Synthesis and Structure–Activity Relationships of Acetylcholinesterase Inhibitors: 1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine Hydrochloride and Related Compounds

Hachiro Sugimoto,*,† Youichi Iimura, Yoshiharu Yamanishi, and Kiyomi Yamatsu

Tsukuba Research Laboratories, Eisai Company, Ltd., 1-3, Tokodai 5-chome, Tsukubashi, Ibaraki 300-26, Japan

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Following the discovery of a new series of anti-acetylcholinesterase (anti-AChE) inhibitors such as 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine (**1**), we reported that its rigid analogue, 1-benzyl-4-(2-isoindolin-2-ylethyl)piperidine (**5**), had more potent activity. We have extended the structure–activity relationship (SAR) study for the rigid analogue and found that the 2-isoindoline moiety in compound **5** can be replaced with a indanone moiety (**8**) without a major loss in potency. Among the indanone derivatives, 1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (**13e**) (E2020) (IC_{50} = 5.7 nM) was found to be one of the most potent anti-AChE inhibitors. Compound **13e** showed a selective affinity 1250 times greater for AChE than for butyrylcholinesterase. *In vivo* studies demonstrated that **13e** has a longer duration of action than physostigmine at a dose of 5 mg/kg (po) and produced a marked and significant increase in acetylcholine content in rat cerebral cortex. We report the synthesis, SAR, and a proposed hypothetical binding site of **13e** (E2020).

A selective loss of choline acetyltransferase (ChAT), the enzyme for acetylcholine (ACh) synthesis, has been demonstrated in the cortex of patients with Alzheimer disease (AD), suggesting that a specific cholinergic deficiency may be characteristic of this disease.[1–5] Evidence of a decreased ACh concentration in postmortem cortical tissue[6] and the decreased ACh synthesis in the biopsy material from Alzheimer patients[7] have recently lent support to this hypothesis.

There has been much recent interest[8,9] in agents which enhance cortical cholinergic transmission as potential therapeutics in the treatment of AD or senile dementia of the Alzheimer type. The so-called "cholinergic hypothesis" for AD is based on a wide body of clinical and neurochemical evidence[10,11] which indicates that the marked deficits in cognitive function which accompany AD can be most consistently related to selective degeneration of cholinergic neurons projecting from the nucleus basalis of Meynert into cortical regions. Two clear-cut strategies to accentuate cholinergic transmission have been evaluated extensively in the clinic:[12,13] (1) inhibition of acetylcholinesterase (AChE) to potentiate the effects of endogenous acetylcholine and (2) therapeutic use of directly acting agonists at postsynaptic muscarinic receptors in the cortex.

Several clinical studies with AChE inhibitors such as physostigmine and more recently with tacrine[14] have revealed modest, though statistically significant, improvements in cognitive function in AD patients. However, variable oral activity, short duration of action, and side effects have continued to restrict the acceptance of these agents. The purpose of our study was to find a new type of AChE inhibitor that would overcome the deficits of physostigmine (short duration) and tacrine (liver toxicity) (Figure 1).

In our previous paper,[15] the design, synthesis, and structure–activity relationships (SAR) for a series of

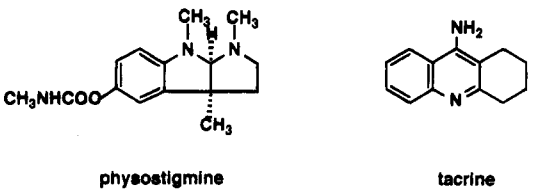

Figure 1.

1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine (**1**) derivatives were described. Introduction of a lower alkyl group at the nitrogen atom of the amide moiety in compound **2** enhanced anti-AChE activity (IC_{50} = 170 nM). The positional effect of substitution in the benzoyl moiety in compound **1** was examined, and the 4-position-substituted benzamide derivative 1-benzyl-4-[2-[*N*-[4-(benzylsulfonyl)benzoyl]-*N*-methylamino]ethyl]piperidine hydrochloride (**3**) showed the highest potency (IC_{50} = 0.6 nM). We then examined the rigid analogue **5** and found that its potency was slightly increased (IC_{50} = 98 nM) in comparison with **2**.[15] As a rigid analogue, we also synthesized phthalimide derivative **6** which was significantly more potent than **5**. In this phthalimide series, 1-benzyl-4-[2-[4-(benzoylamino)phthalimid-1-yl]-ethyl]piperidine hydrochloride (**7**) (IC_{50} = 1.2 nM) was the most potent AChE inhibitor.[16]

In the present study, we synthesized indanone derivatives **8** and **9** to determine the significance of the nitrogen atom of the amide moiety of rigid analogue **5**. The synthesis, SAR, and pharmacology of these compounds are described (Figure 2).

Chemistry

The general synthesis of indanone derivatives **8** and **9** is shown in Scheme 1. The compounds were prepared by the following process. α,β-Unsaturated ketones **12** were prepared from substituted 1-indanone **10** and aldehydes **11** by aldol condensation followed by dehydration. The resulting unsaturated products **12** were catalytically reduced (H_2/Pd–C) to afford compound **13**. Other synthetic routes to indanone derivatives are

† Current address: Eisai Co., Ltd., Koishikawa 4-6-10, Bunkyo-ku, Tokyo 112-88, Japan.

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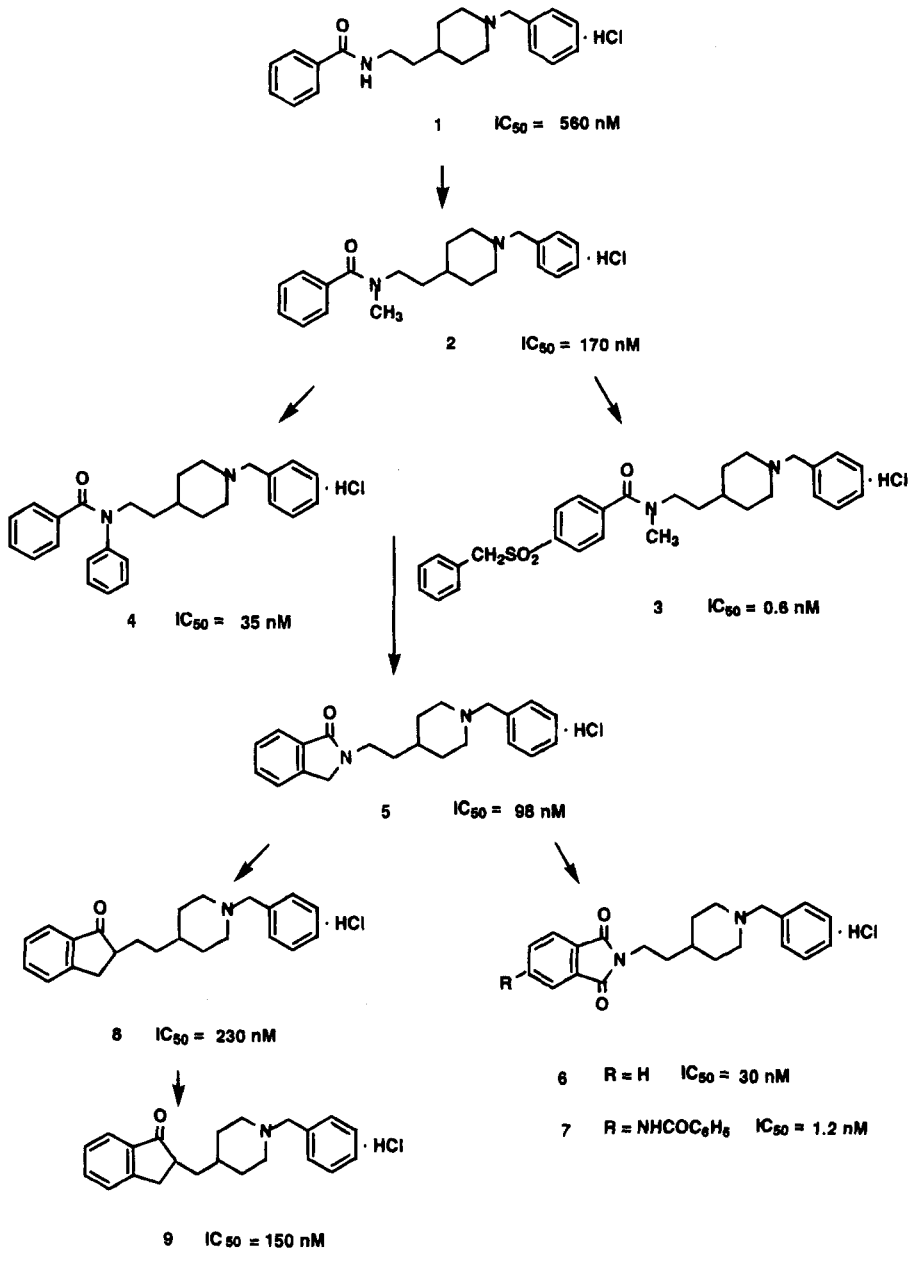
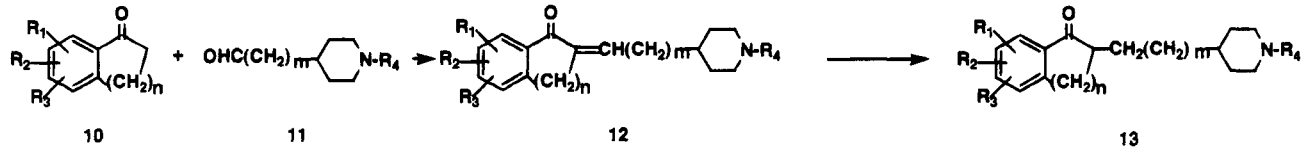


Figure 2.

Scheme 1



shown in Scheme 2. Condensation of 5,6-dimethoxy-1-indanone (**10a**) and amines **14** with paraformaldehyde gave **15**. 1-Benzoyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)-methyl]piperidine (**13a**) was hydrolyzed with aqueous HCl to give 4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]-piperidine hydrochloride (**13b**). Reaction of compound **13b** with substituted benzyl halides (R_4-X) gave 4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]-1-substituted-piperidine **16**.

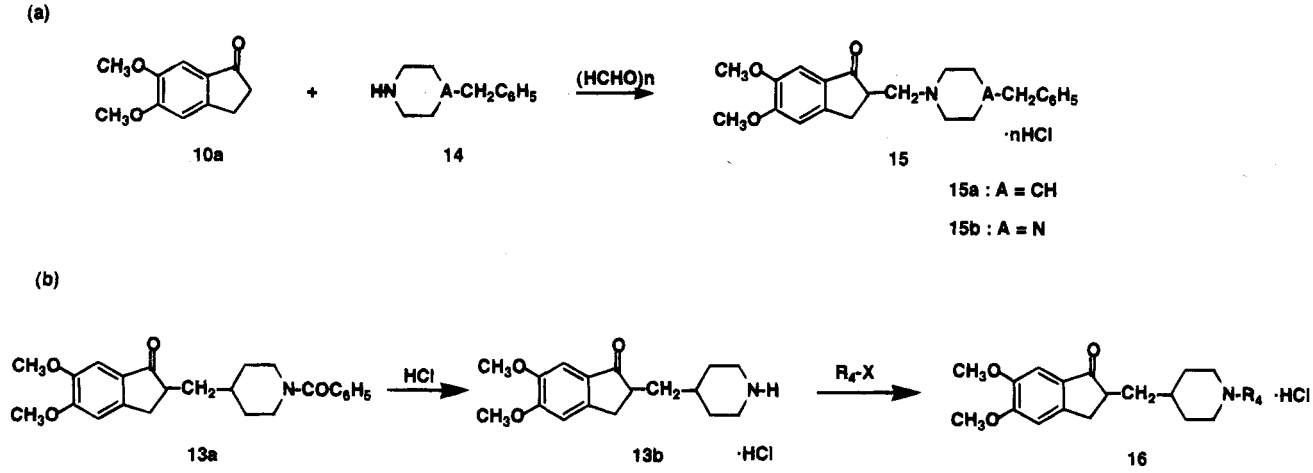
All indanone derivatives with an asymmetric carbon atom (2-position) were studied as the racemates. Compound **17**, prepared by $NaBH_4$ reduction of **13e**, is a mixture of diastereomers.

Structure–Activity Relationships

The new series of indanone derivatives was tested for *in vitro* inhibition of acetylcholinesterase. A mouse brain homogenate was used as the AChE source, and the esterase activity was determined according to the method of Ellman et al.[17]

As shown in Figure 2, unsubstituted indanone derivative **8** (IC_{50} = 230 nM) was found to be slightly less potent than **5** (IC_{50} = 98 nM), suggesting that the nitrogen atom of the amide moiety can be substituted for the carbon atom without a major loss in potency. When the bridging moiety of **8** was changed from an ethylene group to a methylene group (**9**) (IC_{50} = 150

Scheme 2



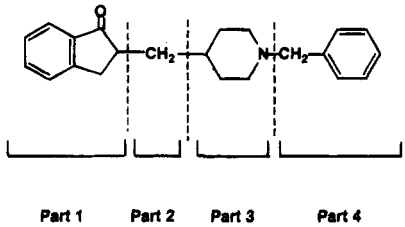
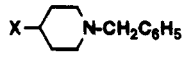
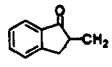
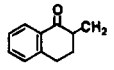
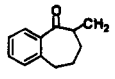
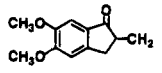
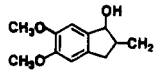
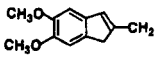


Figure 3. Four parts of compound **9**.

Table 1. Anti-AChE Activity of 1-Benzyl-4-substituted-piperidine Derivatives



no.	X	mp, °C	formula	inhibition of AChE IC_{50}, nM[a]
9		196-197	$C_{22}H_{25}NO·HCl$	150
13c		195-196	$C_{22}H_{27}NO·HCl$	2100
13d		oil	$C_{24}H_{29}NO$	15000[b]
13e		211-212 dec	$C_{24}H_{29}NO_3·HCl$	5.7
17		oil	$C_{24}H_{31}NO_3$	300[c]
18		216-217 dec	$C_{24}H_{29}NO_2·HCl$	4400

[a] Deviation of measurement of IC_{50} value is 10−20%. [b] The value of the hydrochloride is shown. [c] The value of the fumarate is shown.

nM), the potency was slightly increased. Compound **9** was selected as a lead compound of the indanone series. The lead compound was divided into four parts as shown in Figure 3: part 1 (an indanone moiety), part 2 (a linking group), part 3 (a piperidine moiety), and part 4 (a benzyl moiety). We examined each part in detail.[18-20]

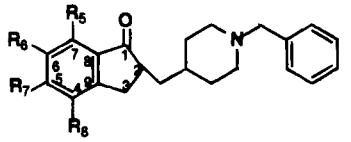
Part 1. Table 1 shows anti-AChE activity of 1-benzyl-4-substituted-piperidine derivatives which have various bicyclic rings in place of the indanone moiety. The effect of replacement of the indanone moiety with α-tetralone (**13c**), 1-benzosuberanone (**13d**), 5,6-dimethoxy-1-indanone (**13e**, E2020), 5,6-dimethoxy-1-indanol (**17**), and 5,6-dimethoxyindene (**18**) was examined. Ring expansion of the cyclic ketone (i.e., compounds **13c,d**) resulted in largely decreased anti-AChE activity. Introduction of a methoxy group at the 5,6-positions (**13e**) in the indanone moiety of compound **9** increased the anti-AChE activity by more than 25-fold. The carbonyl group of the indanone moiety is essential for anti-AChE activity since the 5,6-dimethoxy-1-indanol derivative **17** and the 5,6-dimethoxyindene derivative **18** both had decreased potencies.

The effect of introducing one or more methoxy groups in the indanone moiety in compound **9** is shown in Table 2. Introduction of a methoxy group at the 5-position (R_7) (**13g**) increased the anti-AChE activity by more than 20-fold. A methoxy substituent at the 4-position (R_8) (**13h**) increased the anti-AChE activity by more than 10-fold, while substitution at the 6-position (R_6) (**13f**) resulted in slightly increased activity. Among dimethoxyindanone derivatives, the order of anti-AChE activity was **13e** > **13l** = **13j** = **13k** > **13i** and the 5,6-dimethoxy-1-indanone derivative **13e** showed the highest activity. The anti-AChE activity of compound **13e** was greater than that of the 5,6,7-trimethoxy-1-indanone derivative **13m**.

We previously reported[15,16] that introduction of a methoxy group at the 4-position in the benzoyl moiety of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine hydrochloride (**1**) and at the 5-position in the phthalimide moiety of 1-benzyl-4-(2-*N*-phthalimid-1-ylethyl)piperidine hydrochloride (**6**) enhanced anti-AChE activity 6- and 4-fold, respectively, in comparison with the original corresponding unsubstituted compounds. In the present study, the 5-methoxy-1-indanone derivative **13g** showed dramatically increased activity. These results suggests that the 4-methoxy substituent of the benzoyl moiety greatly enhanced binding to the active site of the enzyme.

Part 2. Various bridging groups between the indanone moiety and the piperidine moiety were examined as shown in Table 3. Direct combination of indanone and piperidine moieties (**13n**) resulted in dramatically reduced potency. The effect of the length of the bridging moiety on the potency varied in the following order: propylene (**13p**) (IC_{50} = 1.5 nM) > methylene (**13e**) (IC_{50} = 5.7 nM) > pentylene (**13r**) (IC_{50} = 14 nM) > ethylene (**13o**) (IC_{50} = 30 nM), butylene (**13q**) (IC_{50} = 35 nM). The introduction of an *exo*-

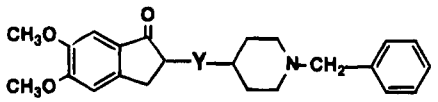
Table 2. Anti-AChE Activity of 1-Benzyl-4-[(substituted-1-oxoindan-2-yl)methyl]piperidine Derivatives



no.	R_5	R_6	R_7	R_8	mp, °C	formula	inhibition of AChE IC_{50}, nM[a]
9	H	H	H	H	196–197	$C_{22}H_{25}NO \cdot HCl$	150
13e	H	OCH_3	OCH_3	H	211–212 dec	$C_{24}H_{29}NO_3 \cdot HCl$	5.7
13f	H	OCH_3	H	H	oil	$C_{23}H_{27}NO_2$	81[b]
13g	H	H	OCH_3	H	203–204 dec	$C_{23}H_{27}NO_2 \cdot HCl$	6.4
13h	H	H	H	OCH_3	oil	$C_{23}H_{27}NO_2$	12[b]
13i	OCH_3	OCH_3	H	H	198–199	$C_{24}H_{29}NO_3 \cdot HCl$	85
13j	OCH_3	H	OCH_3	H	oil	$C_{24}H_{29}NO_3$	25[b]
13k	OCH_3	H	H	OCH_3	oil	$C_{24}H_{29}NO_3$	36[b]
13l	H	H	OCH_3	OCH_3	199–200 dec	$C_{24}H_{29}NO_3 \cdot HCl$	20
13m	OCH_3	OCH_3	OCH_3	H	200–201	$C_{25}H_{31}NO_4 \cdot HCl$	13

[a] Deviation of measurement of IC_{50} value is 10–20%. [b] The value of the hydrochloride is shown.

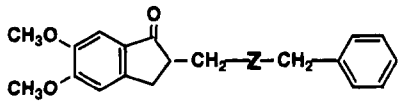
Table 3. Anti-AChE Activity of 1-Benzyl-4-substituted-piperidine Derivatives



no.	Y	mp, °C	formula	inhibition of AChE IC_{50}, nM[a]
13n		247–248 dec	$C_{23}H_{27}NO_3 \cdot HCl$	3300
13e	CH_2	211–212 dec	$C_{24}H_{29}NO_3 \cdot HCl$	5.7
12a[c]	=CH	237–238 dec	$C_{24}H_{27}NO_3 \cdot HCl$	13
13o	CH_2CH_2	201–202 dec	$C_{25}H_{31}NO_3 \cdot HCl \cdot {}^1/_2H_2O$	30
13p	$CH_2CH_2CH_2$	oil	$C_{26}H_{33}NO_3$	1.5[b]
13q	$CH_2CH_2CH_2CH_2$	oil	$C_{27}H_{35}NO_3$	35[b]
13r	$CH_2CH_2CH_2CH_2CH_2$	oil	$C_{28}H_{37}NO_3$	14[b]

[a] Deviation of measurement of IC_{50} value is 10–20%. [b] The value of the hydrochloride is shown. [c] Compound **12a** is the *E* geometric isomer.

Table 4. Anti-AChE Activity of 5,6-Dimethoxyindanone Derivatives



no.	Z	mp, °C	formula	inhibition of AChE IC_{50}, nM[a]
13e		211-212 dec	$C_{24}H_{29}NO_3 \cdot HCl$	5.7
15a		oil	$C_{24}H_{29}NO_3$	480[b]
15b		227-228 dec	$C_{23}H_{28}N_2O_3 \cdot 2HCl$	94

[a] Deviation of measurement of IC_{50} value is 10–20%. [b] The value of the hydrochloride is shown.

methylene double bond (**12a**) (IC_{50} = 13 nM) on the indanone moiety of compound **13e** decreased the anti-AChE activity more than 2-fold.

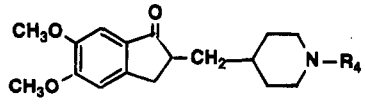
Part 3. The nitrogen atom at the 1-position on the benzylpiperidine moiety in **13e** (IC_{50} = 5.7 nM) is very important, since the activity of the isomeric 4-benzylpiperidine derivative **15a** (IC_{50} = 480 nM) was greatly decreased. Replacement of the piperidine group in **13e** with a piperazine group (**15b**) also resulted in significantly decreased anti-AChE activity (IC_{50} = 94 nM), supporting the previous observation[20] that the distance between the carbonyl group and the nitrogen atom is critical for anti-AChE activity and that the second nitrogen atom, in the case of the piperazine ring, may adversely alter this arrangement (Table 4).

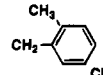
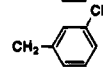
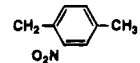
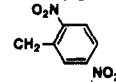
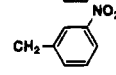
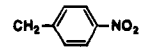
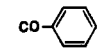
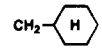
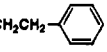
Part 4. The effect of the methyl and nitro substituents in the benzyl moiety (R_4) of **13e** was examined (Table 5). The 3-position-substituted benzyl derivatives **16b,e** showed the highest potency among the 2-, 3-, and 4-substituted regioisomers. These compounds showed nearly equal potency to that of **13e** in the anti-AChE assay. Interestingly **16b**, substituted with an electron-donating group (methyl group), and **16e**, substituted with an electron-withdrawing group (nitro group), showed a similar effect on anti-AChE activity. The basicity of the nitrogen atom in the piperidine ring appears to have an important effect on activity, since the *N*-benzoylpiperidine derivative **13a** was almost inactive. Removal of the benzyl group (**13b**) caused a great reduction in the potency, but comparable anti-AChE activity with respect to **13e** was retained after replacement with a cyclohexylmethyl group (**16g**). However, replacement of the R_4 benzyl moiety with a phenethyl group (**16h**) resulted in significantly decreased potency. Compound **13e** (E2020) is one of the most potent compounds of anti-AChE activity among the indanone derivatives.

Pharmacology

The inhibitory effects of **13e** of the cholinesterase (ChE) activity *in vitro* and *ex vivo* were compared with those of two other ChE inhibitors, physostigmine and

Table 5. Anti-AChE Activity of *N*-Substituted-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine Derivatives



no.	R_4	mp, °C	formula	inhibition of AChE IC_{50}, nM[a]
16a		220-221	$C_{25}H_{31}NO_3 \cdot HCl$	10
16b		212-213	$C_{25}H_{31}NO_3 \cdot HCl$	2.0
16c		229-230 dec	$C_{25}H_{31}NO_3 \cdot HCl$	40
16d		oil	$C_{24}H_{28}N_2O_5$	160[b]
16e		210-211	$C_{24}H_{28}N_2O_5 \cdot HCl$	4.0
16f		234-236	$C_{24}H_{28}N_2O_5 \cdot HCl$	100
13a		151-152	$C_{24}H_{27}NO_4$	>10000
13b	H	249-250 dec	$C_{17}H_{23}NO_3 \cdot HCl$	5400
16g		225-226	$C_{24}H_{35}NO_3 \cdot HCl$	8.9
16h		253-256 dec	$C_{25}H_{31}NO_3 \cdot HCl$	180

[a] Deviation of measurement of IC_{50} value is 10–20%. [b] The value of the hydrochloride is shown.

Table 6. Inhibitory Effects of **13e** and Reference Compounds on AChE and BuChE Activity (*in Vitro*)[a]

compd	activity IC_{50}, nM		ratio of IC_{50} (BuChE/AChE)
	AChE	BuChE	
13e	5.7 ± 0.2	7138 ± 133	1252
physostigmine	0.68 ± 0.02	8.1 ± 0.3	11.9
tacrine	80.6 ± 2.5	73.0 ± 0.9	0.9

[a] Values represent the mean ±SE from four dose–response curves for each test drug.

tacrine, with respect to their selectivity and duration of action. The first experiment was designed to determine the relative inhibitory effects of **13e** on AChE and butyrylcholinesterase (BuChE), in comparison with other ChE inhibitors.[21] In these experiments, rat brain homogenate was used as the source of AChE and rat plasma served as the source of BuChE. ChE activity was determined according to the method of Ellman et al.[17] Both enzymes were incubated with different concentrations of each inhibitor.

As shown in Table 6, the IC_{50} of **13e** for AChE was 5.7 nM. The anti-AChE activity of **13e** was 14 times more potent than that of tacrine but 8 times less potent than that of physostigmine. In contrast, the IC_{50} of **13e** for BuChE was 7138 nM. Among the ChE inhibitors tested, **13e** was the least potent against BuChE. The selectivity of the drugs for the two enzymes is shown as a "ratio of IC_{50}" in Table 6. The ratio of **13e** for BuChE:AChE (1252) is much greater than that of physostigmine (11.9) or tacrine (0.9). These results indicate that **13e** is a highly selective inhibitor of AChE compared with physostigmine and tacrine.

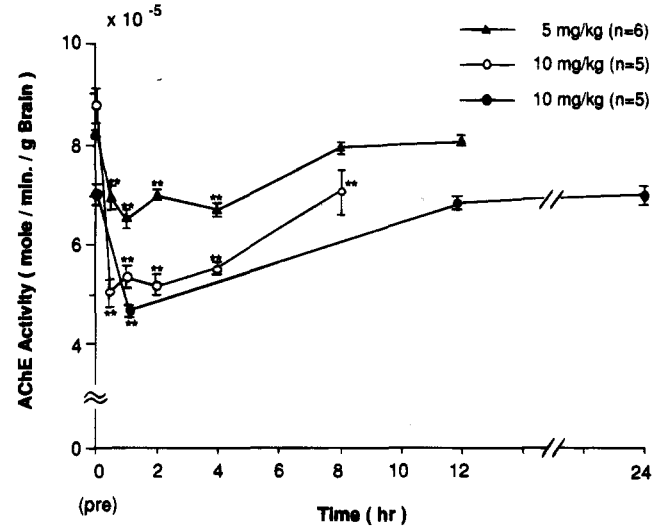


Figure 4. Time course of the effect of oral administration of **13e** on *ex vivo* brain AChE activity in rats. The values are the mean ± SE ($n = 5–6$). * and **: $p < 0.05$ and $p < 0.01$ vs "pre" (saline control, $n = 10$) (Dunnett's *t*-test).

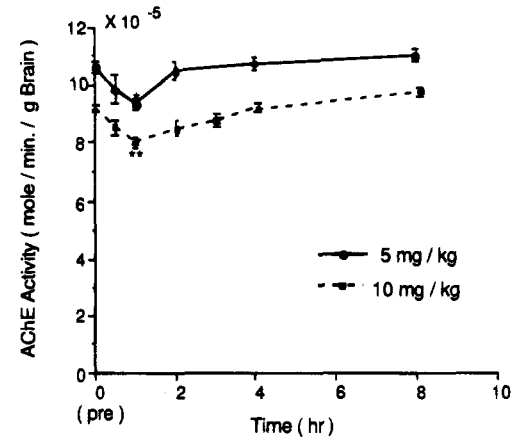


Figure 5. Time course of the effect of oral administration of physostigmine on *ex vivo* brain AChE activity in rats. The values are the mean ± SE ($n = 5–6$). * and **: $p < 0.05$ and $p < 0.01$ vs "pre" (saline control, $n = 10$) (Dunnett's *t*-test).

The second experiment was designed to compare *in vivo* anti-AChE activity of **13e**, physostigmine, and tacrine. Test compounds were given orally to rats, and then they were sacrificed at various intervals after administration. AChE activity in the brain was determined as in the first experiment. The results indicate that **13e** effectively inhibited AChE at the dose of 5 mg/kg at least 4 h after treatment and at the dose of 10 mg/kg at least 8 h (Figure 4). In contrast, AChE activity after treatment with 5 mg/kg physostigmine returned to control levels in 2 h (Figure 5). Tacrine at 10 mg/kg did not inhibit AChE activity at any time point. At 30 mg/kg, tacrine exhibited a slight but significant inhibitory effect for 12 h (Figure 6). These results indicate that, at equal doses, **13e** is orally active and has a longer duration of action than physostigmine or tacrine.[22] **13e** did not show any serious toxicity in a short term toxicity study (data not shown).

Binding Site of 13e and AChE

In a previous paper,[19] we described the hypothetical binding site of **13e** on AChE. The carbonyl oxygen of the indanone moiety is supposed to interact through hydrogen bonding with an acceptor hydroxy group of a

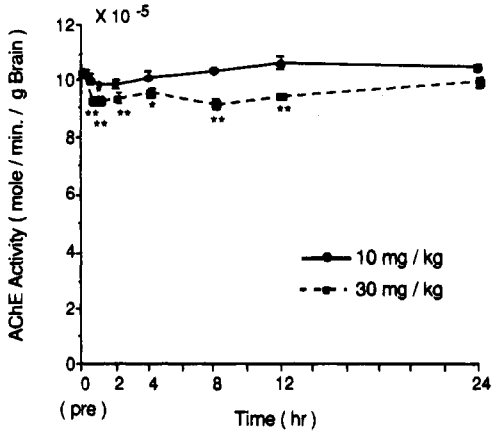


Figure 6. Time course of the effect of oral administration of tacrine on *ex vivo* brain AChE activity in rats. The values are the mean ± SE ($n = 6$). * and **: $p < 0.05$ and $p < 0.01$ vs "pre" (saline control, $n = 6$) (Dunnett's t-test).

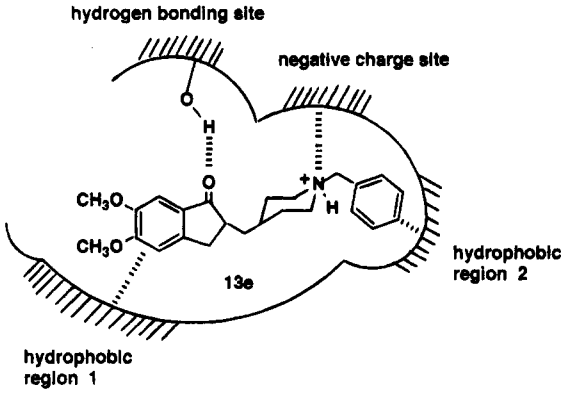


Figure 7. Proposed model of the acetylcholinesterase active site shown with **13e** interacting at the hydrogen-bonding site, negative charge site, and hydrophobic regions 1 and 2 on the binding protein.

serine residue in AChE, and the nitrogen atom of the piperidine moiety is proposed to have a charge–charge interaction with a carboxylic group, possibly an aspartic acid moiety in the anionic site of the enzyme. Therefore, the spatial locations of these "pharmacophoric" atoms are important for inhibitory activity. Recently, Sussman et al.[23] have described the three-dimensional structure of AChE from *Torpedo californica* electric organ as determined by X-ray analysis to 2.8 Å. Their results suggest that (a) the active site contains Glu, not Asp, in the Ser–His–acid catalytic triad and (b) the relation of the triad to the rest of the protein approximates a mirror image of that seen in the serine protease. Furthermore, the active site was reported to lie near the bottom of a deep and narrow cleft that reaches halfway into the protein. A modeling study for ACh binding to the enzyme suggested that the quarternary ammonium ion is bound not to a negatively charged "anionic" site but rather to some of the 14 aromatic residues that line the cleft. The crystal structure of AChE also clearly revealed that the choline-binding anionic subsite is misnamed, as it contains at most one formal negative charge. Instead the quarternary moiety of choline appears to bind chiefly through interactions with the electrons in the aromatic residue. Thus, the revised hypothetical binding site of **13e** on AChE is proposed as illustrated in Figure 7. We have renamed that "choline-binding anionic site" as the

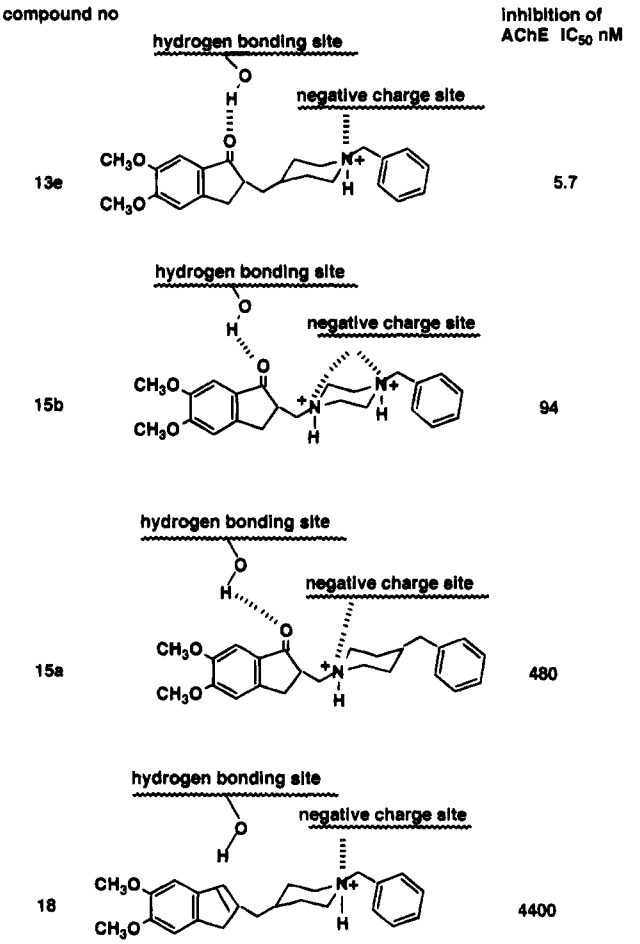


Figure 8. Schematic illustration of the hydrogen-bonding and negative charge site interactions for **13e, 15a,b,** and **18.**

"negative charge site" since it contains at most one formal negative charge.[23] Inhibitors are thus suggested to interact with AChE through the hydrogen-bonding site and negative charge sites. In addition, a hydrophobic region[24] was thought to be closely adjacent to both the negative charge and the hydrogen-bonding sites.[25,26] The hydrophobic region might correspond to hydrophobic region 1 in Figure 7. Quinn's review[27] suggests that there may exist at least one more hydrophobic region at a defined distance away from the negative charge site, which we have designated "hydrophobic region 2" in Figure 7.

Compound **15b** is 15-fold less active than **13e**. The marginal inhibitory activity of **15b** is mentioned because it is difficult to explain why **15b** is less active than **13e**. One possible explanation for the observed activity profile of **15b** is based on the active centers hypothesis of **13e** (Figure 8). The available structure–activity data suggest that both the carbonyl oxygen and the piperidinic nitrogen atom are essential for AChE inhibitory activity. The former is suggested to interact through hydrogen bonding with an acceptor hydroxy group of a serine residue in the hydrogen-bonding site of AChE, and the nitrogen atom is proposed to have a charge–charge interaction with the negative charge site. Therefore, the spatial locations of these "pharmacophoric" atoms are critical for inhibitory activity. Spatial considerations are particularly important for the carbonyl group since the hydrogen-binding interaction is extremely dependent on the distance and the relative orientation of the acceptor and donor groups. Compound **15b** has two

possible cationic nitrogen centers. On the basis of the pharmacophore model, the two nitrogens may compete for the negative charge site of the enzyme, which is regarded as the orienting site.[28] These competitive interactions may lead **15b** into a binding state for which both N atoms are at similar distances from the negative charge site. Such a virtual "displacement" may move the carbonyl group of the bicyclic ring from its optimum binding position so that the carbonyl oxygen can not fit properly at the hydrogen-bonding site. This idea is supported by the biological results obtained for other analogues. The highly active compound **13e** can adopt a position in space near to the optimum interaction geometry. Compound **15b**, less active than **13e**, may have a weak hydrogen-bonding interaction as explained above. Finally, compound **18**, one of the most inactive of the series, can have no hydrogen-bonding interaction (Figure 8). These results also suggest that the hydrogen-bonding interaction is very important to increase anti-AChE activity. Compound **15a**, with both binding centers but with the carboxylic group located at a shorter distance from the cationic head, is about 100-fold less active than **13e**. On the other hand, compound **18**, with only the cationic head as a possible binding center, is almost 1000-fold less active than **13e**.

Experimental Section

Chemistry. 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 are within 0.4% of theoretical values.

1-Benzyl-4-[2-(1-oxoindan-2-yl)ethyl]piperidine Hydrochloride (8). NaH (60% mineral oil dispersion, 0.32 g, 8.0 mmol) was washed with hexane, and tetrahydrofuran (THF) (20 mL) was added thereto. A solution of diethyl (1-oxoindan-2-yl)phosphonate (2.12 g, 7.9 mmol) in THF (30 mL) was dropwise added at 0 °C. The mixture was stirred at room temperature for 30 min and again cooled to 0 °C followed by addition of a solution of 1-benzyl-4-piperidine acetoaldehyde (3.43 g, 15.7 mmol) in dimethylformamide (10 mL). The mixture was stirred at room temperature for 2 h and at 50 °C for 2 h and then refluxed for 2 h. MeOH and 20% H_2SO_4 were added at 0 °C to the reaction mixture; 10 min after the addition, the reaction mixture was made basic with dilute NaOH and extracted with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 500:1) to give 1.64 g (61.9%) of 1-benzyl-4-[2-(1-oxoindan-2-ylidenyl)ethyl]piperidine: ^{1}H NMR ($CDCl_3$) δ 1.10–2.13 (7H, m), 2.26 (2H, t), 2.88 (2H, bd), 3.48 (2H, s), 6.72–7.07 (2H, m), 7.30 (5H, s), 7.10–8.00 (5H, m); MS *m/e* 331 (M^+). Anal. ($C_{23}H_{25}NO$) C, H, N.

A solution of 1-benzyl-4-[2-(1-oxoindan-2-ylidenyl)ethyl]piperidine (0.37 g, 1.1 mmol) in MeOH (10 mL) was hydrogenated over 5% rhodium on carbon at room temperature under 1 atm for 24 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 (CH_2Cl_2:MeOH = 200:1). The eluate was evaporated *in vacuo*, and the residue was treated with 10% HCl–EtOAc. The resulting solid was recrystallized from MeOH–isopropyl ether to give 0.33 g (80%) of **8**: mp 224–225 °C; MS *m/e* 333 (M^+). Anal. ($C_{23}H_{27}NO$·HCl) C, H, N.

1-Benzyl-4-[(1-oxoindan-2-yl)methyl]piperidine hydrochloride (9): yield 80% from 1-benzyl-4-[(1-oxoindan-2-ylidenyl)methyl]piperidine; mp (EtOH–isopropyl ether) 196–197 °C; MS *m/e* 319 (M^+). Anal. ($C_{22}H_{25}NO$·HCl) C, H, N.

1-Benzyl-4-(α-tetralon-2-ylidenylmethyl)piperidine and 1-benzyl-4-(1-benzosuberanon-2-ylidenylmethyl)piperidine were prepared in the same manner (1-benzyl-4-[2-(1-oxoindan-2-ylidenyl)ethyl]piperidine) and purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1).

1-Benzyl-4-(α-tetralon-2-ylmethyl)piperidine hydrochloride (13c): yield 67% from 1-benzyl-4-(α-tetralon-2-ylidenylmethyl)piperidine; mp (CH_2Cl_2–isopropyl ether) 195–196 °C; MS *m/e* 333 (M^+). Anal. ($C_{23}H_{27}NO$·HCl) C, H, N.

1-Benzyl-4-(1-benzosuberanon-2-ylmethyl)piperidine (13d): yield 95% from 1-benzyl-4-(1-benzosuberanon-2-ylidenylmethyl)piperidine; ^{1}H NMR ($CDCl_3$) δ 1.10–2.10 (13H, m), 2.60–3.08 (5H, m), 3.41 (2H, s), 7.00–7.85 (9H, m); MS *m/e* 347 (M^+).

1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-ylidenyl)-methyl]piperidine Hydrochloride (12a). This reaction was conducted in an argon atmosphere. Diisopropylamine (2.05 mL) was added to 10 mL of THF followed by addition of 9.12 mL of a 1.6 M solution of *n*-butyllithium in hexane at 0 °C. The mixture was stirred at 0 °C for 10 min and then cooled to −78 °C, and a solution of 2.5 g (13 mmol) of **10a** in 30 mL of THF and 2.31 mL of hexamethylphosphoric triamide were added thereto. The mixture was stirred at −78 °C for 15 min, and a solution of 1.6 g (7.9 mmol) of 1-benzyl-4-formylpiperidine[29,30] in 30 mL of THF was added thereto. The temperature of the mixture was gradually raised to room temperature followed by stirring for 2 h. An aqueous 1% ammonium chloride solution was added thereto, and the reaction mixture was extracted with ether. The ether layer was washed with a saturated NaCl solution, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1). The eluate was concentrated *in vacuo*, and the residue was treated with 10% HCl–EtOAc. The resulting solid was recrystallized from MeOH–isopropyl ether to give 2.0 g (62%) of **12a**: mp 237–238 °C dec; MS *m/e* 377 (M^+). Anal. ($C_{24}H_{27}NO_3$·HCl) C, H, N.

Geometric Isomer of 12a. The 400 MHz ^{1}H NMR spectra were measured using a JEOL JNM-GX 400 NMR spectrometer. Geometric isomerism of **12a** was assigned by decoupling experiments and NOESY (mixing time 1500 ms). The doublet triplet signal at 6.67 ppm is the olefinic proton, assigned to 4-CH=. When this olefinic proton is irradiated, the doublet signal at 3.59 ppm is changed to a singlet and the multiplet signal at 2.34 ppm is decoupled. The signals at 3.59 and 2.34 ppm are assigned to the indanone 3-proton and the piperidine 4-proton, respectively. The NOE cross-peak between the olefinic proton and the indanone 3-proton is not observed, but the indanone 3-proton and the piperidine 4-proton are observed. This confirms the double bond is the *E* form.

1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine Hydrochloride (13e, E2020). A solution of **12a** (0.4 g, 1.1 mmol) in THF (16 mL) was hydrogenated over 10% palladium on carbon (0.1 g) at room temperature under atmosphere pressure for 6 h. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:2). The eluate was evaporated *in vacuo*, and the residue was treated with 10% HCl–EtOAc. The resulting solid was recrystallized from MeOH–isopropyl ether to give 0.36 g (86.4%) of **13e**: mp 211–212 °C dec; MS *m/e* 379 (M^+). Anal. ($C_{24}H_{29}NO_3$·HCl) C, H, N.

13f–r were prepared in the same manner and purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1) or recrystallization. **13e** and other indanone derivatives with an asymetric carbon atom (2-position) did not optical resolve.

1-Benzyl-4-[(6-methoxy-1-oxoindan-2-yl)methyl]piperidine (13f): yield 68%; ^{1}H NMR ($CDCl_3$) δ 1.00–3.40 (14H, m), 3.47 (2H, s), 3.78 (3H, s), 6.90–7.50 (8H, m); MS *m/e* 349 (M^+).

1-Benzyl-4-[(5-methoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (13g): yield 70%; mp (EtOH–isopropyl ether) 203–204 °C dec; MS *m/e* 349 (M^+). Anal. ($C_{23}H_{27}NO_2$·HCl) C, H, N.

1-Benzyl-4-[(4-methoxy-1-oxoindan-2-yl)methyl]piperidine (13h): yield 70%; ^{1}H NMR ($CDCl_3$) δ 1.05–2.12 (9H,

m), 2.50–3.00 (4H, m), 3.12 (1H, m), 3.48 (2H, s), 3.88 (3H, s), 7.15–7.23 (8H, m); MS *m/e* 349 (M^+).

1-Benzyl-4-[(6,7-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (13i): yield 82%; mp (CH_2Cl_2–isopropyl ether) 198–199 °C; MS *m/e* 379 (M^+). Anal. ($C_{24}H_{29}NO_3$·HCl) C, H, N.

1-Benzyl-4-[(5,7-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (13j): yield 64%; ^1H NMR ($CDCl_3$) δ 1.10–2.15 (9H, m), 2.45–3.05 (4H, m), 3.18 (1H, s), 3.48 (2H, s), 3.81 (3H, s), 3.85 (3H, s), 6.25 (1H, bs), 6.42 (1H, bs), 7.25 (5H, s); MS *m/e* 379 (M^+).

1-Benzyl-4-[(4,7-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (13k): yield 76%; ^1H NMR δ 1.05–2.10 (9H, m), 2.40–3.00 (4H, m), 3.15 (1H, dd), 3.45 (2H, s), 3.80 (3H, s), 3.85 (3H, s), 6.62 (1H, d), 6.90 (1H, d), 7.22 (5H, s); MS *m/e* 379 (M^+).

1-Benzyl-4-[(4,5-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (13l): yield 91%; mp (EtOH–isopropyl ether) 199–200 °C dec; MS *m/e* 379 (M^+). Anal. ($C_{24}H_{29}NO_3$·HCl) C, H, N.

1-Benzyl-4-[(5,6,7-trimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (13m): yield 89%; mp (EtOH–isopropyl ether) 200–201 °C; MS *m/e* 409 (M^+). Anal. ($C_{25}H_{31}NO_4$·HCl) C, H, N.

1-Benzyl-4-(5,6-dimethoxy-1-oxoindan-2-yl)piperidine hydrochloride (13n): yield 95%; mp (CH_2Cl_2–isopropyl ether) 247–248 °C dec; MS *m/e* 365 (M^+). Anal. ($C_{23}H_{27}NO_3$·HCl) C, H, N.

1-Benzyl-4-[2-(5,6-dimethoxy-1-oxoindan-2-yl)piperidine hydrochloride (13o): yield 79% from 1-benzyl-4-[2-(5,6-dimethoxy-1-oxoindan-2-ylidenyl)ethyl]piperidine; mp (MeOH–isopropyl ether) 201–202 °C dec; MS *m/e* 393 (M^+). Anal. ($C_{25}H_{31}NO_3$·HCl) C, H, N.

1-Benzyl-4-[3-(5,6-dimethoxy-1-oxoindan-2-yl)propyl]piperidine (13p): yield 84% from 1-benzyl-4-[3-(5,6-dimethoxy-1-oxoindan-2-ylidenyl)propyl]piperidine; ^1H NMR ($CDCl_3$) δ 1.00–2.15 (13H, m), 2.60–3.05 (4H, m), 3.20 (1H, dd), 3.43 (2H, s), 3.85 (3H, s), 3.90 (3H, s), 6.77 (1H, s), 7.05 (1H, s), 7.20 (5H, s); MS *m/e* 407 (M^+).

1-Benzyl-4-[4-(5,6-dimethoxy-1-oxoindan-2-yl)butyl]piperidine (13q): yield 67% from 1-benzyl-4-[4-(5,6-dimethoxy-1-oxoindan-2-ylidenyl)butyl]piperidine; ^1H NMR ($CDCl_3$) δ 1.00–2.10 (15H, m), 2.58–3.05 (4H, m), 3.32 (1H, dd), 3.50 (2H, s), 3.90 (3H, s), 3.97 (3H, s), 6.88 (1H, s), 7.18 (1H, s), 7.31 (5H, s); MS *m/e* 421 (M^+).

1-Benzyl-4-[5-(5,6-dimethoxy-1-oxoindan-2-yl)pentyl]piperidine (13r): yield 58% from 1-benzyl-4-[5-(5,6-dimethoxy-1-oxoindan-2-ylidenyl)pentyl]piperidine; ^1H NMR ($CDCl_3$) δ 1.05–2.15 (17H, m), 2.40–3.00 (4H, m), 3.21 (1H, dd), 3.45 (2H, s), 3.85 (3H, s), 3.90 (3H, s), 6.78 (1H, s), 7.08 (1H, s), 7.21 (5H, s); MS *m/e* 435 (M^+).

1-Benzyl-4-[(5,6-dimethoxy-1-hydroxyindan-2-yl)methyl]piperidine (17). To a solution of the free base of **13e** (0.3 g, 0.8 mmol) in MeOH (3 mL) was added dropwise a solution of $NaBH_4$ (0.04 g, 1.0 mmol) in MeOH (2 mL) at 0 °C. This mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. The mixture was acidified with AcOH (pH 4) and evaporated. The residue was diluted with CH_2Cl_2 (30 mL) and washed with 10% Na_2CO_3 (20 mL) and saturated NaCl (20 mL). After drying ($MgSO_4$), the organic layer was evaporated to afford a colorless oil. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1) to give 0.22 g (71%) of the free base **17** as a mixture of diasteroisomers: ^{H} NMR ($CDCl_3$) δ 1.05–3.15 (15H, m), 3.48 and 3.52 (total 2H), 3.88 (6H, s), 4.72 and 4.82 (total 1H, s), 6.72 and 6.75 (total 1H, s), 6.92 and 6.95 (total 1H, s), 7.32 (5H, s); MS *m/e* 381 (M^+).

1-Benzyl-4-[(5,6-dimethoxyinden-2-yl)methyl]piperidine Hydrochloride (18). A solution of **17** (0.24 g, 0.63 mmol) and 10% HCl–EtOAc in CH_2Cl_2 was evaporated *in vacuo*. The residue was recrystallized from CH_2Cl_2–isopropyl ether to give 0.24 g (95%) of **18**: mp 216–217 °C dec; MS *m/e* 363 (M^+). Anal. ($C_{24}H_{29}NO_2$·HCl) C, H, N.

4-Benzyl-1-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (15a). 10a (1.00 g, 5.2 mmol), 0.31 g of paraformaldehyde, and 0.91 mL of 4-benzylpiperidine were suspended in a mixture comprised of EtOH (30 mL) and water. The pH of the suspension was adjusted to 3 with concentrated HCl. The mixture was refluxed for 3 h and filtered to obtain a white solid. The solid was suspended in CH_2Cl_2, washed with 10% Na_2CO_3 (aq) and water, dried over ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeO = 100:1). The eluate was evaporated *in vacuo* to give 0.71 g (36%) of **15a**: ^1H NMR ($CDCl_3$) δ 1.10–3.50 (16H, m), 3.87 (3H, s), 3.98 (3H, s), 6.80 (1H, s), 7.00–7.25 (6H, m); MS *m/e* 379 (M^+).

1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperazine dihydrochloride (15b): yield 23%; mp (MeOH) 227–228 °C dec; MS *m/e* 380 (M^+). Anal. ($C_{23}H_{28}N_2O_3$·2HCl) C, H, N.

1-Benzoyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (13a). 10a (0.85 g, 4.4 mmol) and 1.38 g (6.4 mmol) of 1-benzoyl-4-piperidinecarbaldehyde were dissolved in THF (20 mL). Sodium methoxide (1.02 g, 28 wt % solution in MeOH) was added to the solution at 0 °C. The mixture was stirred at room temperature for 2 h, and diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1) to give 1.23 g (71%) of 1-benzoyl-4-[(5,6-dimethoxy-1-oxoindan-2-ylidenyl)methyl]piperidine. This compound (1.23 g, 3.1 mmol) was dissolved in THF (20 mL), and hydrogenated with 10% palladium on carbon (0.3 g) at room temperature for 24 h. The catalyst was filtered out, and the filtrate was evaporated *in vacuo*. The residue was recrystallized from CH_2Cl_2–hexane to give 1.10 g (89%) of **13a**: mp 151–152 °C; MS *m/e* 393 (M^+). Anal. ($C_{24}H_{27}NO_4$) C, H, N.

4-[(5,6-Dimethoxy-1-oxoindan-2-yl)methyl]piperidine Hydrochloride (13b). 13a (9.05 g, 23 mmol) was dissolved in dioxane (90 mL) followed by the addition of 6 N HCl (90 mL). The mixture was heated under reflux for 10 h and evaporated *in vacuo*. The residue was diluted with water and extracted with EtOAc. The pH of the water layer was adjusted to 12 with dilute NaOH and the mixture extracted with CH_2Cl_2. The organic layer was washed with water, dried over ($MgSO_4$), and evaporated *in vacuo*. The residue was treated with 10% HCl–EtOAc; the resulting solid was recrystallized from MeOH–EtOH to give 6.30 g (85%) of **13b**: mp 249–250 °C dec; MS *m/e* 279 (M^+). Anal. ($C_{17}H_{23}NO_3$·HCl) C, H, N.

1-(2-Methylbenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine Hydrochloride (16a). A mixture of free base **13b** (0.25 g, 0.86 mmol), triethylamine (0.13 mL), and 2-methylbenzyl chloride (0.12 g, 0.86 mmol) in THF was refluxed for 2 h. The mixture was evaporated *in vacuo*. The residue was diluted with EtOAc. The organic layer was washed 10% aqueous Na_2CO_3, dried over $MgSO_4$, and evaporated *in vacuo*. The obtained residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:1). The eluate was evaporated *in vacuo*, and the residue was treated with 10% HCl–EtOAc. The resulting solid was recrystallized from EtOH–isopropyl ether to give 0.37 g (58%) of **16a**: mp 220–221 °C; MS *m/e* 393 (M^+). Anal. ($C_{25}H_{31}NO_3$·HCl) C, H, N.

1-(3-Methylbenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (16b): yield 42%; mp (EtOH–isopropyl ether) 212–213 °C; MS *m/e* 393 (M^+). Anal. ($C_{25}H_{31}NO_3$·HCl) C, H, N.

1-(4-Methylbenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (16c): yield 52%; mp (EtOH–isopropyl ether) 229–230 dec; MS *m/e* 393 (M^+). Anal. ($C_{25}H_{31}NO_3$·HCl) C, H, N.

1-(2-Nitrobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine (16d): yield 52%; ^1H NMR δ 1.00–2.20 (9H, m), 2.50–2.92 (4H, m), 3.73 (2H, s), 3.86 (3H, s), 3.93 (3H, s), 6.81 (1H, s), 7.11 (1H, s), 7.22–7.85 (4H, m); MS *m/e* 424 (M^+).

1-(3-Nitrobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (16e): yield 46%; mp (EtOH–isopropyl ether) 210–211 °C; MS *m/e* 424 (M^+). Anal. ($C_{24}H_{28}N_2O_5$·HCl) C, H, N.

1-(4-Nitrobenzyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)-methyl]piperidine hydrochloride (16f): yield 63%; mp (EtOH–CH_2Cl_2–isopropyl ether) 234–236 °C dec; MS *m/e* 424 (M^+). Anal. ($C_{24}H_{28}N_2O_5$·HCl) C, H, N.

1-(Cyclohexyl)-4-[(5,6-dimethoxy-1-oxoindan-2-yl)-methyl]piperidine hydrochloride (16g): yield 26%; mp (EtOH–isopropyl ether) 225–226 °C dec; MS *m/e* 385 (M^+). Anal. ($C_{24}H_{35}NO_3$·HCl) C, H, N.

1-Phenethyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine hydrochloride (16h): yield 24%; mp (EtOH–CH_2Cl_2–isopropyl ether) 253–256 dec; MS *m/e* 393 (M^+). Anal. ($C_{25}H_{31}NO_3$·HCl) C, H, N.

Determination of Cholinergic Parameters. *In Vitro* Anticholinesterase Activity. The inhibitory effects of **13e** (E2020) 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. Mouse brain homogenates (100 mg of brain/mL of 0.1 M sodium potassium phosphate buffer, pH 8.0) and rat plasma were used as sources of AChE and 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. E2020 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 at −80 °C until assayed. The frozen tissues were homogenized in 10 vol 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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