Synthesis and Structure-Activity Relationships of Acetylcholinesterase Inhibitors: 1-Benzyl-4-(2-phthalimidoethyl)piperidine and Related Derivatives

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Following the discovery of a new series of 1-benzyl-4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]piperidine (**2**) derivatives with a potent anti-acetylcholinesterase (anti-AChE) activity, we extended the structure-activity relationships (SAR) to rigid analogues (**4**) and 1-benzyl-4-[2-(*N*-benzoyl-*N*-phenylamino)ethyl]piperidine derivatives (**3**). Introduction of a phenyl group on the nitrogen atom of the amide moieties resulted in enhanced activity. The rigid analogue containing isoindolone (**9**) was found to exhibit potent anti-AChE activity comparable to that of **2**. Furthermore, replacement of the isoindolone with other heterobicyclic ring systems was examined. Among the compounds prepared in these series, 1-benzyl-4-[2-[4-(benzoylamino)phthalimido]ethyl]piperidine hydrochloride (**19**) (IC_{50} = 1.2 nM) is one of the most potent inhibitors of AChE. Compound **19** showed a definite selectivity to AChE over the BuChE (about 34700-fold) and, at dosages of 10–50 mg/kg, exerted a dose-dependent inhibitory effect on AChE in rat brain.

Multiple central nervous system dysfunctions have been identified in senile dementia of the Alzheimer's type (SDAT), including changes in the concentration of norepinephrine, serotonin, dopamine, and various peptides,[1] but by far the most consistent finding is a large reduction of choline acetyltransferase (ChAT) activity. ChAT is an enzyme located exclusively within cholinergic neurons and thus serves as biochemical marker for these cells. This marked decrease of ChAT activity has been demonstrated in the hippocampus and cortex, areas of the brain believed to be associated with learning and memory.[2-11]

These findings suggest that impaired cortical cholinergic transmission may be at least partly responsible for the symptoms of SDAT. 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 action of ACh by inhibiting the degrading enzyme acetylcholinesterase (AChE), can bring about memory improvement in SDAT.[12,13]

In our previous paper,[14] the design, synthesis, and structure-activity relationships (SAR) for a series of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine (**1**) were described. Among these compounds 1-benzyl-4-[2-[*N*-[(4'-benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine hydrochloride (**5**) was one of the most potent inhibitors of AChE and showed an affinity 18 000 times greater for AChE than for BuChE. At a dose of 3 mg/kg, compound **5** produced a marked and significant increase ACh content in the rat brain.

It was also shown that introduction of a methyl group (**2**) and a phenyl group (**3a**) on the nitrogen atom of the amide resulted in increased anti-AChE activity. We then focused on the rigid analogues (**4**) of 1-benzyl-4-[2-(*N*-benzoyl-*N*-methylamino)ethyl]piperidine (**2**) and 1-benzyl-4-[2-(*N*-benzoyl-*N*-phenylamino)ethyl]piperidine analogues (**3**). We report herein the results of our study on the syntheses and the structure-activity relationships of the series of **3** and **4** (Figure 1).

Chemistry

The compounds **3a–o** were synthesized by methods that we described in the previous paper.[14] The rigid analogue of **2**, 1-benzyl-4-[2-(1-oxoisoindolin-2-yl)ethyl]piperidine hydrochloride (**9**) was synthesized by the route shown in Scheme I. 1-Benzyl-4-(2-hydroxyethyl)piperidine (**6**) was prepared by the modified Stokbroekx's method.[15] The reaction of compound **6** with thionyl chloride gave 1-benzyl-4-(2-chloroethyl)piperidine hydrochloride (**7**), which on treatment with 1-oxoindoline[16] gave the desired compound **9**. 1-Benzyl-4-(2-phthalimidoethyl)piperidine hydrochloride (**10**) and its derivatives **11–16**, **20–26** were synthesized by either the condensation of phthalic anhydride and 4-(2-aminoethyl)-1-benzylpiperidine (**8**)[14] in *n*-BuOH or the reaction of phthalimide and compound **7** in *N*,*N*-dimethylformamide.

(1) Perry, E. K.; Gibson, P. H.; Blessed, G.; Tomlinson, B. E. *Neurobiol. Aging* **1981**, *2*, 251.
(2) Bartus, R. T.; Deen, R. L.; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408–417.
(3) Davies, P. *Brain Res.* **1978**, *171*, 319–326.
(4) Davies, P.; Malonney, A. J. F. *Lancet* **1976**, 403.
(5) Perry, E. K.; Gibson, P. H.; Blessed, G.; Perry, R. H.; Tomlinson, B. E. *J. Neurol. Sci.* **1977**, *34*, 247–265.
(6) Perry, E. K.; Perry, R. H.; Blessed, G.; Tomlinson, B. E. *Lancet 1* **1977**, 189–191.
(7) Perry, E. K.; Perry, R. H.; Gibson, P. H.; Blessed, G.; Tomlinson, B. E. *Neurosci. Lett.* **1977**, *6*, 85–89.
(8) Reisine, T. D.; Yamamura, H. I.; Bird, E. D.; Spoke, E.; Enna, S. J. *Brain Res.* **1978**, *149*, 447–481.
(9) Sims, N. R.; Smith, C. C. T.; Davison, A. M.; Bowen, D. M.; Flack, R. H. A. *Lancet* **1980**, 333–336.
(10) White, P.; Goodhardt, M. J.; Kent, J. P.; Hiley, C. R.; Carrasco, L. H.; Williams, J. E.; Bowen, D. M. *Lancet* **1977**, 668–671.
(11) Perry, K. E.; Tomlinson, B. E.; Blessed, G.; Bergman, K.; Gibson, P. H.; Perry, R. H. *Br. Med. J.* **1979**, *2*, 1457.
(12) Davis, K. L.; Mohs, R. C.; Timklenberg, J. R. *N. Engl. J. Med.* **1978**, *301*, 946.
(13) Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Aachiki, K.; Kling, A. *N. Engl. J. Med.* **1986**, *315*, 1241.
(14) Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Kawakami, Y.; Nakamura, Y.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1990**, *33*, 1880.
(15) Stokbroekx's, R. A.; Van der Aa, M. J. M.; Luyckx, M. G. M.; Grauwels, G. A. J. European Patent 320,032, 1989.
(16) Chapman, J. M., Jr.; Cocolas, G. H.; Hall, I. H. *J. Med. Chem.* **1983**, *26*, 243–246.
(17) Sakamoto, T.; Kondo, Y.; Yamanaka, H. *Chem. Pharm. Bull.* **1985**, *33* (2), 626.

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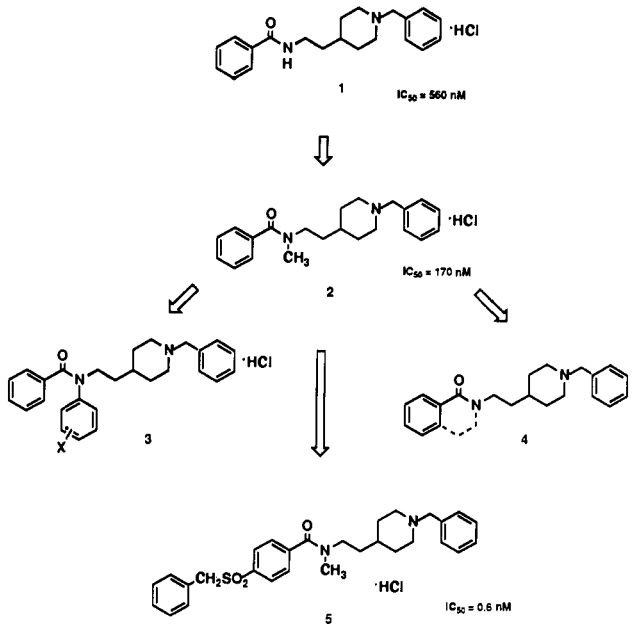
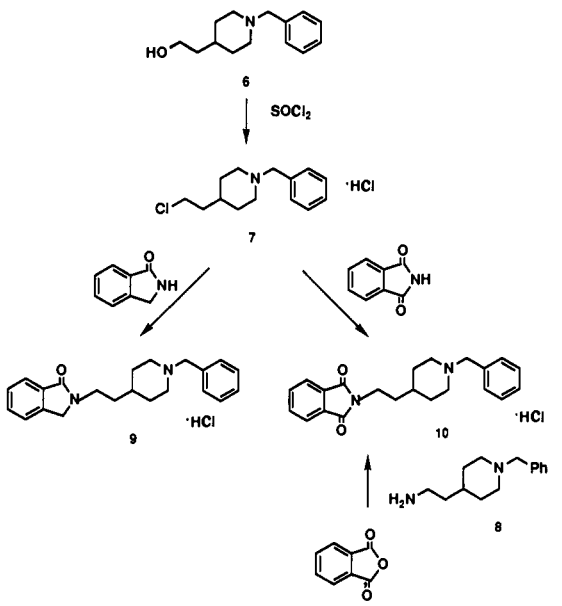


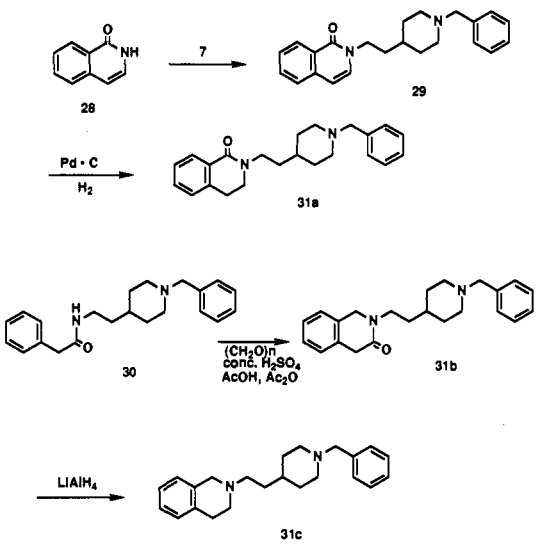
Figure 1

Scheme I

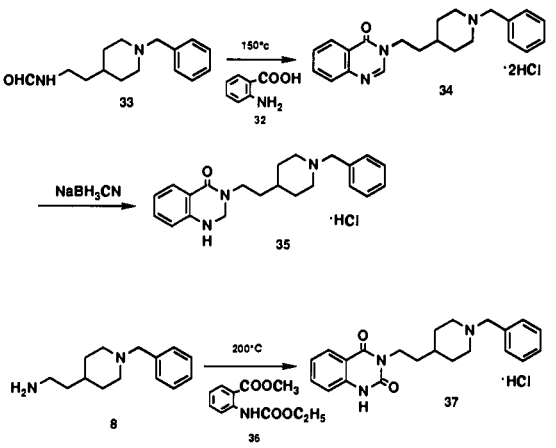


Condensation of 1 (2*H*)-oxoisoquinoline (**28**)[17] and compound **7** in *N*,*N*-dimethylformamide gave compound **29**, which was subjected to catalytic reduction (H_2/Pd–C) to yield 1-benzyl-4-[2-[3,4-dihydro-1-isoquinolon-2-yl]-ethyl]piperidine (**31a**). The reaction of 1-benzyl-4-[2-(*N*-phenylacetylamino)ethyl]piperidine (**30**) with paraformaldehyde gave 1-benzyl-4-[2-[1,4-dihydro-3-isoquinolon-2-yl]ethyl]piperidine (**31b**). Compound **31b** was reduced with $LiAlH_4$ in tetrahydrofuran to yield 1-benzyl-4-[2-(1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]piperidine (**31c**) (Scheme II). 1-Benzyl-4-[2-(*N*-formylamino)ethyl]-piperidine (**33**) was allowed to react with anthranilic acid (**32**) at 150 °C to give 1-benzyl-4-[2-[4-oxoquinazolin-3(4*H*)-yl]ethyl]piperidine (**34**), which was subjected to reduction with sodium cyanoborohydride to yield 1-benzyl-4-[2-[1,2-dihydro-4-oxoquinazolin-3(4*H*)-yl]ethyl]piperidine (**35**). Condensation of methyl *N*-(ethoxycarbonyl)-anthranilate (**36**) with compound **8** at 200 °C gave 1-benzyl-4-[2-[1,2-dihydro-2,4-dioxoquinazolin-3(4*H*)-yl]ethyl]-piperidine (**37**) (Scheme III).

Scheme II



Scheme III



Structure–Activity Relationships

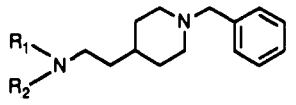
A new series of piperidine derivatives was tested for biological activity in vitro using AChE from mouse brain homogenates. Esterase activity was determined according to the method of Ellman et al.[18] The anti-AChE activities of the series of 1-benzyl-4-[2-(*N*-benzoyl-*N*-phenylamino)-ethyl]piperidine derivatives (**3**) are presented in Table I.

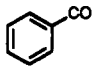
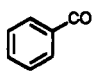
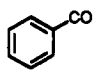
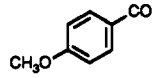
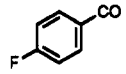
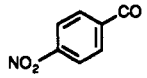
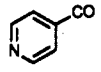
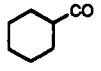
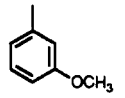
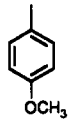
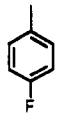
Substitution at position 4 of the phenyl ring of the benzoyl group (R_1) with an electron-accepting group (fluoro, **3c**; nitro, **3d**) led to an increase in anti-AChE activity. Replacement of the benzoyl group with a propionyl (**3h**) or cyclohexylcarbonyl (**3f**) group caused a remarkable decrease in activity, but replacement with an acetyl group did not (**3g**). When the amide moiety of compound **3g** was reduced by $LiAlH_4$ to an amino moiety (**3m**), anti-AChE activity nearly disappeared. These results suggest that the carbonyl and aromatic planarity are essential for activity.

Substitution at the meta position of the *N*-phenyl ring group of compound **3a** by a methoxy group (**3i**) or fluoro group (**3k**) caused little change in anti-AChE activity, whereas substitution with a methoxy group (**3j**) at the para position resulted in decreased activity. We tried to incorporate an ortho substitution to the *N*-phenyl ring group, but the ortho substitution derivatives could not be

(18) Ellman, G.; Courtney, D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

Table I. Structures and Activities for Various 1-Benzyl-4-[2-(substituted amino)ethyl]piperidines



no.	R_1	R_2	yield, %	inhibn of AChE: IC_{50}, nM[a]
1		H	65	560
2		CH_3	67	170
3a			38	35
3b			28	590
3c			73	18
3d			71	5.4
3e			81	64
3f			79	9400
3g	CH_3CO		37	52
3h	CH_3CH_2CO		69	830
3i	CH_3CO		50	46
3j	CH_3CO		43	700
3k	CH_3CO		72	65
3l	CH_3CO		98	205
3m	CH_3CH_2		63	12000
3n	CH_3CO		73	108
3o	CH_3CO	CH_3	95	660

[a] The value of the hydrochloride is shown.

obtained by the same synthetic method. Replacement of the *N*-methyl group of the benzamide derivative (2) with a phenyl group (3a) increased the anti-AChE activity by about 5-fold. However, in the case of the acetamide derivative 3o, the activity increased by about 13-fold (3g). Thus either R_1 or R_2 must be an aromatic ring group to retain the activity.

Compound 9 was synthesized as the cyclic analogue of 2 and showed slightly greater anti-AChE activity. However, another cyclic analogue, the phthalimide derivative (10), showed about 6-fold stronger activity than compound 2. We then synthesized phthalimide derivatives to investigate their structure–activity relationships. Table II shows the anti-AChE activity of phthalimide derivatives. Substitution at the 3 or 4 position of the phthalimide ring moiety by a nitro group (13), amino group (17), or methoxy group (20) produced about a 3–4-fold increase in activity compared to that of compound 10. Introduction of a bulky moiety (acetylamino, 18; benzoylamino, 19; (benzylamino)-carbonyl, 21; benzoyl, 22) at the 4-position of the phthalimide derivatives further increased the activity. In contrast, introduction of the benzoylamino group (25) at position 3 of the phthalimide ring moiety significantly decreased its activity. We previously reported that substitution at the para position of the phenyl ring of the benzamide moiety with a bulky moiety led to a substantial increase in activity for the series of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine derivatives (1). This observation is consistent for the phthalimide derivatives, since the 4-position of phthalimide ring could be regarded as equivalent to the para position of the phenyl ring of the benzamide moiety in terms of positional relationships with the carbonyl group. Anti-AChE activity decreased with decreasing length of the bridging alkyl chain in the phthalimide derivatives (IC_{50} of 11 = 27 000 nM, 12 = 3000 nM, and 13 = 12.5 nM).

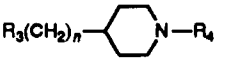
Introduction of a methoxy group (14) or chloro group (15) at the para position on the phenyl ring of R_4 reduced the activity. Replacement of R_4 with a methyl group (16) caused a great reduction in activity.

We next examined derivatives with six-membered ring systems in place of the five-membered ring of the phthalimide moieties. Table III shows the anti-AChE activity of these compounds. The six-membered ring derivatives 29, 31a, 34, and 35 were much less potent, while 31b and 31d had enhanced activity. However, when the carbonyl group of the amide group of the tetrahydroisoquinolone ring was reduced to amino (3c), anti-AChE activity was significantly decreased. The activity of 1,2-dihydroquinazolin-2-one derivative (38) was comparable to that of compound 31b, while the quinazoline-2,4-(1*H*,3*H*)-dione derivative (37) showed slightly greater activity. The valerolactam derivative (40) was less potent than compound 31b, which suggested that the aromatic ring moiety is needed to increase the anti-AChE activity. These results not only support the previous observation that the carbonyl and aromatic moieties of this series of AChE inhibitors are necessary for activity but also suggest that the positional relationship between the carbonyl and aromatics is important.

Biological Results

Among compounds prepared in these series, 1-benzyl-4-[2-[4-(benzoylamino)phthalimido]ethyl]piperidine hydrochloride (19) (IC_{50} = 1.2 nM) is the most potent inhibitor of AChE. Compound 19 also was found to show a definite selectivity to AChE over butyrylcholinesterase (BuCHE)

Table II. Structures and Activities for Various 1-Benzyl-4-[ω-(substituted phthalimido)alkyl]piperidines



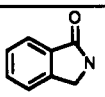
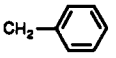
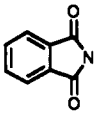
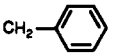
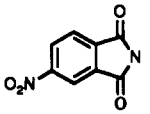
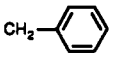
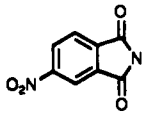
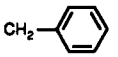
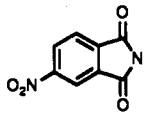
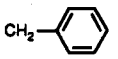
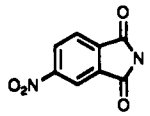
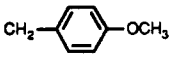
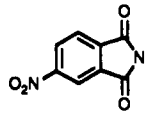
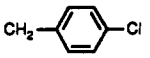
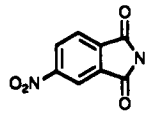
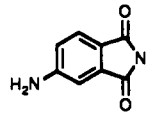
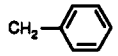
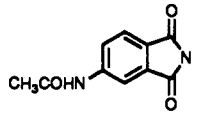
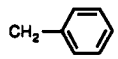
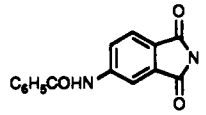
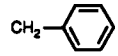
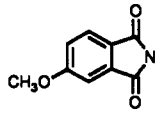
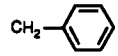
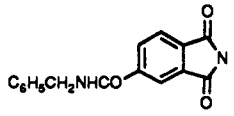
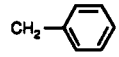
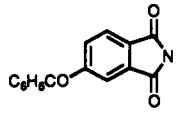
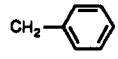
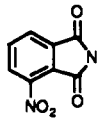
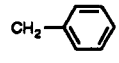
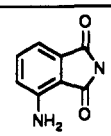
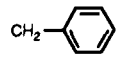
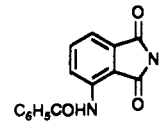
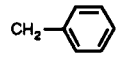
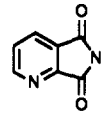
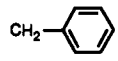
no.	R_3	n	R_4	yield, %	mp, °C	formula	inhibn of AChE: IC_{50}, nM
9		2		21	183–184	$C_{22}H_{26}N_2O$·HCl	98
10		2		62	216–218	$C_{22}H_{24}N_2O_2$·HCl	30
11		0		55	254–255	$C_{20}H_{19}N_3O_4$·HCl	27000
12		1		65	271–273	$C_{21}H_{21}N_3O_4$·HCl	3000
13		2		45	224–227	$C_{22}H_{23}N_3O_4$·HCl	12.5
14		2		35	205–206	$C_{23}H_{25}N_3O_5$·HCl	440
15		2		35	229–231	$C_{22}H_{22}N_3O_4Cl$·HCl	240
16		2	CH_3	74	256–258 dec	$C_{16}H_{19}B_3O_4$·HCl	6800
17		2		96	140–142	$C_{22}H_{25}N_3O_2$	8.8[a]
18		2		74	238–239 dec	$C_{24}H_{27}N_3O_3$·HCl	2.8
19		2		65	151–153	$C_{29}H_{29}N_3O_3$·HCl	1.2
20		2		38	221–222 dec	$C_{23}H_{26}N_2O_3$·HCl	8.0
21		2		34	227–228 dec	$C_{30}H_{31}N_3O_3$·HCl	2.2
22		2		52	162–163	$C_{29}H_{26}N_2O_3$·HCl	2.4
23		2		33	177–179	$C_{22}H_{23}N_3O_4$·HCl	9.0

Table II. (Continued)

no.	R_3	n	R_4	yield, %	mp, °C	formula	inhibn of AChE: IC_{50}, nM
24		2		98	amorphous	$C_{22}H_{25}N_3O_2$	11.0[a]
25		2		76	246–248 dec	$C_{29}H_{29}N_3O_3$·HCl	340
26		2		52	178–181	$C_{21}H_{23}N_3O_2$·HCl	13

[a] The value of the hydrochloride is shown.

(34700-fold). Physostigmine has a preference for AChE over BuChE (11.7-fold), and tacrine has a less selective inhibitory effect (Table IV). The selectivity of compound 19 for AChE was higher than that of 1-benzyl-4-[2-[*N*-[4-(benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine hydrochloride (5).

The results of the ex vivo inhibitory effects on AChE of compound 19 are shown in Figure 2. In rat brain, compound 19 at doses of 10 and 50 mg/kg exerted a dose-dependent inhibitory effect on AChE.

On the basis of these biological studies, it is concluded that compound 19 has a highly selective and very potent inhibitory effect on AChE, and the structure of compound 19 qualifies as a new type of acetylcholinesterease inhibitor.

Experimental Section

All melting points were determined using a Yanagimoto micromelting apparatus unless otherwise specified and are uncorrected. ^{1}H NMR spectra were taken with a JEOL FX-90Q spectrometer using Me_4Si as an internal standard. Mass spectra were obtained on a JEOL HX-100 spectrometer using a direct insertion probe. Elemental analysis is indicated only by symbols of the elements; analytical results were within 0.4% of theoretical values.

1-Benzyl-4-[2-(*N*-acetyl-*N*-phenylamino)ethyl]piperidine (3g). Acetyl chloride (0.40 g, 5.1 mmol) was added dropwise to a mixture of 1-benzyl-4-[2-(*N*-phenylamino)ethyl]piperidine[14] (0.70 g, 2.4 mmol), triethylamino (2.00 g, 19.8 mmol), and 20 mL of tetrahydrofuran while the mixture was cooled with ice with stirring. The reaction was allowed to proceed at room temperature for 3 h, and 20 mL of water was then added, followed by extraction with methylene chloride. The extract was washed with a saturated saline solution and dried over anhydrous magnesium sulfate. The solvent was then evaporated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 95:5), thereby preparing the title compound **3g**: yield 0.31 g (37%); ^{1}H NMR ($CDCl_3$) δ 1.0–2.1 (m, 12 H), 2.6–3.0 (m, 2 H), 3.39 (s, 2 H), 3.67 (t, 2 H), 6.9–7.5 (m, 10 H); MS *m/e* 336 (M^+).

1-Benzyl-4-(2-chloroethyl)piperidine Hydrochloride (7). To a solution of 1-benzyl-4-(2-hydroxyethyl)piperidine (6) (14.9 g, 68.2 mmol) in CH_2Cl_2 (400 mL), was added dropwise $SOCl_2$ (9.92 mL, 0.136 mol) with ice cooling, and the mixture was stirred for 4 h at 50 °C. After the mixture was evaporated, Et_2O was added to the residue and the precipitate was collected by filtration to give 15.6 g (83%) of 1-benzyl-4-(2-chloroethyl)piperidine hydrochloride (7): ^{1}H NMR ($CDCl_3$) 1.32 (t, 3 H), 1.20–2.08 (m, 6 H), 2.78–2.94 (db, 2 H), 3.45 (s, 2 H), 3.52 (t, 2 H), 7.24 (s, 5 H) (free base).

1-Benzyl-4-[2-(2-oxoisoindolin-2-yl)ethyl]piperidine Hydrochloride (9). A mixture of isoindolin-1-one (1.0 g, 7.5 mmol), compound 7 (free base) (2.0 g, 8.41 mmol), KI (0.01 g), and NaH (60% dispersion in oil, from which the oil was removed by rinsing three times with hexane in DMF 40 mL) (0.6 g, 15 mmol), was stirred at 80 °C for 5 h. After cooling, 100 mL of water was added, and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was then washed with water, dried with anhydrous magnesium sulfate, and evaporated in vacuo. The residue was purified by silica gel flash column chromatography ($CHCl_3$–MeOH = 100:1) to give 0.68 g of free base compound 9. It was treated with 10% HCl–AcOEt, and the resulting crystals were recrystallized from EtOH–AcOEt to give 0.57 g (21%) of compound 9, mp 183–184 °C. Anal. ($C_{22}H_{26}N_2O$·HCl) C, H, N.

1-Benzyl-4-[2-(4-nitrophthalimido)ethyl]piperidine Hydrochloride (13). A mixture of 4-(2-aminoethyl)-1-benzylpiperidine (8) (1.10 g, 4.7 mmol) and 4-nitrophthalic anhydride (1.0 g, 5.2 mmol) in dioxane (30 mL) was refluxed for 2 h. Then 50 mL of water was added, and the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried with anhydrous sodium sulfate and evaporated in vacuo. The residue was purified by silica gel column chromatography. The product was eluted with $CHCl_3$–MeOH (20:1) to give 1.3 g of free base compound 13. It was treated with 10% HCl–EtOAc, and the resulting crystals were recrystallized from acetone–isopropyl ether to give 0.98 g (49%) of 13, mp 224–227 °C. Anal. ($C_{22}H_{23}N_3O_4$·HCl) C, H, N.

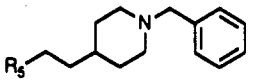
1-Benzyl-4-[2-(4-aminophthalimido)ethyl]piperidine (17). A mixture of the free base of compound 13 (7.90 g, 20 mmol), concentrated HCl–MeOH (14 mL:14 mL), and iron powder (3.40 g) was stirred at 60–70 °C for 1.5 h. Then 200 mL of water was added, and the reaction mixture was alkalized with a saturated sodium bicarbonate solution and extracted wtih $CHCl_3$. The $CHCl_3$ layer was dried with K_2CO_3 and evaporated to give 7.0 g (96%) of compound 17, mp 140–142 °C. Anal. ($C_{22}H_{25}N_3O_2$) C, H, N.

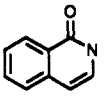
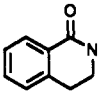
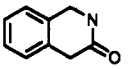
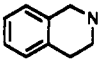
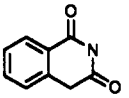
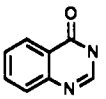
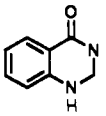
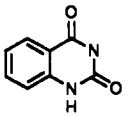
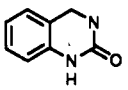
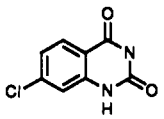
1-Benzyl-4-[2-(4-benzoylamino)phthalimido]ethyl]-piperidine Hydrochloride (19). A mixture of compound 17 (2.0 g, 5.5 mmol), benzoyl chloride (1.0 g, 7.1 mmol), and 1 g of triethylamine in tetrahydrofuran (20 mL) was stirred overnight at room temperature. Saturated sodium bicarbonate solution was added, and the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried with anhydrous magnesium sulfate, and evaporated to give the free base of compound 19. It was treated with 10% HCl–EtOAc, and the resulting crystals were recrystallized from EtOH–Et_2O to give 1.8 g (65%) of compound 19, mp 151–153 °C. Anal. ($C_{29}H_{29}N_3O_3$·HCl) C, H, N.

1-Benzyl-4-[2-[1-isoquinolon-2-yl]ethyl]piperidine (29). To a suspension of NaH (0.17 g, 4.25 mmol), from which the oil was removed by rinsing the NaH (60% dispersion in oil) three times with hexane in DMF 10 mL, a solution of 1-isoquinolone (0.40 g, 2.75 mmol) in DMF 20 mL was added. 1-Isoquinolone was synthesized from 2-(β-ethoxyethenyl)benzamide as described in our previous paper.[14]

The reaction mixture was stirred for 1 h at 60 °C. A solution of compound 7 (0.70 g, 2.55 mmol) in DMF (10 mL) was added, and the reaction mixture was stirred for 3 h at 70 °C. The reaction mixture was poured into ice water and extracted with EtOAc. The EtOAc layer was washed with water, dried with anhydrous

Table III. Structures and Activities for 1-Benzylpiperidine Derivatives



no.	R_5	yield, %	mp, °C	formula	inhibn of AChE: IC_{50}, nM
29		72	amorphous	$C_{23}H_{26}N_2O$	1100[a]
31a		42	oil	$C_{23}H_{28}N_2O$	1000[a]
31b		63	amorphous	$C_{23}H_{28}N_2O$	17[a]
31c		37	oil	$C_{23}H_{30}N_2$	1600[a]
31d		63	210–214 dec	$C_{23}H_{26}N_2O_2$·HCl	23
34		67	135–140 dec	$C_{22}H_{25}N_3O$·2HCl	1200
35		73	80–82 dec	$C_{22}H_{27}N_3O$·2HCl	800
37		15	208–210	$C_{22}H_{25}N_3O_2$·HCl	4.2
38		73	amorphous	$C_{22}H_{27}N_3O$	13[a]
39		55	212–213	$C_{22}H_{24}N_3O_2Cl$·HCl	4.5
40		18	oil	$C_{19}H_{28}N_2O$	270[a]

[a] The value of hydrochloride is shown.

Table IV. Inhibitory Effect of **19** and Reference Compounds on AChE and BuChE Activity (in Vitro)

compounds	IC_{50}, nM[a] AChE activity	IC_{50}, nM[a] BuChE activity	ratio of IC_{50} (BuChE/AChE)
19	1.22 (0.94–1.53)	42325 (41589–44156)	34700
5	0.56 (0.42–0.71)	10100 (8196–11971)	18000
physostigmine	0.69 (0.63–0.74)	8.1 (7.80–8.40)	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 plasma. N = 4. Numbers in a parentheses represent a range of 95% confidence limits.

magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 20:1) to give 0.70 g (79%) of compound **29**: ^{1}H NMR ($CDCl_3$) δ 1.10–2.20 (m, 9 H), 2.70–3.10 (m, 2 H), 3.50 (s, 2 H), 4.03 (t, 2 H), 6.50 (m, 1 H), 6.90–7.90 (m, 9 H) 8.47 (d, 1 H); MS *m/e* 346 (M^+).

1-Benzyl-4-[2-(*N*-phenylacetylamino)ethyl]piperidine (30). To a solution of compound **8** (1.93 g, 8.84 mmol) in 20 mL of tetrahydrofuran were added triethylamine (1.79 g, 17.7 mmol) and phenylacetyl chloride (1.64 g, 10.6 mmol) at 0 °C. This mixture was stirred at 0 °C for 1 h. The mixture was evaporated to remove the solvent, and the resulting residue was diluted with CH_2Cl_2 (150 mL), washed with a 10% sodium carbonate solution (100 mL), and a saturated sodium chloride solution (100 mL). After drying with anhydrous magnesium sulfate, the CH_2Cl_2 layer was evaporated to give a yellow oil. The yellow oil was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 50:1) to give 2.97 g (100%) of compound **30**: ^{1}H NMR ($CDCl_3$) δ 1.10–2.21 (s, 9 H), 2.72–3.00 (m, 4 H) 3.13–3.61 (m, 2 H), 3.55 (s, 2 H), 5.50 (1 H), 7.30 (s, 10 H); MS *m/e* 336 (M^+).

1-Benzyl-4-[2-[1,4-dihydro-3-isoquinolon-2-yl]ethyl]piperidine (31b). To a mixture of compound **30** (1.15 g, 3.42 mmol) in AcOH (2 mL), Ac_2O (2 mL), and paraformaldehyde (0.215 g, 7.16 mmol) was added concentrated H_2SO_4 (0.44 g, 4.48 mmol) at 75 °C. This solution was stirred at 100 °C for 3 h. The mixture was alkalized with 1 N NaOH, diluted with 30 mL of H_2O, and extracted with 50 mL of CH_2Cl_2. This CH_2Cl_2 layer was washed with 30 mL of 10% sodium carbonate solution. After drying with anhydrous magnesium sulfate, the CH_2Cl_2 layer was

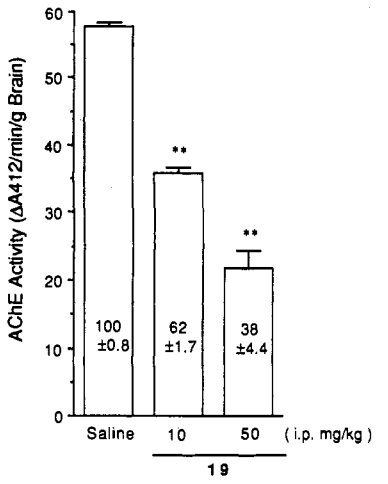


Figure 2. Effect of **19** on rat brain AChE (ex vivo). The compound was administered intraperitoneally at two doses 1 h before decapitation. Ordinate indicates AChE activity (ΔA412 min/g). Each column represents the mean ± SE for six determinations. (**) $p < 0.01$.

evaporated to produce a yellow oil. The yellow oil was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 50:1) to give 0.75 g (63 %) of **31b**: ^1H NMR ($CDCl_3$) δ 1.10–2.20 (m, 9 H), 2.93 (bd, 2 H), 3.40–3.65 (m, 6 H), 4.43 (s, 2 H), 7.00–7.50 (m, 4 H), 7.31 (s, 5 H); MS *m/e* 348 (M^+).

1-Benzyl-4-[2-(1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]-piperidine (31c). To a solution of compound **31a** (0.14 g, 0.40 mmol) in tetrahydrofuran (4 mL) was added $LiAlH_4$ (0.03 g, 0.79 mmol) at 0 °C. This mixture was stirred under reflux for 1 h. Then, 5 drops of water, 5 drops of 1 N NaOH, and 15 drops of water were added. The mixture was filtered, and the filtrate was evaporated to afford a yellow oil (0.05 g, 37%) of compound **31c**: ^1H NMR ($CDCl_3$) δ 1.10–2.20 (m, 9 H), 2.22–2.97 (m, 8 H), 3.45 (s, 2 H), 3.55 (s, 2 H), 6.90–7.20 (m, 4 H), 7.21 (s, 5 H); MS *m/e* 334 (M^+).

1-Benzyl-4-[2-(*N*-formylamino)ethyl]piperidine (33). A mixture of compound **8** (5.0 g, 22.9 mmol) in 99% formic acid (25 mL) was stirred at 100 °C for 6 h. The mixture was diluted with 200 mL of ice water and alkalized with 1 N NaOH. The mixture was extracted with ether. After being dried with anhydrous magnesium sulfate, the ether layer was evaporated to give a yellow oil (3.47 g). The yellow oil was purified by silica gel flash column chromatography (CH_2Cl_2–MeOH = 10:1) to give 2.13 g (38%) of compound **33**.

1-Benzyl-4-[2-[4-oxaquinazolin-3(4*H*)-yl]ethyl]piperidine Hydrochloride (34). A mixture of compound **33** (1.92 g, 7.78 mmol) and anthranilic acid (**32**) (2.14 g, 15.6 mmol) was heated at 150 °C for 4 h. The mixture was diluted with 0.1 N NaOH (100 mL) and extracted with CH_2Cl_2. After being dried with anhydrous magnesium sulfate, the CH_2Cl_2 layer was evaporated to give a brown oil (3.29 g). The oil was purified by silica gel flash column chromatography (hexane–EtOAc = 1:1) to give 2.33 g of the free base of compound **34**. It was treated with 10% HCl–EtOAc, and the resulting crystals were recrystallized from MeOH–isopropyl ether to give 2.20 g (67%) of compound **34**, mp 135–140 °C dec. Anal. ($C_{22}H_{25}N_3O$·2HCl) C, H, N.

1-Benzyl-4-[2-[1,2-dihydro-4-oxoquinazolin-3(4*H*)-yl]ethyl]piperidine Hydrochloride (35). To a solution of compound **34** (free base) (0.52 g, 1.49 mmol) in MeOH (6 mL) were added 2 N HCl–MeOH and then $NaBH_3CN$ (0.103 g, 1.64 mmol). The solution was adjusted to pH 4 by addition of 2 N HCl–MeOH. This mixture was stirred at room temperature for 1 h, poured into 0.1 N NaOH (50 mL), and extracted with CH_2Cl_2. After being dried with anhydrous magnesium sulfate, the CH_2Cl_2 layer was evaporated to give a pale yellow oil. The oil was purified by silica gel flash column chromatography ($CHCl_3$–MeOH = 20:1) to give 0.52 g of the free base of compound **35**. It was treated with 10% HCl–EtOAc, and the resulting crystals were recrystallized from MeOH–isopropyl ether to give 0.46 g (73%) of compound **35**, mp 80–82 °C dec. Anal. ($C_{22}H_{27}N_3O$·2HCl) C, H, N.

1-Benzyl-4-[2-[1,2-dihydro-2,4-dioxoquinazolin-3(4*H*)-yl]ethyl]piperidine Hydrochloride (37). A mixture of methyl *N*-(ethoxycarbonyl)anthranilate (**36**) (50 g, 0.224 mol) and compound **8** (49 g, 0.224 mol) was stirred at 190–200 °C for 6 h to produce MeOH and EtOH, which were then removed. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH = 50:1) to give the free base of compound **37**. It was treated with 10% HCl–EtOH, and the resulting crystals were recrystallized from EtOH–H_2O to give 13.3 g (15%) of compound **37**, mp 208–210 °C. Anal. ($C_{22}H_{25}N_3O_2$·HCl) C, H, N.

1-Benzyl-4-[2-(2-piperidonyl)ethyl]piperidine (40). To a suspension of NaH (60% dispersion in oil) (0.89 g, 22.0 mmol), from which the oil was removed by rinsing the NaH three time with hexane in DMF 10 mL, was added a solution of δ-valerolactam (0.69 g, 6.96 mmol) in DMF (10 mL). The suspension was stirred at 70 °C for 2 h and then a solution of compound **7** (free base) (1.10 g, 4.63 mmol) in DMF (10 mL) was added dropwise, and the mixture was left to stand at room temperature. After the mixture was stirred at 70 °C for 2 h, it was evaporated to remove the solvent. $CHCl_3$ was added to the residue, and the $CHCl_3$ layer was washed with water. After being dried with anhydrous magnesium sulfate, the $CHCl_3$ layer was evaporated to give a brown oil. The brown oil was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 100:1) to give 0.25 g (18%) of compound **40**: ^1H NMR ($CDCl_3$) δ 1.10–2.16 (m, 13 H), 2.16–2.50 (m, 2 H), 2.87 (bd, 2 H), 3.03–3.43 (m, 4 H), 3.48 (s, 2 H), 7.27 (s, 5 H); MS *m/e* 300 (M^+).

Materials and Methods. Determination of Cholinergic Parameters. In Vitro Anti-Cholinesterase Activity. The inhibitory effects of compound **19** on AChE and BuChE were compared with those of physostigmine and tacrine in in vitro experiments. Cholinesterase activity was measured by the spectrophotometric method of Ellman et al.[18] Mouse brain homogenates (100 mg of brain per mL of 0.1 M sodium–potassium phosphate buffer, pH 8.0) and rat plasma were used as sources of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), respectively. Acetylthiocholine (AthCh) and butyrylthiocholine (ButhCh) were used as the substrates for measurement of AChE activity and BuChE activity, respectively. In brief, 300 μL of compound solution and 1.0 mL of the enzyme were mixed and preincubated for 60 min at 37 °C. At the end of the preincubation period, the enzyme reaction was conducted by mixing a 130-μL aliquot of the preincubated mixture with 0.5 mM AthCh or 1.0 mM ButhCh and 0.33 mM 5,5′-dithiobis(2-nitrobenzoic acid) in the phosphate buffer, pH 8.0 at 25 °C. Different concentrations of the compounds were assayed, and IC_{50} values were determined graphically from log concentration–inhibition curves.

AChE Activity in Ex Vivo Experiments in Rat Brain. Male Wistar rats weighing 275–344 g were used. Compound **19** was suspended in 5% gum arabic solution and administered intraperitoneally. Control animals were given 5% gum arabic solution. Rats were sacrificed by decapitation 1 h after drug administration. The whole brain was removed, immediately frozen in liquid nitrogen, and stored in at −80 °C until assayed. The frozen tissues were homogenized in 10 volumes of ice-cold 0.1 M sodium–potassium phosphate buffer, pH 8.0, and AChE activity was measured using AthCh as the substrate.

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Supplementary Material Available: A listing of NMR and MS data and elemental analysis for compounds **3a–o**, **24**, **29**, **31a–c**, **38**, and **40** (2 pages). Ordering information is given on any current masthead page.