Syntheses and Anticholinesterase Activity of Tetrahydrobenzazepine Carbamates

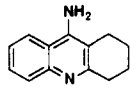
Yuhpyng L. Chen,* Dane Liston, Jann Nielsen, Douglas Chapin, Audrey Dunaiskis, Kirk Hedberg, Jeffery Ives, Jonathan Johnson, Jr., and Shawn Jones

Departments of Medicinal Chemistry and Neuroscience, Central Research Division, Pfizer Inc., Groton, Connecticut 06340

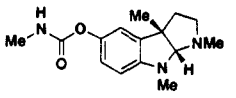
Received February 14, 1994[⊕]

The synthesis of a series of alkylcarbamates of 1,5-methano-2,3,4,5-tetrahydro-1*H*-2-benzazepin-7-ol is reported. Many of these compounds are potent acetylcholinesterase (AChE) inhibitors. The *in vitro* AChE inhibition, cholinergic effects, acute toxicity, and elevation of brain acetylcholine levels *in vivo* of this series of compounds are described. A representative compound, **1d** (5.6 mg/kg, po), was able to reverse hemicolinium-3-induced amnesia in the mouse passive avoidance assay.

Senile dementia of the Alzheimer's type (SDAT) is a common neurodegenerative disorder which results in profound memory loss and cognitive decline. Several neurotransmitter systems are affected in the disease, but the earliest and most dramatic neurochemical lesions occur in the cholinergic system.[1,2] Consequently, there is great interest in the use of cholinomimetic agents for the palliative treatment of Alzheimer's disease. Inhibition of acetylcholinesterase (AChE) is perhaps the most direct approach to this end, and tetrahydroaminoacridine (tacrine; Warner-Lambert) is the first drug of this type approved for use against SDAT in the United States. A high incidence of hepatotoxicity has been noted in the clinic with tacrine. The liver toxicity observed may be structure dependent rather than mechanism related. Several structurally distinct AChE inhibitors, including a controlled-release formulation of the naturally occurring carbamate physostigmine (Cogmine), are in clinical development.

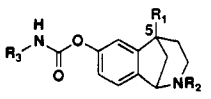


tacrine

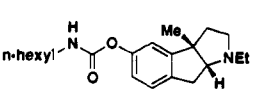


physostigmine

Previously,[3,4] we described a series of 8-carbaphysostigmine analogues (e.g., **2**) as potent acetylcholinesterase inhibitors. On the basis of the hypothesis that the spatial relationship between the carbamate and the protonated-amine region in these compounds is important, a series of alkylcarbamates of 1,5-methano-2,3,4,5-tetrahydro-1*H*-2-benazepin-7-ol **1** was prepared and evaluated as AChE inhibitors. Modifications of the substituents at the N_2



1



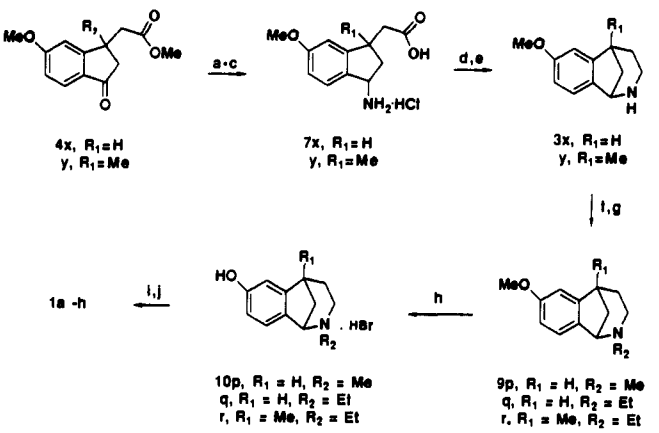
2

and C_5 positions as well as the substituents of the 7-carbamate region were explored in series **1**. In the present paper, we describe the synthesis of this series of compounds and report on *in vitro* and *in vivo* biological activities relevant to alterations in acetylcholine (ACh) metabolism.

Chemistry

Compounds in series **1** were prepared *via* a key intermediate, **3x**[5] or **3y**. Compounds **3x,y** were prepared according to known procedures[5] starting from indanone acetate **4x,y**[4] as shown in Scheme 1. Treatment of **4x** or **4y** with hydroxylamine and sodium acetate in methanol afforded the oximes **5x** or **5y**, respectively, as a mixture of *E* and *Z* isomers. Hydrogenation of the oxime mixture **5x** or **5y** followed by acid hydrolysis gave the amine **7x** or **7y**, respectively. Treatment of **7x,y** with water-soluble carbodiimide hydrochloride in pyridine afforded the lactam **8x,y** which was reduced with borane–tetrahydrofuran complex to give the benzazepine amine **3x,y**. *N*-Alkyl derivatives **9p–r** were prepared by acylation of **3x,y** followed by reduction with borane–methyl sulfide complex. Demethylation of **9p–r** followed by reaction with an alkyl isocyanate in the presence of a catalytic amount of sodium or sodium hydride in dry benzene afforded the final benzazepine carbamates **11a–h**. All of these compounds were prepared as oily racemates. The corresponding di-*p*-toluoyl-L-tartrate salt (BY) or *p*-tosylate salt was prepared as amorphous solid salts for biological assays. The physicochemical properties of the final carbamate analogues are listed in Table 1.

Scheme 1[a]



[a] (a) NH_2OH·HCl, NaOAc·$3H_2O$; (b) 10% Pd/C in HCl(g)/MeOH, H_2 (50 psi); (c) 2 N HCl; (d) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate in pyridine; (e) BH_3·THF; (f) R_2COCl/pyridine; (g) BH_3·DMS; (h) 48% HBr; (i) i. NaH or Na, ii. R_3NCO; (j) di-*p*-toluoyl-L-tartaric acid or *p*-TsOH.

Biological Results and Discussion

Early kinetic studies proposed that AChE has two active subsites, the "esteratic" and "anionic" subsites, which presumably bind to the carbamate and the quaternary nitrogen of acetylcholine, respectively. Recently, the three-dimensional structure of acetylcholinesterase from *Torpedo californica* electric organ has been determined by

[⊕] Abstract published in *Advance ACS Abstracts*, June 1, 1994.

0022-2623/94/1837-1996$04.50/0

Table 1. Physicochemical Properties of Benzazepine Carbamate Derivatives

compd	R_1	R_2	R_3	yield[a] (%)	formula[b]	anal.
1a	Me	Et	*n*-heptyl	79	$C_{22}H_{34}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1c	H	Me	*n*-heptyl	78	$C_{20}H_{30}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.4H_2O$	C,H,N
1d	H	Me	*n*-hexyl	56	$C_{19}H_{28}N_2O_2 \cdot C_{20}H_{18}O_8$	C,H,N
1e	H	Et	*n*-heptyl	81	$C_{21}H_{32}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot H_2O$	C,H,N
1f	H	Et	*n*-hexyl	79	$C_{20}H_{30}N_2O_2 \cdot C_7H_8O_3S$	C,H,N
1g	H	Et	*n*-butyl	93	$C_{18}H_{28}N_2O_2 \cdot C_{20}H_{18}O_8$	C,H,N
1h	H	Et	methyl	64	$C_{15}H_{20}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.8H_2O$	C,H,N

[a] Yields were not optimized. [b] $C_{20}H_{18}O_8$ = di-*p*-toluoyl-L-tartaric acid; $C_7H_8O_3S$ = *p*-toluenesulfonic acid. The parent compound is an oil, and the corresponding salt was prepared as an amorphous solid after trituration with diethyl ether or isopropyl ether. Compounds failed to give crystals.

Table 2. AChEIC_{50},[a] Side Effects Profile,[b] and LD_{50}[c] of Physostigmine, Heptylphysostigmine, an 8-Carbaphysostigmine, 2, and Benzazepine Carbamate Analogues

compd[d]	R_1	R_2	R_3	IC_{50} (nM)	dia[b] (mg/kg)	sal[b] (mg/kg)	tre[b] (mg/kg)	LD_{50}[c] (mg/kg)
(−)-physostigmine				128 ± 14	0.32	0.32	0.32	0.88
(−)-heptylphysostigmine				110 ± 20	1.8	10	10	24
(−)-2				20 ± 7	1.8	3.2	3.2	4
1a	Me	Et	*n*-heptyl	159 ± 211	1.0	ND[e]	1.8	4
1b	Me	Et	*n*-hexyl	133 ± 15	NT[f]	NT[f]	NT[f]	9
1c	H	Me	*n*-heptyl	143 ± 18	ND[e]	10	10	18
1d	H	Me	*n*-hexyl	72 ± 7	ND[e]	5.6	3.2	6
1e	H	Et	*n*-heptyl	169 ± 2	3.2	17.8	17.8	22
1f	H	Et	*n*-hexyl	125 ± 25	1.8	3.2	3.2	9
1g	H	Et	*n*-butyl	206 ± 16	NT[f]	NT[f]	NT[f]	NT[f]
1h	H	Et	methyl	171 ± 30	NT[f]	NT[f]	NT[f]	1

[a] IC_{50} values shown are mean ± standard deviation of three assays against human acetylcholinesterase. [b] Minimal dose to produce side effects; dia = diarrhea; sal = salivation; tre = tremor. [c] LD_{50} values are from lethality measured at 24 h in mice following ip administration. [d] Compounds 1a–h are racemates. [e] ND = not detected at doses ≤ LD_{50}. [f] NT = not tested.

X-ray analysis.[7] These studies showed that the quaternary nitrogen of ACh does not, in fact, seem to bind to the anionic component, identified as Glu 199; instead, it binds strongly with the π electrons of the Trp 84 group. Thus, we may infer that the carbamate group of physostigmine or 8-carbaphysostigmine series interacts with the catalytic triad (Ser-His-Glu). Previously, we reported that structural modification of physostigmine provided a more potent AChE inhibitor, 2, which possessed a more basic quaternary nitrogen than physostigmine. Our initial working hypothesis assumed that the quaternary nitrogen is essential for the activity. In continuation of this work, we initiated our synthesis in the benzazepine carbamate series to find the optimal spatial placement of the quaternary nitrogen relative to the carbonyl center of the carbamate.

The racemic tetrahydrobenzazepine carbamates were not only potent inhibitors of AChE in vitro (e.g., compound 1d with IC_{50} of 72 nM, Table 2) but also showed reduced toxicity *in vivo*, with LD_{50}s as much as 20-fold greater than that of physostigmine. This reduced toxicity was also reflected in a reduction in potency at producing peripheral cholinergic effects (e.g., diarrhea and salivation) and central cholinergic effects (e.g., tremor) (Table 2). The structure–activity relationships in series 1 at N_2 and the carbamate side chain seem to be similar to those in series 2, as discussed previously.[3] Similarly, extending the chain length of the carbamate side chain from methyl to *n*-heptyl diminished acute toxicity while retaining potent AChE inhibitory activity. The presence of a methyl group, R_1, at C_5 in series 1 did not affect potency. In contrast, the presence of a methyl group at C_{3a} in series 2 increased potency by a factor of 6 to 12. Most of the compounds in series 1, presented in Table 2, showed similar *in vitro* AChE inhibitory activity with varying degrees of cholinergic side effects *in vivo* and acute lethality.

Table 3. Percent Elevation of ACh Level in Mouse Forebrain following Administration of an Acetylcholinesterase Inhibitor

compd	dose[a] (mg/kg)	ACh (% control[b]) mean ± SEM
vehicle		100
THA	6.6 (ip)	136 ± 4
heptylphysostigmine	4.8 (ip)	148 ± 8
2	0.8 (ip)	148 ± 9
1c	3.6 (ip)	159 ± 5
1d	1.2 (ip)	148 ± 15
1d	5.0 (po)	122 ± 6
1e	4.4 (ip)	153 ± 5
1f	1.8 (ip)	156 ± 11

[a] The dose used is equivalent to 20% of LD_{50} *via* intraperitoneal (ip) or oral (po) administration. [b] Refer to the Experimental Section for test procedures and methods of reporting results. Statistically different from vehicle with $p < 0.01$ (Student's *t*-test) for all compounds presented in the table. The percentage of ACh elevation varied somewhat from experimental to experiment.

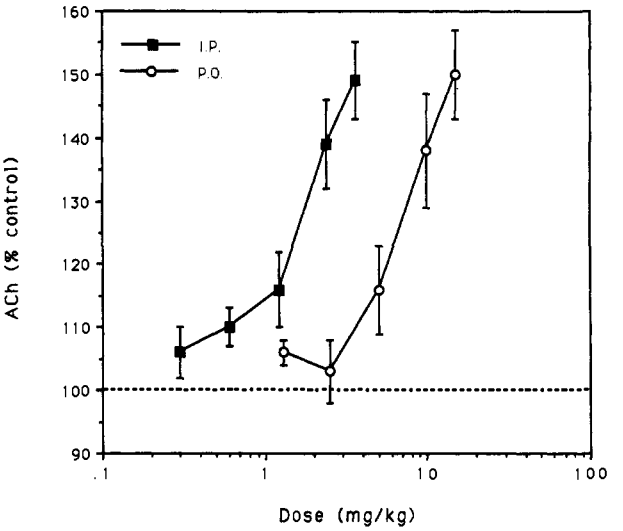


Figure 1. Elevation of mouse forebrain ACh levels by 1d. Refer to the Experimental Section for test procedures.

Compounds which produced central cholinergic mediated effects, such as tremor, also showed elevations in brain ACh levels (Table 3). Compound 1d was tested more extensively *in vivo*. In a separate experiment, a dose-dependent increase in mouse forebrain ACh levels following ip or po administration was found for compound 1d (Figure 1). The duration of this elevation in brain ACh levels was relatively prolonged, extending for at least 2 h following a moderate oral dose (Figure 2). Compound 1d was also found to cause hypothermia which is believed to be a central cholinergic effect in mice for at least 2 h (Figure 3).

In order to determine the behavioral relevance of the observed elevation in brain ACh levels, mice were tested in a cholinergic-deficit passive avoidance model which measures cognitive performance.[6] As can be seen in Figure 4, 1d was able to improve performance in mice at an oral

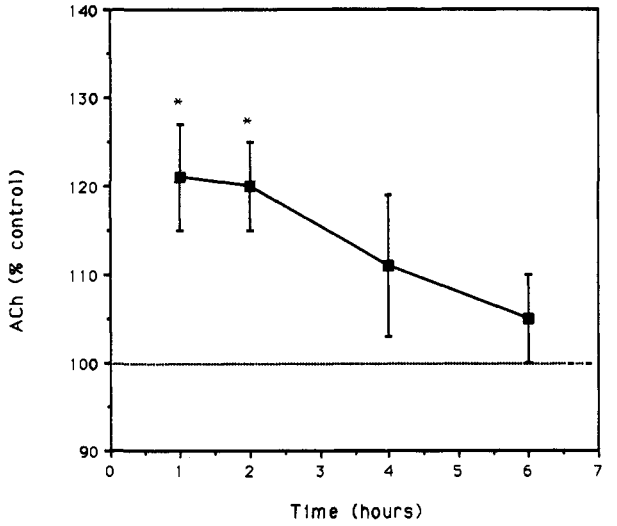


Figure 2. Duration of ACh elevation in mouse brain after oral administration of compound **1d** at 5 mg/kg. Refer to the Experimental Section for test procedures. Statistically different from vehicle with * = $p < 0.05$ (Student's t-test).

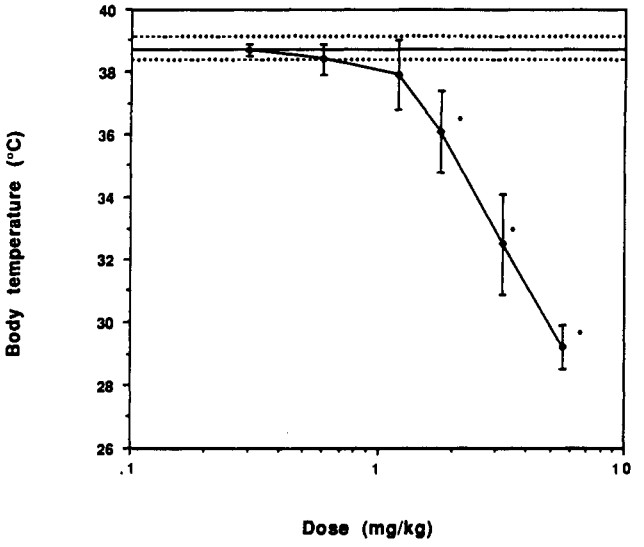


Figure 3. Dose response of hypothermia in mice after ip administration of compound **1d**. Refer to the Experimental Section for test procedures. Statistically different from vehicle with * = $p < 0.05$ (analysis of variance test).

dose of 5.6 mg/kg. An inverted-U dose–response curve was observed, as behavioral disruption due to cholinergic side effects occurred at higher doses. We have observed similar narrow therapeutic windows in this model with all other AChE inhibitors tested.

Conclusions

According to the current model for the active site of AChE, we hypothesized that the carbonyl center of the carbamate group in series 1 interacts with the enzyme *via* the Ser-His-Glu catalytic triad and the quaternary nitrogen interacts with the neighboring aromatic residues. Using this initial working hypothesis, we identified compounds in the benzazepine carbamate series as a novel class of potent AChE inhibitors. All compounds produced peripheral cholinergic effects (e.g., salvation and diarrhea) and central cholinergic effects (e.g., tremor). Several compounds of this series elevated mouse brain ACh levels. Although cholinergic side effects limited the therapeutic efficacy, hemicholinium-3-induced amnesia was reversed

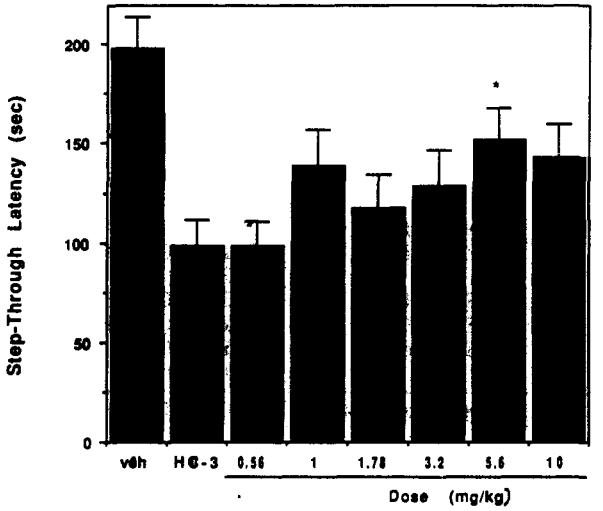


Figure 4. Reversal of hemicholinium-3-induced amnesia in the mouse passive avoidance assay by po administration of **1d**: veh = vehicle. Mann–Whitney U test: * = $p < 0.05$. Refer to the Experimental Section for test procedures.

after oral administration of **1d** in the passive avoidance assay. While a potent, selective AChE inhibitor presents a potential therapeutic treatment of Alzheimer's disease, a requirement of second-generation palliative drugs will be greater central selectivity for a specific cholinergic receptor subtype in order to reduce undesired cholinergic side effects and thereby improve the therapeutic window.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian XL-300 or Bruker 300 spectrometer, and chemical shifts are reported in δ with tetramethylsilane as internal reference. IR spectra were obtained on a Perkin-Elmer 1420 spectrophotometer. Elemental analyses were performed by the Analytical Laboratory of Pfizer Central Research, and the analytical results obtained for those elements were within ±0.4% of theoretical values.

Methyl 5-Methoxy-3-methyl-1-oximinoindan-3-acetate (5y). A mixture of 5-methoxy-3-methyl-1-indan-3-acetate (8.850 g, 35 mmol), NH_2OH·HCl (3.100 g, 44.6 mmol), and NaOAc·$3H_2O$ (6.070 g, 44.6 mmol) in 30 mL of methanol and 40 mL of water was heated to reflux for 3 h. The reaction was quenched with water and the mixture extracted with ethyl acetate. The organic layer was separated, dried, and concentrated to give 9.348 g (100%) of predominantly one isomer of oxime **5y** as a yellow oil. The crude material was used directly for the next hydrogenation: ^{1}H NMR ($CDCl_3$) δ 8.20 (br s, 1H), 7.60 (d, 1H), 6.82 (dd, 1H), 6.77 (d, 0.1H), 6.75 (d, 0.9H), 3.84 (s, 0.3H), 3.83 (s, 2.7H), 3.62 (s, 0.3H), 3.61 (s, 2.7H), 3.05 (ABq, 2H), 2.6 (q, 2H), 1.4 (s, 3H).

Methyl 1-Amino-3-methyl-5-methoxyindan-3-acetate Hydrochloride (6y). A solution of oxime **5y** (9.200 g, 34.98 mmol) in 100 mL of saturated HCl(g) in MeOH with 10% Pd/C (1.000 g) was hydrogenated at 50 psi overnight. TLC showed starting material left. More 10% Pd/C (1.000 g) was added, and the resulting mixture was hydrogenated for an additional 15 h. The mixture was filtered through Celite, and the filtrate was concentrated to dryness to give a pale yellow oil. After addition of 50 mL of acetone, solid formed and was filtered. The solid was recrystallized from acetone/methanol to give 4.000 g of **6y** as a white crystal: mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 8.7 (br s, 3H), 7.6 (d, 1H), 6.82 (m, 2H), 4.7 (t, 1H), 3.7 (s, 3H), 3.5 (s, 3H), 2.75 (dd, 1H), 2.5 (d, 2H), 1.8 (dd, 1H), 1.4 (s, 3H). Anal. ($C_{14}H_{19}NO_3$·HCl·$0.3H_2O$) C, H, N.

1-Amino-3-methyl-5-methoxyindan-3-acetic Acid Hydrochloride (7y). A solution of **6y** (3.950 g, 13.81 mmol) in 100 mL of 2 N HCl was heated at reflux for 3 h. Removal of solvent under reduced pressure and pumping *in vacuo* gave a solid. The

solid was triturated with isopropyl ether to give 3.700 g (98.6% yield) of **6y** as a white solid, mp 188–189 °C. Two sets of peaks with a ratio of 56:44 were observed on the ^1H and ^{13}C NMR spectra. We did not attempt to characterize the stereochemistry, and the material was used directly for the next cyclization step: ^1H NMR (DMSO-d_6) δ 7.53 (dd, 1H), 6.8–7.0 (m, 2H), 4.66 (t, 1H), 3.75 (s, 3H), 2.8–2.95 (m, 1H), 2.38–2.5 (m, 2H), 2.19 (dd, 0.44H), 1.80 (dd, 0.56H), 1.41 (s, 1.68H), 1.23 (s, 1.32H). Anal. ($C_{13}H_{17}NO_3$·HCl·0.3H_2O) C, H, N.

1,5-Methano-3-oxo-5-methyl-7-methoxy-2,3,4,5-tetrahydro-1*H*-2-benzazepine (8y). A mixture of **7y** (3.600 g, 13.25 mmol) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (5.610 g, 13.25 mmol) in 500 mL of pyridine was stirred at room temperature over 9 days. The mixture was concentrated to dryness, and the residue was diluted with water and extracted with chloroform. The organic layer was dried and concentrated to give an orange oil. The oil was purified by silica gel column chromatography using chloroform as eluent to give white solid which was recrystallized from acetone to give 1.100 g (38% yield) of **8y** as white crystals: mp 166–167 °C; ^1H NMR ($CDCl_3$) δ 8.0 (m, 1H), 7.02 (d, 1H), 6.67 (d, 1H), 6.58 (dd, 1H), 4.3 (t, 1H), 3.7 (s, 3H), 2.38 (ABq, 2H), 2.0 (m, 2H), 1.4 (s, 3H). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

1,5-Methano-5-methyl-7-methoxy-2,3,4,5-tetrahydro-1*H*-2-benzazepine (3y). To a solution of **8y** (1.030 g, 4.75 mmol) in 10 mL of dry THF was added dropwise 48 mL of 1 M BH_3·THF complex. The mixture was stirred at 0 °C for 1 h and then heated at reflux overnight; 6 N HCl (25 mL) was added dropwise to the cooled reaction mixture, and the resulting mixture was stirred at room temperature for 1 h and then heated at reflux for 1 h. The mixture was concentrated to dryness, and the residue was basified with 2 N NaOH and extracted with methylene chloride. The organic layer was dried and concentrated to give 0.962 g (100% yield) of **3y** as a colorless oil which was used directly for the next reaction: ^1H NMR ($CDCl_3$) δ 7.08 (d, 1H), 6.64 (dd, 1H), 6.6 (d, 1H), 4.12 (d, 1H), 3.78 (s, 3H), 2.66 (dd, 1H), 2.02 (m, 1H), 1.5–1.8 (m, 3H), 1.28 (s, 3H).

1,5-Methano-2-ethyl-5-methyl-7-methoxy-2,3,4,5-tetrahydro-1*H*-2-benzazepine (9r). A mixture of **3y** (0.900 g, 4.43 mmol), acetyl chloride (0.35 mL, 4.88 mmol), and pyridine (0.39 mL, 4.88 mmol) in 50 mL of methylene chloride was stirred at room temperature overnight. The reaction was quenched with water, and the organic layer was separated, dried, and concentrated to give 1.050 g (97% yield) of the corresponding *N*-acetyl derivative as a yellow oil.

A 0.900-g (3.67-mmol) sample of the oil was dissolved in 50 mL of dry THF and cooled to 0 °C; 2 M BH_3·DMS (4.6 mL, 9.20 mmol) was added dropwise, and the resulting reaction mixture was heated at reflux for 3 h and cooled and the reaction quenched with 10 mL of methanol. The resulting reaction was then quenched with 50 mL of 2 N HCl and the mixture heated at reflux for 1 h, cooled, basified with 2 N NaOH to pH 14, and, then, extracted with ether. The organic layer was dried and concentrated to give 0.830 g (99% yield) of **9r** as a colorless oil which was used directly for the next reaction: ^1H NMR ($CDCl_3$) δ 6.95 (m, 1H), 6.6 (m, 2H), 3.84 (d, 1H), 3.72 (s, 3H), 2.57 (dd, 1H), 2.2–2.4 (m, 1H), 2.0–2.2 (m, 1H), 1.9–2.0 (m, 1H), 1.63–1.84 (m, 3H), 1.2–1.4 (m, 5H).

1,5-Methano-2-ethyl-5-methyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-7-ol (10r). A mixture of **9r** (0.830 g, 3.62 mmol) and 15 mL of 48% HBr was heated at reflux for 3 h. The mixture was concentrated to dryness, triturated with 2 mL of isopropyl alcohol, and filtered to give 0.500 g (46% yield) of **10r** as a white solid: ^1H NMR (D_2O) δ 7.3–7.45 (m, 1H), 6.8–6.9 (m, 2H), 4.69 (d, 1H), 3.31 (dd, 1H), 3.1–3.2 (m, 1H), 2.8–2.95 (m, 1H), 2.2–2.3 (m, 1H), 1.9–2.2 (m, 3H), 1.6–1.7 (m, 1H), 1.42 (s, 3H), 1.32 (t, 3H); ^{13}C NMR (D_2O) δ 160.98, 154.86, 129.88, 127.58, 116.48, 111.91, 66.34, 52.20, 50.69, 49.70, 47.81, 44.92, 36.33, 28.71, 23.21, 11.64. An additional 0.314 g of impure product could be obtained from recrystallization of the filtrate.

1,5-Methano-2-ethyl-5-methyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-7-ol, Hexylcarbamate, Di-*p*-toluoyl-L-tartaric Acid (1b). A solution of **10r** (0.200 g, 0.67 mmol) in 15 mL of benzene was treated with NaH (60% in oil, 0.029 g, 0.75 mmol). After the mixture was stirred for 30 min, hexyl isocyanate (0.0953 g, 0.75 mmol) was added and the resulting mixture was stirred at room temperature overnight. The mixture was diluted with ether and washed with brine. The organic layer was dried and concentrated to give 0.305 g of oil which was purified through silica gel column chromatography to give 0.170 g of **11b** as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.0 (d, 1H), 6.8–6.9 (m, 2H), 5.24 (t, 1H, NH), 3.9 (d, 1H), 3.15 (q, 2H), 2.5–2.7 (m, 2H), 2.2–2.4 (m, 1H), 2.0–2.2 (m, 1H), 1.9–2.0 (m, 1H), 1.6–1.9 (m, 2H), 1.4–1.6 (m, 2H), 1.1–1.4 (m, 11H), 1.06 (t, 3H), 0.8 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 154.8, 151.06, 150.8, 135.5, 124.4, 118.9, 114.5, 61.8, 51.2, 49.3, 47.2, 43.7, 41.3, 37.1, 31.5, 29.8, 26.4, 22.53, 22.45, 14.0, 12.7.

The corresponding di-*p*-toluoyl-L-tartaric acid salt **1b** was prepared as a white solid after triturating with diethyl ether. This solid was used for biological studies. Anal. ($C_{21}H_{32}N_2O_2$·$C_{20}H_{18}O_8$·0.7H_2O) C, H, N.

Biological Methods. Acetylcholinesterase Inhibition. AChE activity was determined as described by Ellman.[8] The assay solution consisted of a 0.1 M sodium phosphate buffer, pH 8.0, with the addition of 100 mM tetraisopropylpyrophosphoramide, 100 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.02 units/mL AChE (Sigma Chemical Co.; derived from human erythrocytes), and 200 mM acetylthiocholine iodide. The final assay volume was 0.25 mL. Test compounds were added to the assay solution prior to enzyme addition, and a 20-min preincubation period with enzyme was followed by addition of substrate. Changes in absorbance at 412 nM were recorded for 5 min. The reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated.

***In Vivo* Physiological Measurements.** Male CD-1 mice (15–27 g; Charles River) were allowed tap water and Agway Prolab RMH 3000 chow (Agway, Syracuse, NY) *ad libitum* for at least 4 days before testing to allow for acclimation to the facility. The animal rooms were 21 ± 1 °C with lights on 0700 to 1900. For testing, animals were fasted overnight and injected intraperitoneally (ip) or orally (po) with drugs (10 mL/kg of body weight) and placed in novel cages individually. Salivation, tremor, and diarrhea were scored on a 5-point scale at 15, 30, and 60 min postdosing. Body temperature was measured with a rectal thermocouple. Lethality was scored over a 24-h time period. The LD_{50} was determined using a minimum of four dose levels with nine animals per dose and calculated using the Spearman–Karber statistics.

Mouse Brain Acetylcholine Measurement. AChE inhibitors were administered ip or po, and animals were sacrificed 1 h later. The forebrains were removed and homogenized in 20 mM sodium phosphate buffer, pH 5.3. Homogenates were centrifuged 20 min at 12000 *g*; supernatants (10–20 μL) were used for determination of ACh with the ACh analysis system from Bioanalytical Systems (West Lafayette, IN). Briefly, a polymeric anion-exchange column separated ACh from choline, and a postcolumn reactor column containing immobilized AChE and choline oxidase converted ACh and choline to betaine and hydrogen peroxide; the hydrogen peroxide was readily measured with an electrochemical detector at 500 mV vs the Ag/AgCl reference electrode and a platinum working electrode. Sensitivity was approximately 3–5 pmol of ACh. Typical ACh control values were 18–25 nmol/g in mouse forebrain. Data for drug treated animals are reported as percent control values. In general, the treatment consisted of eight animals per group. Statistical significance was determined by Student's one-tailed *t*-test.

Passive Avoidance Method. The one trial step-through passive avoidance procedure in mice was similar to that used by Bammer.[6] The training session involved placing the mouse in the lighted side of the shuttle cage (Coulbourn Instruments Inc., Lehigh Valley, PA) facing opposite to the dark side. The mouse was allowed to cross over to the other side where a constant current (0.7 mA, unscrambled) footshock was delivered until the mouse escaped to the original side. The mouse was then removed from the apparatus. Mice that did not cross over to the shock side within 90 s or that did not escape the footshock within 20 s were discarded from the experiment. Mice were tested for their retention of the avoidance task 24 h after the training procedure. Intracerebroventricular (icv) hemicholinium-3 was administered 2 h and test drug 1 h prior to the training session.

They were placed in the light side of the chamber, and the time to cross to the other side was measured. Animals not entering the shock side within 300 s were removed and assigned a score of 300. Between training and testing procedures, animals were housed 12 per cage and not handled.

Supplementary Material Available: Experimental data for compounds **9q**, **10q**, and **11a,c–h** (3 pages). Ordering information is given on any current masthead page.

References

(1) Rosenberg, D. R.; Wright, B. A.; Gershon, S. Cognitive Enhancing Agents for the Treatment of Senile Dementia of the Alzheimer's Type. *Drugs Today* **1990**, *26*, 449.
(2) Kumar, V.; Calache, M. Treatment of Alzheimer's Disease with Cholinergic drugs. *Int. J. Clin. Pharmacol., Ther. Toxicol.* **1991**, *29*, 23.
(3) Chen, Y. L.; Hedberg, K. Total Synthesis of an Optically Active 8-Carbaphysostigmine Analog: A Potent Acetylcholinesterase Inhibitor. *Bio. Med. Chem. Lett.* **1991**, *1*, 47.
(4) Chen, Y. L.; Nielsen, J.; Hedberg, K.; Dunaiskis, A.; Jones, S.; Russo, L.; Johnson, J.; Ives, J.; Liston, D. Syntheses, Resolution, and Structure-Activity Relationships of Potent Acetylcholinesterase Inhibitors: 8-Carbaphysostigmine Analogues. *J. Med. Chem.* **1992**, *35*, 1429.
(5) Jacobson, A.; Mokotoff, M. Azabicyclo Chemistry. I. Synthesis of 1,5-Methano-7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepines. B-Norbenzomorphans. *J. Med. Chem.* **1970**, *13*, 7.
(6) Bammer, G. Pharmacological Investigations of Neurotransmitter Involvement in Passive Avoidance Responding: A Review and Some New Results. *Neurosci. Biobehav. Rev.* **1982**, *6*, 247.
(7) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. Atomic Structure of Acetylcholinesterase from *Torpedo californica*: A Prototypic Acetylcholine-Binding Protein. *Science* **1991**, *253*, 872.
(8) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* **1961**, *7*, 88.