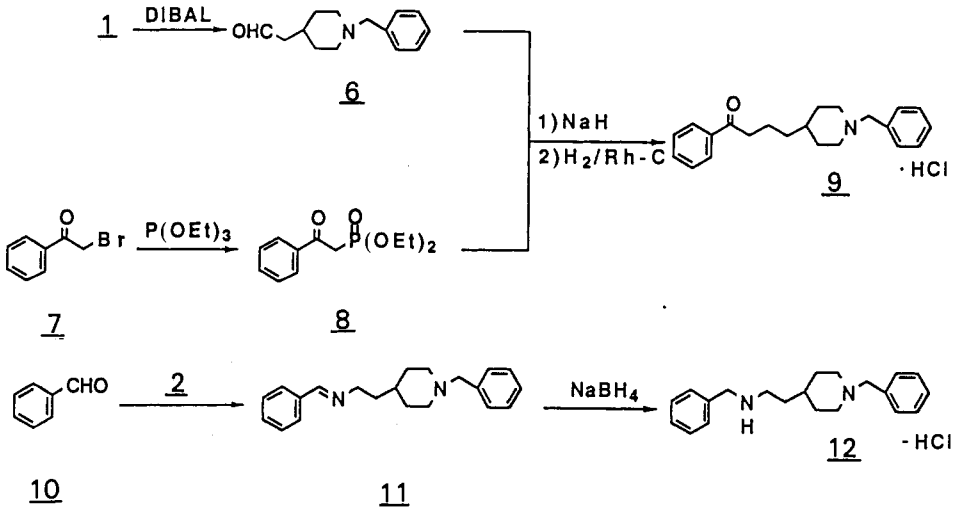
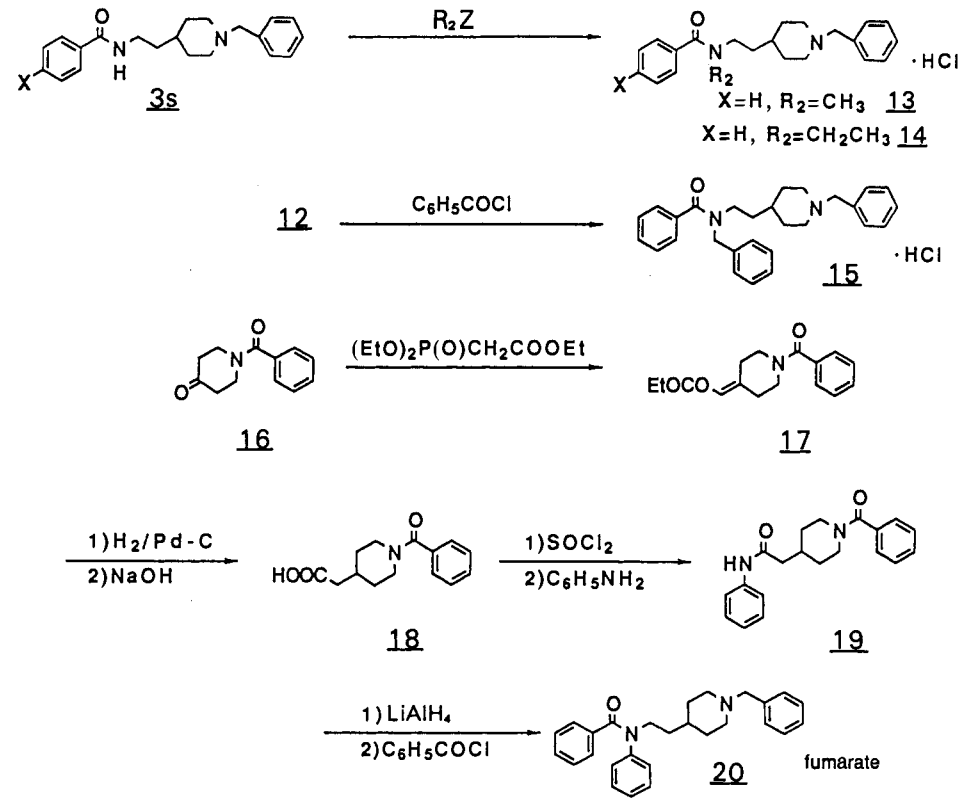
Scheme I



Scheme II



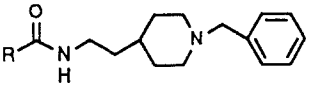
Scheme III

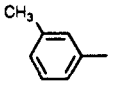
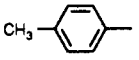
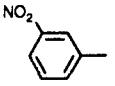
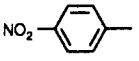
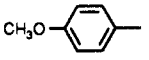
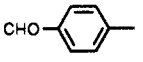
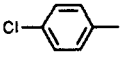
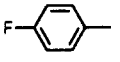
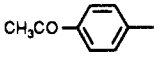
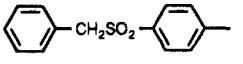
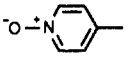


To study the structure–activity relationships, we divided the parent structure of **3a** into four regions: the phenyl group, the amide group, the piperidine ring, and the benzyl group. First, we varied the substituent group at the 2-, 3-, and 4-positions of the phenyl ring of the benzoyl moiety. The phenyl ring was then replaced by other rings.

The effect of the substituent R_1 in 1-benzyl-4-[2-[*N*-(substituted-acyl)amino]ethyl]piperidine derivatives on the

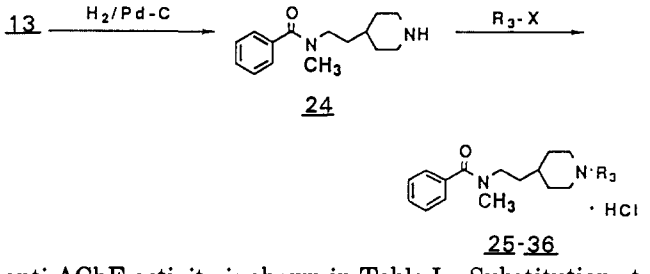
Table I. 1-Benzyl-4-[2-[*N*-(substituted-acyl)amino]ethyl]piperidine Derivatives



no.	R	yield %	mp, °C (recrytn solv[a])	formula[b]	inhibn of AChE:[c] IC_{50}, nM
3a		65	205–206 (A)	$C_{21}H_{26}N_2O$·HCl	560
3b		60	128–130 (A)	$C_{22}H_{28}N_2O$	1000[d]
3c		51	83–84 (B)	$C_{22}H_{28}N_2O$	470[d]
3d		82	98–99 (C)	$C_{22}H_{28}N_2O$	180[d]
3e		70	152–154 (A)	$C_{21}H_{25}N_3O_3 \cdot C_4H_4O_4$[e]	880
3f		61	148–149 (C)	$C_{21}H_{25}N_3O_3$·HCl	230
3g		85	114–115 (B)	$C_{21}H_{25}N_3O_3$	55[d]
3h		53	168–169 (A)	$C_{22}H_{28}N_2O_2$·HCl	88
3i		65	176–177 (B)	$C_{22}H_{26}N_2O_2$·HCl	120
3j		51	196–198 (B)	$C_{21}H_{25}N_2OCl$·HCl	180
3k		55	145–147 (B)	$C_{21}H_{25}N_2OF$·HCl	85
3l		64	186–188 (A)	$C_{23}H_{28}N_2O_2$·HCl	51
3m		49	187–188 (D)	$C_{28}H_{32}N_2O_3S$·HCl	29
3n		60	191–192 (C)	$C_{20}H_{25}N_3O$·2HCl	800
3o		41	126–128 (C)	$C_{20}H_{25}N_3O$·2HCl	69
3p		70	155–156 (C)	$C_{20}H_{25}N_3O \cdot C_4H_4O_4$[e]	39
3q		41	119–121 (B)	$C_{20}H_{25}N_3O_2$·HCl	155
3r		57	188–190 (C)	$C_{21}H_{32}N_2O \cdot C_4H_4O_4$[e]	1600

[a] A = EtOH–Et_2O, B = EtOH–IPE, C = MeOH–AlOEt, D = washed with acetone. [b] All compounds were analyzed within ±0.4% of the theoretical value for C, H, and N. [c] The inhibitory activities of acetylcholinesterase (AChE) were shown by IC_{50}. [d] The value of the hydrochloride. [e] Fumarate.

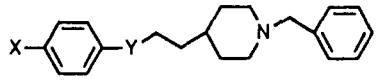
Scheme IV

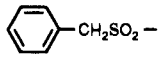
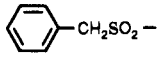
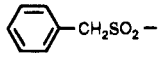


anti-AChE activity is shown in Table I. Substitution at the para position of the phenyl ring of R (**3d,g**) produced higher activity than substitution at the ortho and meta positions (Table I). When R was replaced by a cyclic alkyl group (**3r**), the activity of anti-AChE was decreased. The bulky and lipophilic moiety at the para position in the phenyl ring of R (**3l,m**) enhanced the inhibitory effect on AChE. The benzylsulfonyl moiety at the para position in the phenyl group of benzamide **21–23** brought about dramatic enhancement of activity (Table II).

For the amide moiety, when the CONH group was replaced by a $COCH_2$ group (**9**) there was no change in the activity, but when a CH_2NH group (**12**) was used the activity nearly disappeared. Introduction of a lower alkyl group (**13** and **14**) and a phenyl group (**20**) on the nitrogen

Table II. 1-Benzyl-4-[2-[*N*-(4′-substituted-benzoyl)-*N*-substituted-amino]ethyl]piperidine Derivatives



no.	X	Y	% yield	mp, °C (recrystn solv[a])	formula[b]	inhibn of AChE:[c] IC_{50}, nM
9	H	$-COCH_2-$	66	149–150 (B)	$C_{22}H_{27}NO\cdot HCl$	530
12	H	$-CH_2NH-$	58	273–276 (A)	$C_{21}H_{28}N_2\cdot HCl$	46000
13	H	$-CON(CH_3)-$	67	183–184 (A)	$C_{22}H_{28}N_2O\cdot HCl$	170
14	H	$-CON(C_2H_5)-$	73	223–224 (B)	$C_{23}H_{30}N_2O\cdot HCl$	130
15	H	$-CON(CH_2C_6H_5)-$	86	185–187 (B)	$C_{28}H_{32}N_2O\cdot HCl$	940
20	H	$-CON(C_6H_5)-$	98	100–103 (C)	$C_{27}H_{30}N_2O\cdot C_4H_4O_4$[d]	35
21		$-CON(CH_3)-$	78	200–201 (D)	$C_{29}H_{34}N_2O_3S\cdot HCl$	0.6
22		$-CON(C_2H_5)-$	84	106–108 (F)	$C_{30}H_{36}N_2O_3S$	0.3[e]
23		$-CON(C_6H_5)-$	98	211–213 (C)	$C_{33}H_{34}N_2O_3S\cdot HCl$	0.6

[a] A = EtOH–Et_2O, B = EtOH–IPE, C = MeOH–AcOEt, D = EtOH, E = washed with acetone, F = washed with IPE. [b] All compounds were analyzed within ±0.4% of the theoretical values for C, H, and N. [c] The inhibitory activities of AChE were shown by IC_{50}. [d] Fumarate. [e] The value of the hydrochloride.

atom of amide increased anti-AChE activity, whereas introduction of a benzyl group (**15**) decreased anti-AChE activity.

The basicity of the nitrogen atom of the piperidine group appears to have an important role in the degree of activity, since *N*-benzoylpiperidine analogue **33** was almost inactive. The effects on activity of introduction of substituents on the phenyl ring of *N*-benzylpiperidine are shown in Table III. Substitution at the ortho or para position of the phenyl ring caused a remarkable decrease in the activity of **25**, **27**, **28**, and **30**, but substitution at the meta position of the phenyl ring does not cause a decrease in the activity of **26** and **29**. When the distance between the nitrogen atom of piperidine group and the phenyl group of the benzyl moiety became greater than that of **13**, activity was lost dramatically (**31** and **32**). Replacement of R with hydrogen (**24**), cyclopropylmethyl (**34**), and adamantylmethyl caused a great reduction in potency, but replacement with cyclohexylmethyl caused retention of anti-AChE activity.

1-Benzyl-4-[2-[*N*-(4′-(benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine hydrochloride (**21**) (IC_{50} = 0.56 nM) is one of the most potent inhibitors of AChE in this series.

Biological Results

Compound **21** was the most potent inhibitor of AChE and showed a definite preference for AChE over the BuChE (about 18000-fold). On the other hand, physostigmine showed a preference for AChE over BuChE (11.7-fold). Tacrine showed a less selective inhibitory effect on AChE (Table IV). Compound **21** showed a greater affinity for AChE than either physostigmine or tacrine. It can therefore be expected that **21** may show a desirable effect in clinical studies of its use for SDAT, because AChE is distributed in the brain, which is the target organ.

The inhibitory effect of physostigmine on AChE was measured with and without preincubation. The IC_{50} of physostigmine was 0.69 nM with preincubation and 340 nM without preincubation. The reason for this phenomenon may depend on carbamylation of the enzyme.[9]

Compound **21** inhibited AChE reversibly in vitro in a concentration-dependent manner. After dialysis, the inhibition almost disappeared. This result indicates that the inhibition of AChE by this compounds is reversible and is the same as those of tacrine and physostigmine (Table V).[10-12]

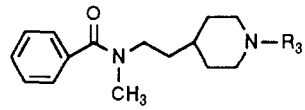
The results of ex vivo inhibitory effects on AChE in rat brain are shown in Figure 1. In rats, **21** at a dose of 1.0–3.0 mg/kg exerted a dose-dependent inhibitory effect on AChE in the cortex and hippocampus, both of which are reported to be involved in memory. Physostigmine at a dose of 0.3–1.0 mg/kg had a significant inhibitory effect in rat brain. This difference might be explained by the lower penetrating ability of **21** than that of physostigmine (unpublished data).

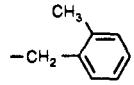
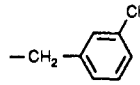
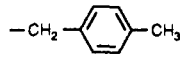
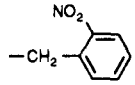
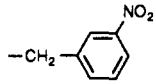
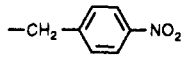
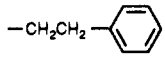
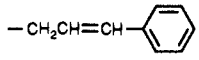
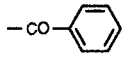
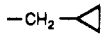
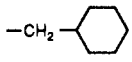
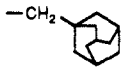
As we shown in Figure 2, physostigmine at a dose of 0.3–1.0 mg/kg increased the ACh content of rat brain. At a higher dose of 3.0 mg/kg, **21** produced a marked and significant increase in the rat cerebral cortex and hippocampus. These data reflect the result of ex vivo inhibitory effects on AChE in rat brain.

On the basis of these biological studies, it is concluded that **21** has a highly selective and very pronounced inhibitory effect on AChE, and that the parent structure of

(9) Groff, W. A.; Ellin, R. I.; Skalsky, R. L. *J. Pharm. Sci.* **1977**, *66* (3), 389.
(10) Patocka, J.; Bajgor, J.; Bielavsky, J.; Fusek, J. *Coll. Chech. Chem. Commun.* **1976**, *41*, 816.
(11) Tonkopil, V. D.; Ardabeva, T. V. *Bill. Eksp. Biol. Med.* **1978**, *86*, 441.
(12) Main, A. R. In *Essays in Toxicology*; ed. Wayland and Hagen, Eds.; Academic Press: New York, 1973; Vol. 4, pp 59–105.

Table III. 1-Substituted-4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]piperidine Derivatives



no.	R_3	% yield	mp, °C (recryst solv[a])	formula[b]	inhibn of AChE:[c] IC_{50}, nM
25		62	216–218 (D)	$C_{23}H_{30}N_2O\cdot HCl$	770
26		45	226–227 (B)	$C_{23}H_{30}N_2O\cdot HCl$	145
27		53	181–182 (B)	$C_{23}H_{30}N_2O\cdot HCl$	41000
28		65	176–177 (B)	$C_{22}H_{27}N_3O_3\cdot HCl$	14000
29		44	216–217 (B)	$C_{22}H_{27}N_3O_3\cdot HCl$	370
30		84	214–215 (B)	$C_{22}H_{27}N_3O_3\cdot HCl$	3300
31		47	197–198 (A)	$C_{23}H_{30}N_2O\cdot HCl$	13000
32		48	150–151 (C)	$C_{24}H_{30}N_2O\cdot HCl$	54000
33		88	oil	$C_{22}H_{26}N_2O_2$	52000
24		86	169 (C)	$C_{15}H_{22}N_2O\cdot C_4H_4O_4$[d]	26000
34		47	101–102 (A)	$C_{19}H_{28}N_2O\cdot HCl$	38000
35		50	166–168 (D)	$C_{22}H_{34}N_2O\cdot HCl$	410
36		47	223–224 (C)	$C_{26}H_{38}N_2O\cdot HCl$	24000

[a] A = EtOH–Et_2O, B = EtOH–IPE, C = MeOH–AcOEt, D = AcOEt–IPE. [b] All compounds were analyzed within ±0.4% of the theoretical values for C, H, and N. [c] The inhibitory activities were shown by IC_{50}. [d] Fumarate.

Table IV. Inhibitory Effect of 21 and Reference Compounds on AChE and BuChE Activity (in Vitro)

compound	IC_{50},[a] nM: AChE activity	IC_{50},[a] nM: BuChE activity	ratio of IC_{50} (BuChE/AChE)
21	0.56 (0.42–0.71)	10100 (8196–11971)	18000
physostigmine	0.69 (0.63–0.74)	8.1 (7.8–8.4)	11.7
tacrine	81 (72.6–88.6)	73 (72.1–73.9)	0.9

[a] AChE source was rat brain homogenate; BuChE source was rat serum. $N = 4$. Numbers in a parentheses represent a range of 95% confidence limits.

Table V. Reversibility of AChE Inhibition by 21

sample	before dialysis: AChE activity[a]	before dialysis: percentage of control	after dialysis: AChE activity[a]	after dialysis: percentage of control
control	16.37	100	17.9	100
21 (0.27 μM)	9.10	56	17.7	99
21 (0.91 μM)	5.42	33	17.8	99
21 (91 nM)	2.59	16	16.5	92

[a] Activity of AChE is expressed as ΔA_{412} min × 10^2.

21 is a leading new candidate for treatment of SDAT.

Experimental Section

All melting points were determined on a Yanagimoto micro-melting apparatus, unless otherwise specified, and are uncorrected. ^1H NMR spectra were taken with a JEOL FX-90Q spectrometer using Me_4Si as an internal standard. Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values.

4-(2-Aminoethyl)-1-benzylpiperidine (2). To a suspension of $LiAlH_4$ (49.4 g, 1.30 mol) in THF (2000 mL) was added 1-(2-cyanoethyl)-4-benzylpiperidine (1, 232.2 g, 1.08 mol). The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water and the resulting slurry was filtered off. The filtrate was dried over $MgSO_4$. The solvent was removed to give 215.5 g (91.1%) of 2: ^1H NMR ($CDCl_3$) δ 1.30–1.95 (m, 11 H, piperidine), 2.76 (m, 4 H, NH_2, piperidine), 3.45 (s, 2 H, CH_2), 7.15 (s, 5 H, aromatics).

1-Benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine Hydrochloride (3a). To a mixture of 2 (5.50 g, 25.0 mmol) in 10% potassium carbonate aqueous (35 mL) and $CHCl_3$ (35 mL) was added dropwise a solution of benzoyl chloride (3.90 g, 27.5 mmol) in $CHCl_3$ at 0 °C. After stirring for 30 min, the $CHCl_3$ layer was washed with water, dried over K_2CO_3, and evaporated. The crude residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 100:1) to give 8.20 g of free base (3a). This was treated with EtOAc–HCl, and the resulting crystals were re-

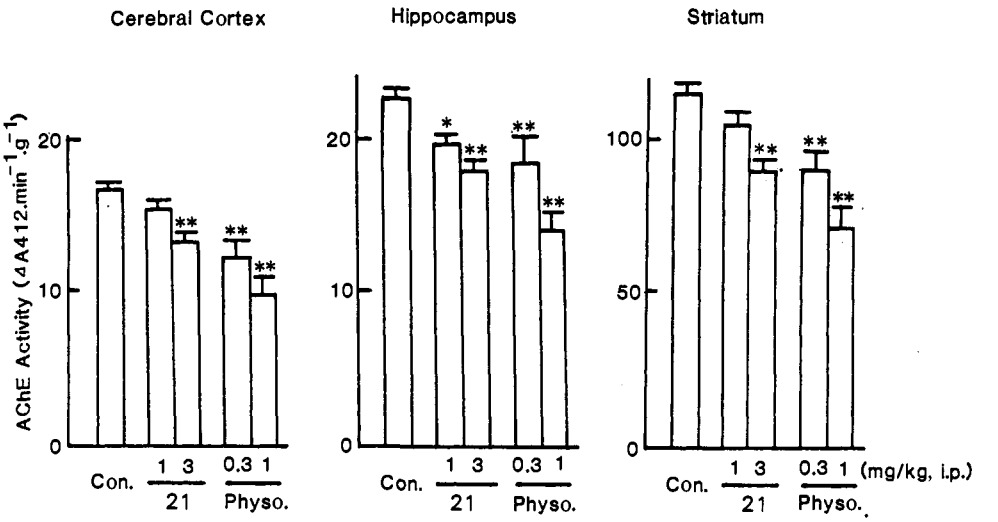


Figure 1. Effect of **21** and physostigmine on rat brain AChE (ex vivo). The compounds were administered intraperitoneally at two doses 1 h before decapitation. Ordinate indicates AChE activity (ΔA_{412} min^{-1} g^{-1}). Each column represents the mean ± SEM for six determinations. *$p < 0.05$, **$p < 0.01$.

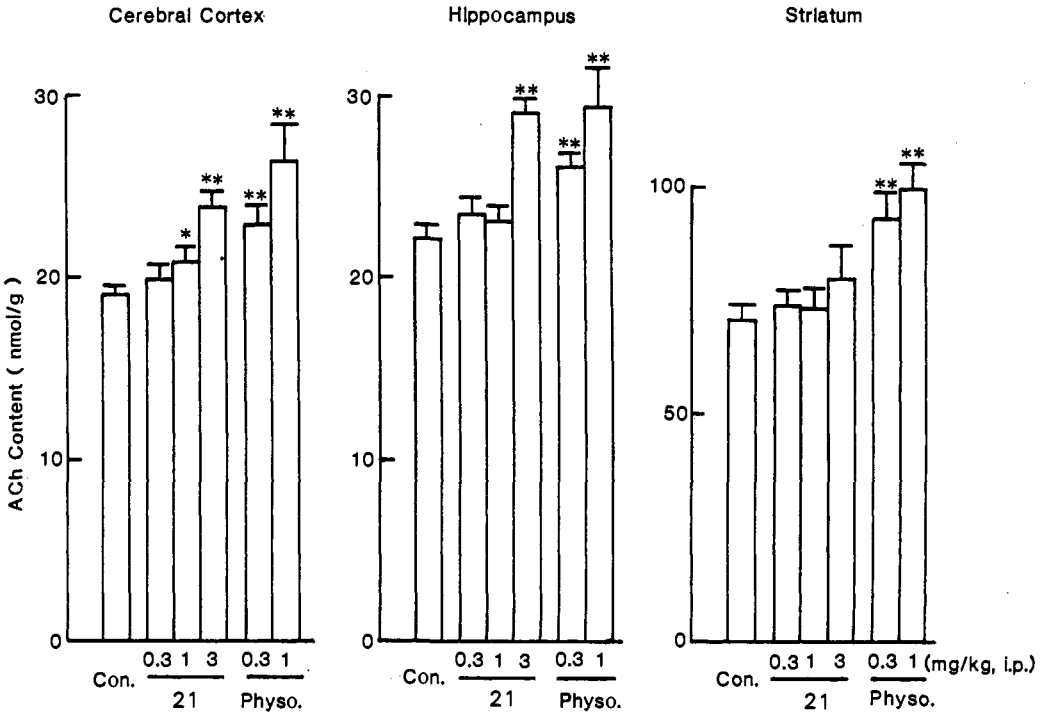


Figure 2. Effect of **21** and physostigmine on AChE content in rat brain. The compounds were administered intraperitoneally at two doses 1 h before microwave irradiation. Ordinate indicates ACh content (nmol/g). Each column represents the mean ± SEM for six determinations. *$p < 0.05$, **$p < 0.01$.

crystallized from EtOH–Et_2O to give 5.80 g (65.1%) of **3a**, mp 205–206 °C. Anal. ($C_{21}H_{26}N_6O$·HCl) C, H, N.

***p*-(Benzylthio)benzoic Acid (5).** To a suspension of *p*-bromothiophenol (**4**, 100 g, 528 mmol) and sodium hydride (60% dispersion in mineral oil, 25.4 g, 634 mmol) in DMF was added benzyl bromide (90.5 g, 528 mmol) at 0 °C. The mixture was stirred for 1 h at 50–60 °C. Water was added to the reaction mixture and it was then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over $MgSO_4$, and the solvent was removed in vacuo. The resultant solid residue was washed with hexane to give 98.3 g (66.7%) of *p*-bromophenyl benzyl thioether: mp 63–64 °C;[13] ^1H NMR ($CDCl_3$) δ 4.04 (s, 2 H, CH_2), 7.24 (m, 9 H, aromatics). Anal. ($C_{13}H_{11}BrS$) C, H.

To a mixture of Mg (6.60 g, 270 mmol) and ethylene dibromide (0.50 g) in THF (100 mL) was added dropwise a solution of *p*-bromophenyl benzyl thioether (62.5 g, 220 mmol) in THF (150 mL). After the mixture was refluxed for 1 h, dry CO_2 gas was passed through the mixture for 3 h. To the mixture was added 1 N HCl and it was extracted with EtOAc. The EtOAc layer was washed with water, dried over $MgSO_4$, and evaporated to give crude *p*-(benzylthio)benzoic acid (**5**). The crude compound was recrystallized from MeOH–H_2O to give 30.5 g (55.0%) of **5**: mp 187–189 °C dec;[14] ^1H NMR (DMSO-d_6) δ 4.32 (s, 2 H, CH_2), 7.34 (m, 7 H, aromatics), 7.80 (d, 2 H, aromatics). Anal. ($C_{13}H_{11}OS$) C, H.

1-Benzyl-4-[2-[*N*-[4′-(benzylsulfonyl)benzoyl]amino]ethyl]piperidine Hydrochloride (3m). To a solution of **5** (29.5 g, 120 mmol) in AcOH (400 mL) was added dropwise 33% H_2O_2 (40 mL) at 60 °C. After stirring for 90 min at 70 °C, the reaction mixture was poured into ice water (300 mL). The precipitate was collected and washed with water to give 28.9 g (87.0%) of *p*-(benzylsulfonyl)benzoic acid: mp 270–273 °C dec; ^1H NMR

(13) Vowinkel, E. *Synthesis* **1974**, *6*, 430.

(14) Pettit, L. D.; Sherrington, C. *J. Chem. Soc. A* **1968**, 3078.

(DMSO-d_6) δ 4.72 (s, 2 H, CH_2), 7.24 (m, 5 H, aromatics), 7.94 (dd, 4 H, aromatics). Anal. ($C_{13}H_{11}O_4S$) C, H.

A mixture of *p*-(benzylsulfonyl)benzoic acid (13.8 g, 50.0 mmol) and thionyl chloride (100 mL) was heated under reflux for 2 h. The excess thionyl chloride was removed under reduced pressure and the residue was washed with *n*-hexane to give 12.8 g (87.0%) of *p*-(benzylsulfonyl)benzoyl chloride.

A mixture of *p*-(benzylsulfonyl)benzoyl chloride (4.10 g, 14.0 mmol), **2** (3.00 g, 14.0 mmol), and triethylamine (2.80 g, 28.0 mmol) in THF (150 mL) was stirred under reflux for 40 min. The solvent was removed and the residue was placed in $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over $MgSO_4$, and evaporated to give the crude product. It was purified by silica gel column chromatography ($CHCl_3$–MeOH 100:1) to give the free base, which was converted to the hydrochloride with HCl–EtOAc and washed with acetone to give 3.55 g (49.4%) of **3m**: mp 186.5–187.5 °C. Anal. ($C_{28}H_{32}N_2O_3S$·HCl) C, H, N.

1-Benzyl-4-(4-oxo-4-phenylbutyl)piperidine Hydrochloride (9). To a solution of **1** (2.00 g, 9.00 mmol) in THF (20 mL) was added DIBAL (9.33 mL) at 0 °C. This mixture was stirred at room temperature for 1 h. To the mixture was added MeOH (1.5 mL), water (1.5 mL), and 20% H_2SO_4 (20 mL) at 0 °C. After 10 min, the mixture was made alkaline with saturated aqueous NaOH and extracted with EtOAc (100 mL). The EtOAc layer was washed with water, dried over $MgSO_4$, and evaporated to give crude 2-(1-benzylpiperidin-4-yl)acetoaldehyde (**6**) as a pink oil. This crude compound was purified by silica gel column chromatography (CH_2Cl_2–MeOH 20:1) to give 1.44 g (71.0%) of **6**: ^{1}H NMR ($CDCl_3$) 1.15–2.15 (m, 7 H, piperidine), 2.43 (dd, 2 H, CH_2), 2.84 (d, 2 H, piperidine), 3.46 (s, 2 H, benzyl), 7.21 (s, 5 H, aromatics), 9.63 (s, 1 H, CHO).

To triethyl phosphate (5.84 g, 35.0 mmol) was slowly added dropwise a solutio of phenacyl bromide (**7**, 5.00 g, 25.0 mmol) in ether (30 mL) at 10 °C for 30 min and evaporated to remove the solvent. The residue was placed in CH_2Cl_2 and the CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc 2:1) to give 4.05 g (63.0%) of diethyl (2-oxo-2-phenylethyl)phosphonate (**8**). The ^{1}H NMR spectrum was in agreement with the reported data.[15]

To a suspension of NaOH (0.23 g, 5.80 mmol) in THF (3 mL) was added a solution of **8** (1.47 g, 5.70 mmol) in THF (10 mL) at 0 °C. After stirring at room temperature for 30 min, a solution of **6** (0.87 g, 4.00 mmol) in DMF (7 mL) was added to the mixture at 0 °C. The mixture was stirred at room temperature for 1 h and was then poured into water (2 mL) and evaporated to remove the solvent. The residue was placed in CH_2Cl_2 and the CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (100:1) to give 0.50 g (39.9%) of 1-benzyl-4-[4-oxo-4-phenyl-2-but-(*E*)-enyl]piperidine: ^{1}H NMR ($CDCl_3$) δ 1.10–2.13 (m, 7 H, piperidine), 2.26 (t, 2 H, CH_2), 2.88 (b d, 2 H, piperidine), 3.48 (s, 2 H, benzyl), 6.72–7.07 (m, 2 H, CH=CH), 7.30 (s, 5 H, aromatics), 7.10–8.00 (m, 5 H, aromatics).

A solution of 1-benyzl-4-[4-oxo-4-phenyl-2-but-(*E*)-enyl]-piperidine (0.30 g, 0.94 mmol) in MeOH (10 mL) was hydrogenated over 5% rhodium on carbon (0.10 g) at 1 atm for 4 h. The catalyst was filtered off and the filtrate was evaporated in vacuo to give the crude product. It was purified by silica gel column chromatography. The product was eluted with hexane–EtOAc (10:1) to give 0.20 g (66.0%) of free base. This was treated with HCl–EtOH, and the resulting crystals were recrystallized from MeOH–isopropyl ether to give 0.22 g (65.0%) of **9**: mp 149–150 °C. Anal. ($C_{22}H_{27}NO$·HCl) C, H, N.

1-Benzyl-4-[2-(*N*-benzylamino)ethyl]piperidine Dihydrochloride (12). A solution of benzyaldehyde (1.06 g, 10.0 mmol) and **2** (2.18 g, 10.0 mmol) in benzene was stirred under reflux for 2 h. The solvent was removed in vacuo. The residue was dissolved in MeOH, and $NaBH_4$ (0.76 g, 20.0 mmol) was added to the mixture. The mixture was evaporated and the residue was placed in CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated to give crude product. It was purified by silica gel column chromatography to give free base. This was converted to the hydrochloride with HCl–EtOAc, and the resulting crystals were recrystallized from EtOH–Et_2O to give 1.60 g (46.4%) of **12**, mp 273–276 °C dec. Anal. ($C_{21}H_{28}N_2$·2HCl) C, H, N.

1-Benzyl-4-[2-(*N*-benzoyl-*N*-benzylamino)ethyl]piperidine Hydrochloride (15). To a suspension of **12** (0.40 g, 1.00 mmol) in pyridine (10 mL) was added benzoyl chloride (0.15 g, 1.00 mmol). The mixture was stirred at room temperature overnight and was then evaporated to remove the solvent. The residue was placed in CH_2Cl_2, and the CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 50:1) to give 0.42 g of free base. It was converted to the hydrochloride with HCl–EtOAc, and the resulting crystals were recrystallized from MeOH–isopropyl ether to give 0.40 g (86.0%) of **15**, mp 185–187 °C. Anal. ($C_{28}H_{32}N_2O$·HCl) C, H, N.

1-Benzyl-4-[2-(*N*-benzoyl-*N*-phenylamino)ethyl]piperidine Fumarate (20). To a solution of 1-benzoyl-4-piperidone (**16**, 9.40 g, 49.7 mmol) and triethyl phosphonoacetate (11.2 g, 49.9 mmol) in EtOH (150 mL) was added EtONa (4.15 g, 61.0 mmol) at 0 °C. After the mixture was stirred for 1 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was pufified by silica gel column chromatography. The product was eluted with benzene–EtOAc (5:2) to give 12.7 g (98.0%) of 1-benzoyl-4-[(ethoxycarbonyl)-methylene]piperidine (**17**): ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3 H, CH_3), 2.2–2.6 (m, 6 H, piperidine), 2.96 (m, 2 H, piperidine), 3.50 (s, 2 H, benzyl), 4.12 (q, 2 H, CH_2), 5.62 (s, 1 H, CH), 7.28 (s, 5 H, aromatics).

A solution of **17** (12.7 g, 49.0 mmol) in EtOH (50 mL) was hydrogenated over 10% palladium on carbon (1.5 g) at 1 atm for 30 min. The catalyst was filtered off and the filtrate was evaporated in vacuo to give 12.0 g (93.7%) of the ethyl ester derivative.

A solution of 12.0 g of ethyl ester derivative in NaOH–EtOH–H_2O (1 g:80 mL:50 mL) was stirred at room temperature for 7 h. The reaction mixture was made acidic (pH 3–4) with concentrated HCl, extracted with CH_2Cl_2, dried over $MgSO_4$, and evaporated to give 4.70 g of (1-benzoylpiperidin-4-yl)acetic acid (**18**).

After a solution of **18** (4.70 g, 20.0 mmol) and thionyl chloride (8 mL) in benzene (30 mL) was refluxed, the solution was evaporated. To the residue was added THF (20 mL), and to the mixture was added a solution of aniline (1.86 g, 20.0 mmol) in THF (20 mL) at 0 °C. The reaction mixture was poured into water and extracted with CH_2Cl_2.

The CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography. The product was eluted with CH_2Cl_2–MeOH (10:1) to give 5.80 g (93.0%) of (1-benzoylpiperidine-4-yl)acetanilide (**19**): ^{1}H NMR ($CDCl_3$) δ 1.1–2.2 (m, 9 H, piperidine), 2.8 (d, 2 H, piperidine), 3.96 (s, 2 H, benzyl), 6.8–7.6 (m, 11 H, aromatics).

A mixture of **19** (0.90 g, 2.90 mmol) and $LiAlH_4$ (0.38 g, 10.0 mmol) in THF (30 mL) was refluxed for 1 h. Water was added to the mixture and it was extracted with EtOAc. The EtOAc layer was washed with water, dried over $MgSO_4$, and evaporated. To a solution of the residue (0.55 g) obtained above and triethylamine (1.20 g) in THF (15 mL) was added benzoyl chloride (0.54 g) dropwise at 0 °C. After the mixture was stirred for 2.5 h, the resulting mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography. The product was eluted with CH_2Cl_2–MeOH (10:1) to give 0.48 g of free base. It was treated with fumaric acid–EtOH, and the resulting crystals were recrystallized from MeOH–EtOAc to give 0.49 g (32.9%) of **20**: mp 100–103 °C; ^{1}H NMR ($CDCl_3$) 1.1–2.2 (m, 9 H, piperidine), 2.7–3.0 (m, 2 H, piperidine), 3.48 (s, 2 H, benzyl), 3.89 (m, 2 H, CH_2), 6.8–7.4 (m, 15 H, aromatics). Anal. ($C_{27}H_{30}N_2O$·$C_4H_4O_4$) C, H, N.

1-Benzyl-4-[2-[*N*-[4′-(benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine Hydrochloride (21). To a suspension of NaOH (0.37 g, 9.30 mmol) in DMF (2 mL) was added a solution of the free base of **3m** (1.38 g, 3.10 mmol) in DMF (8 mL). After 20 min, CH_3I (1.30 g, 9.30 mmol) was added to the mixture. The mixture was stirred at room temperature overnight.

(15) Mathey, F.; Savignac, Ph. *Tetrahedron* **1978**, *34*, 649.

EtOH at 0 °C was added to the mixture and it evaporated to remove the solvent. To the residue was added CH_2Cl_2 (100 mL); the CH_2Cl_2 layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 40:1) to give the free base. It was treated with HCl–EtOAc, and the resulting crystals were recrystallized from EtOH to give 1.19 g (72.8%) of **21**, mp 200–201 °C. Anal. ($C_{29}H_{34}N_2O_3S$·HCl) C, H, N.

1-Cinnamyl-4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]-piperidine Hydrochloride (32). A solution of **13** (3.00 g, 8.90 mmol) in MeOH (100 mL) was hydrogenated over 10% palladium on carbon (0.2 g) at 1 atm overnight. The catalyst was filtered off and the filtrate was evaporated in vacuo to give 2.20 g of 4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]piperidine (**24**). A mixture of **24** (2.00 g, 8.13 mmol), $NaHCO_3$ (0.70 g, 8.33 mmol), and cinnamyl bromide (1.60 g, 8.13 mmol) in EtOH (50 mL) was stirred under reflux for 3 h. After the solvent was evaporated, water was added to the residue and it was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried over K_2CO_3, and evaporated. The residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 50:1) to give 0.70 g (24.1%) of the free base. It was converted to the hydrochloride with HCl–EtOAc, and the resulting crystals were recrystallized from EtOH–EtOAc to give 0.65 (20.0%) of **32**, mp 150–151 °C. Anal. ($C_{24}H_{30}N_2O$·HCl) C, H, N.

Materials and Methods. Determination of Cholinergic Parameters. Acetylcholinesterase Activity in in Vivo Studies. The inhibitory effects of **21** on AChE and BuChE were compared with those of physostigmine and tacrine in in vivo experiments. A mouse brain homogenate was used as the AChE souce and the esterase activity was determined according to the method of Ellman et al.[8] Briefly, acetylcholine iodide (0.5 mM) as a substrate, a test compound, and 3,4-dinitrobenzene were added to the mouse brain homogenate, and the mixture was incubated for 2 min at 25 °C. The amount of a yellow substance formed after incubation was determined at 412 nm, with a spectrophotometer, for the AChE activity.

Reversibility of AChE Inhibition. Reversibility of AChE inhibition by **21** was determined in in vitro experiments. One hundred microliters of **21** (at an appropriate concentration) and 1 mL of mouse brain homogenate were packed into a seamless cellulose tube and dialyzed at 4 °C for 44 h in a phosphate buffer solution (pH 7.4). The AChE in the tube was assayed in triplicate before and after dialysis.

AChE Activity in ex Vivo Experiments and ACh Content in Rat Brain. Male Sprague–Dawley rats weighing 250–380 g were used. Physostigmine and **21** dissolved in distilled water were administered intraperitoneally. ·Control animals were given physiological saline. Rats were killed by decapitation 1 h after drug treatment for determination of AChE activity by focused microwave irradiation (9 kW, 1.0 s) for determination of ACh content. The brain was removed and some regions (cerebral cortex, hippocampus, and striatum) were dissected out and immersed in liquid nitrogen immediately after sacrifice and then were prepared for the experiments. The AChE activity in the tissues was determined by the method of Ellman et al.[8] at 25 °C for 2 min with 0.5 mM of acetylcholine iodide as the substrate. The regional ACh content was determined by high-performance liquid chromatography with electrochemical detection, according to a modification of Asano's method.[16]

Registry No. **1**, 126848-77-9; **2**, 86945-25-7; **3a**, 113027-39-7; **3a** free base, 126848-78-0; **3b**, 126848-79-1; **3b** free base, 126848-80-4; **3c**, 126848-81-5; **3c** free base, 126848-82-6; **3d**, 113027-87-5; **3d** free base, 126848-83-7; **3e**, 126848-85-9; **3e** free base, 126848-84-8; **3f**, 126848-86-0; **3f** free base, 126848-87-1; **3g**, 126848-88-2; **3g** free base, 113027-40-0; **3h**, 113027-85-3; **3h** free base, 126848-89-3; **3i**, 126848-90-6; **3i** free base, 113027-51-3; **3j**, 126848-91-7; **3j** free base, 113027-44-4; **3k**, 113027-94-4; **3k** free base, 126848-92-8; **3l**, 113027-86-4; **3l** free base, 126848-93-9; **3m**, 113045-22-0; **3m** free base, 126848-94-0; **3n**, 126848-95-1; **3n** free base, 126848-96-2; **3o**, 126848-97-3; **3o** free base, 113027-62-6; **3p**, 126848-99-5; **3p** free base, 126848-98-4; **3q**, 113045-52-6; **3q** free base, 126849-00-1; **3r**, 126849-02-3; **3r** free base, 126849-01-2; **4**, 106-53-6; **5**, 22855-95-4; **6**, 120014-32-6; **7**, 70-11-1; **8**, 3453-00-7; **9**, 120012-07-9; **9** free base, 126849-03-4; **10**, 100-52-7; **12**, 126849-04-5; **12** free base, 126849-05-6; **13**, 113045-46-8; **13** free base, 126849-06-7; **14**, 126849-07-8; **14** free base, 126849-08-9; **15**, 120012-96-6; **15** free base, 126849-09-0; **16**, 24686-78-0; **17**, 21363-69-9; **18**, 56772-11-3; **19**, 120014-22-4; **20**, 126849-11-4; **20** free base, 126849-10-3; **21**, 113045-24-2; **21** free base, 126849-12-5; **22**, 113027-97-7; **22** free base, 126849-13-6; **23**, 126849-14-7; **23** free base, 126849-15-8; **24**, 126849-16-9; **24** free base, 120014-28-0; **25**, 120012-78-4; **25** free base, 126849-17-0; **26**, 126849-18-1; **26** free base, 120013-05-0; **27**, 120013-04-9; **27** free base, 126849-19-2; **28**, 126849-20-5; **28** free base, 120013-06-1; **29**, 120013-07-2; **29** free base, 126849-21-6; **30**, 120013-01-6; **30** free base, 126849-22-7; **31**, 120012-84-2; **31** free base, 126849-23-8; **32**, 120012-82-0; **32** free base, 126874-75-7; **33**, 126874-76-8; **33** free base, 120013-00-5; **34**, 120013-15-2; **34** free base, 126849-24-9; **35**, 120011-92-9; **35** free base, 126849-25-0; **36**, 120011-91-8; **36** free base, 126849-26-1; $C_6H_5CH_2Br$, 100-39-0; o-MeC_6H_4COCl, 933-88-0; *m*-MeC_6H_4COCl, 1711-06-4; *p*-MeC_6H_4COCl, 874-60-2; *o*-$O_2NC_6H_4COCl$, 610-14-0; *m*-$O_2NC_6H_4COCl$, 121-90-4; *p*-$O_2NC_6H_4COCl$, 122-04-3; *p*-$MeOC_6H_4COCl$, 100-07-2; *p*-$OHCC_6H_4COCl$, 16173-52-7; *p*-ClC_6H_4COCl, 122-01-0; *p*-FC_6H_4COCl, 403-43-0; *p*-AcC_6H_4COCl, 31076-84-3; $(EtO)_2P(O)CH_2COOEt$, 867-13-0; *o*-$MeC_6H_4CH_2Br$, 89-92-9; *m*-$MeC_6H_4CH_2Br$, 620-13-3; *p*-$MeC_6H_4CH_2Br$, 104-81-4; *o*-$O_2NC_6H_4CH_2Br$, 3958-60-9; *m*-$O_2NC_6H_4CH_2Br$, 3958-57-4; *p*-$O_2NC_6H_4CH_2Br$, 100-11-8; $Ph(CH_2)_2Br$, 103-63-9; $PhCH{=}CHCH_2Br$, 4392-24-9; $AdCH_2Br$, 14651-42-4; benzoyl chloride, 98-88-4; 2-pyridinecarbonyl chloride, 29745-44-6; 3-pyridinecarbonyl chloride, 10400-19-8; 4-pyridinecarbonyl chloride, 14254-57-0; *N*-oxido-4-pyridinecarbonyl chloride, 39178-36-4; cyclohexanecarbonyl chloride, 2719-27-9; *p*-bromophenyl benzyl thioether, 53136-21-3; *p*-(benzylsulfonyl)benzoic acid, 70793-11-2; *p*-(benzylsulfonyl)benzoyl chloride, 113028-06-1; 1-benzyl-4-[4-oxo-4-phenyl-2-but-(*E*)-enyl)piperidine, 126849-27-2; 1-benzoyl-4-[(ethoxycarbonyl)methyl]piperidine, 54108-67-7; (bromomethyl)cyclopropane, 7051-34-5; (bromomethyl)cyclohexane, 2550-36-9.

(16) Asano, M.; Miyauchi, T.; Kato, T.; Fujimori, K.; Yamamoto, K. *J. Liq. Chromatog.* **1986**, *9* (1), 199.