Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2683–2689

BIOORGANIC & MEDICINAL CHEMISTRY

Molecular Recognition by Acetylcholinesterase at the Peripheral Anionic Site: Structure–Activity Relationships for Inhibitions by Aryl Carbamates

Gialih Lin,* Cheng-Yue Lai and Wei-Cheng Liao

Department of Chemistry, National Chung-Hsing University, Taichung 402, Taiwan

Received 1 April 1999; accepted 14 June 1999

Abstract—Substituted phenyl-*N*-butyl carbamates (**1**–**9**) are potent irreversible inhibitors of *Electrophorus electricus* acetylcholinesterase. Carbamates **1**–**9** act as the peripheral anionic site-directed irreversible inhibitors of acetylcholinesterase by the stop-time assay in the presence of a competitive inhibitor, edrophonium. Linear relationships between the logarithms of the dissociation constant of the enzyme–inhibitor adduct (K_i), the inactivation constant of the enzyme–inhibitor adduct (k_2), and the bimolecular inhibition constant (k_i) for the inhibition of *Electrophorus electricus* acetylcholinesterase by carbamates **1**–**9** and the Hammett substituent constant (σ), are observed, and the reaction constants (ρs) are -1.36, 0.35 and -1.01, respectively. Therefore, the above reaction may form a positive charged enzyme–inhibitor intermediate at the peripheral anionic site of the enzyme and may follow the irreversible inactivation by a conformational change of the enzyme.

Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) plays a major role in the termination of impulse transmission at cholinergic synpases, where AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh).[1,2] The three-dimensional (3-D) X-ray structure of AChE from *Torpedo californica* electric organ has been reported.[3] The X-ray structure of a transition state analogue complex of AChE has also been reported recently.[4] The active site of AChE also consists of at least five major binding sites (Fig. 1): (a) an oxyanion hole (OH), Gly118, Gly119 and Ala201,[3] that stabilizes the tetrahedral intermediate; (b) an esteratic site (ES), that is comprised of the catalytic triad Ser200-His440-Glu327;[3] (c) an anionic substrate (AS) binding site, Trp84, Glu199 and Phe330,[3,4] that contains a small number of negative charges but many aromatic residues, where the quaternary ammonium part of ACh and of various active site ligands binds through a preferential interaction of quaternary nitrogens with the π electrons of aromatic groups; (d) an active site-selective aromatic binding site (AACS) that is contiguous with or near ES and AS and that is important in binding aryl substrates and active site ligands;[2] and (e) an acyl binding site (ABS), Phe288 and Phe299, that binds the acetyl group of ACh.[4] Besides five active sites, AChE also has a peripheral anionic site (PAS), Trp279, Tyr70, Tyr121, Asp72, Glu199 and Phe290,[5–7] that may bind to 9-aminoacridine, 9-amino-1,2,3,4-tetrahydroacridine (tacrine) and is >20 Å from the active site.[2]

In Alzheimer's disease, a neurological disorder, cholinergic deficiency in the brain has been reported.[8,9] Therefore, synthesis and study of inhibitors of AChE may help to develop useful drugs to treat such disease. AChE has long been an attractive target for the rational design of mechanism-based inhibitors such as organophosphus compounds,[10,11] fluoroketones,[12,13] carbamates,[14–17] quaternary ammonium salts, propidium, edrophonium, and decamethonium,[2,9,18] and amino compounds, 9-aminoacridine, tacrine and Huperzine-A.[2,6,7,18,19]

The active site binding inhibitors of AChE are well known.[2] 9-Aminoacridine,[5] tacrine and its derivatives,[6] and propidium[7] have been characterized as PAS-directed inhibitors. We have also reported that both

Key words: Acetylcholinesterase inhibition; peripheral site; carbamates.
Abbreviations: AACS, active site-selective aromatic cation binding site; ABS, acyl binding site; ACh, acetylcholine; AChE, acetylcholinesterase; AS, anionic substrate binding site; ATCh, acetylthiocholine; DTNB; 5,5′-dithio-bis-2-nitrobenzoate; ES, esteratic site; K_i, dissociation constant of the enzyme-inhibitor adduct; K_i, bimolecular inhibition constant; k_2, inactivation constant of the enzyme-inhibitor adduct; OH, oxyanion hole; PAS, peripheral anionic site; σ, Hammett substituent constant; QSAR, quantitive structure–activity relationship; ρ, reaction constants.
* Corresponding author.

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00213-8

enantiomers of naphthyl-*N*-*n*-butyl carbamates are PAS-directed inhibitors of AChE.[17] In this paper, we further use substituted phenyl-*N*-*n*-butyl carbamates (**1**–**9**, Fig. 2) to probe the structure–reactivity relationships at the PAS of AChE.

The effects of structure on reactivity can be divided into three major types:[20–23] inductive, resonance and steric effects. In most cases, two or all three of these are operating, and it is usually not easy to tell how much of the rate enhancement or decrease is caused by each of the three effects. Quantitative treatment of the effects of structure on reactivity was first reported by Hammett and known as the Hammett equation (eq (1)).

$$\log k = \log k_0 + \rho\sigma \quad (1)$$

In eq (1), the $\log k_0$ value and the parameters ρ and σ are the reaction constant (the intensity factor of the inductive effect) and the Hammett substituent constant, respectively. The σ values are the scales for the measurement of both inductive and resonance effects of substituents. The sign for an electron-donating group such as *p*-OCH_3 is negative due to the through-resonance effect of the methoxyl group at the *para*-position, but the sign for an electron withdrawing group such as *m*-OCH_3 is positive due to the pure inductive effect of the methoxyl group at the *meta*-position. QSARs in serine protease catalysis[24–29] and acetylcholinesterase[30] have been reported. For enzyme inhibition, less attention has been paid to the correlation with the structure of inhibitors.[25,31] Lin and Lai[32,33] and Feaster et al.[34] have reported the linear free energy relationships for the inhibition of CEase by aryl carbamates.[32–34]

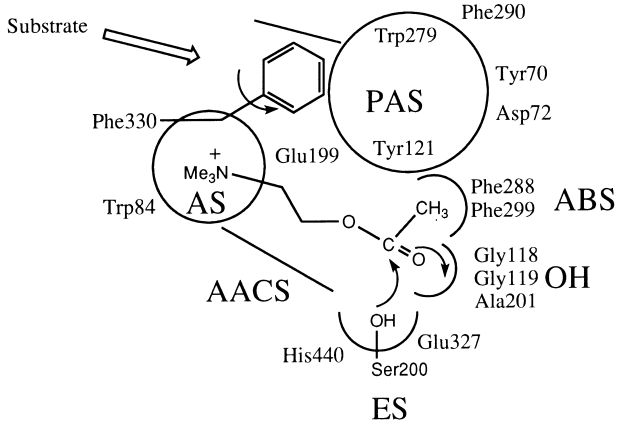


Figure 1. The active site and the PAS of AChE. Numbers refer to residue positions in *Torpedo californica* AChE.[3]

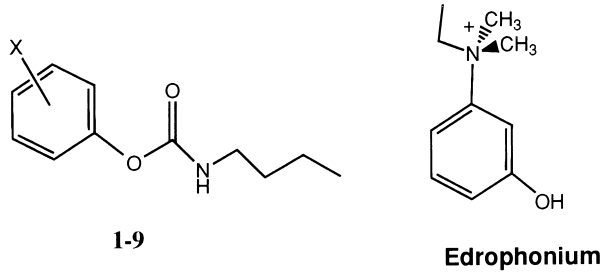


Figure 2. Chemical structures of carbamates **1**–**9** and edrophonium.

Results

Carbamates **1**–**9** (Fig. 2) are prepared from the condensation of the corresponding phenol with 1.2 mol equivalents of *n*-butyl isocyanate in the presence of a catalytic amount of pyridine in dichloromethane at 25°C for 48 h (76–85% yield).

In the presence of substrate, the mechanism of irreversible inhibition of AChE has been proposed in Scheme 1.[10,17]

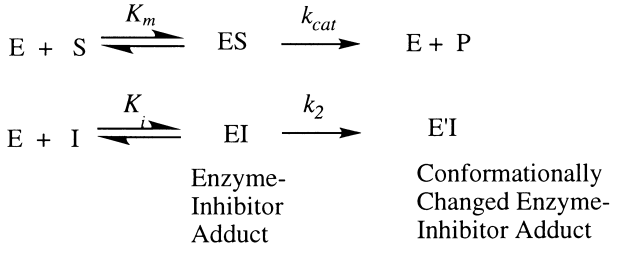
Because the inhibition of AChE follows first-order kinetics over the observed time period, the rate of hydrolysis of E′I must be significantly slower than the rate of formation of E′I. Therefore, values of K_i and k_c can be calculated from eq (2):[10,17,32–34,36–39]

$$k_{app} = \frac{k_2[I]}{K_I(1 + \frac{[S]}{K_m}) + [I]} \quad (2)$$

In eq (2), k_{app} values are the first-order rate constants which can be obtained according to Hosie's method. Bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency.

Carbamates **1**–**9** are characterized as irreversible inhibitors of AChE due to the facts that the inhibition is time-dependent and follows first-order kinetics and that the enzyme displays saturation kinetics with increasing concentration of inhibitor;[40] however, carbamates **1**–**9** do not meet the third criterion for active site-directed irreversible inhibitors as proposed by Abeles and Maycock.[40] In other words, the enzyme is not protected from inhibition by carbamates **1**–**9** in the presence of a reversible inhibitor, edrophonium. Therefore, carbamates **1**–**9** are characterized as PAS-directed inhibitors, like 9-aminoacridine,[6] tacrine,[7] and binaphthyl carbamates.[17]

Table 1 summarizes the inhibition data K_i, k_2, and k_i for the AChE-catalyzed hydrolysis of ATCh in the presence of DTNB and carbamates **1**–**9**. The k_i values for electron-withdrawing groups such as NO_2, CF_3, Cl, and *m*-OCH_3 are less than that for the electron-donating one (*p*-OCH_3). Therefore, an electron-donating substituent facilitates the inhibition, but an electron-withdrawing



Scheme 1. Kinetic scheme for irreversible inhibition of AChE in the presence of substrate.

Table 1. The Hammett substituent constants and kinetic data for the inhibition of AChE by carbamates **1–9**

Compound	$X=$	σ	K_i (μM)	k_2 (10^{-4} s^{-1})	k_i (M^{-1} s^{-1})
2	*p*-OMe	−0.27	1.0 ± 0.1	2.00 ± 0.04	200 ± 20
1	H	0	3.0 ± 0.2	2.90 ± 0.05	97 ± 7
3	*m*-OMe	0.12	3.7 ± 0.4	3.00 ± 0.06	81 ± 9
4	*p*-Cl	0.23	4.0 ± 0.4	3.30 ± 0.04	83 ± 8
5	*m*-Cl	0.37	8.1 ± 0.6	3.60 ± 0.08	44 ± 3
7	*m*-CF_3	0.43	8.6 ± 0.7	3.80 ± 0.07	44 ± 4
6	*p*-CF_3	0.54	14 ± 1	4.00 ± 0.09	29 ± 2
8	*m*-NO_2	0.71	23 ± 2	4.70 ± 0.08	20 ± 2
9	*p*-NO_2	0.78	29 ± 2	5.0 ± 0.1	17 ± 1

substituent detracts the inhibition. From the K_i values, the binding between the electron-donating substituted carbamate **1** is bound more tightly to AChE than the electron-withdrawing substituted carbamates **3–9**. The k_2 values are insensitive to the substituents.

After quantitative structure–activity correlations of the logarithms of the inhibition data (Table 1) by Hammett equation (eq (1)), the results are shown in Figure 3 and summarized in Table 2. The logarithms of the K_i, k_2, and k_i values are correlated with the σ values alone, and the ρ values for these inhibition reactions are −1.36, 0.35 and −1.01, respectively. In other words, the inhibitory potency depends upon the σ values alone.

Discussion

Carbamates **1–9** are characterized as irreversible inhibitors of AChE as proposed by Abeles and Maycock.[40] AChE is not protected from inhibition by carbamates **1–9** in the presence of a reversible inhibitor, edrophonium. It is conceivable that carbamates **1–9** react irreversibly at PAS, not at the active site.

Tables 1 and 2 summarize both inhibition and correlation data for the inhibition of AChE by carbamates **1–9**. All inhibitory potencies depend upon the σ values of substituents alone; therefore, the inhibitory potency is predominated by both inductive and resonance effects of substituents. The inhibitory potency for carbamate **1** toward AChE is the greatest due to the fact that the inhibition is facilitated by the only electron-donating substituent, *p*-OCH_3. By switching the OCH_3 group from *para* to *meta* position, there is a significant difference in inhibition due to the fact that the OCH_3 group donates electrons via the through-resonance[20–23] at *para* position but withdraws electrons via the inductive effect at *meta* position. By switching the NO_2 group from *para* to *meta* position, there is not much difference in inhibition due to the fact that the NO_2 group withdraws electrons at both *para* and *meta* positions. Since the correlations (Table 2) applies for the σ values for both *meta* and *para* positions, the binding at the PAS of AChE by carbamates **1–9** is nonspecific for the position of the substituents. Therefore, the direction for the binding between carbamates **1–9** and the PAS of AChE may be perpendicular to the benzene ring of the inhibitor molecules. Thus, substituents at either *meta* or *para* position do not have much steric difference for this binding.

The mechanism for the inhibition of AChE by carbamates **1–8** is proposed in Figure 4 according to the negative ρ

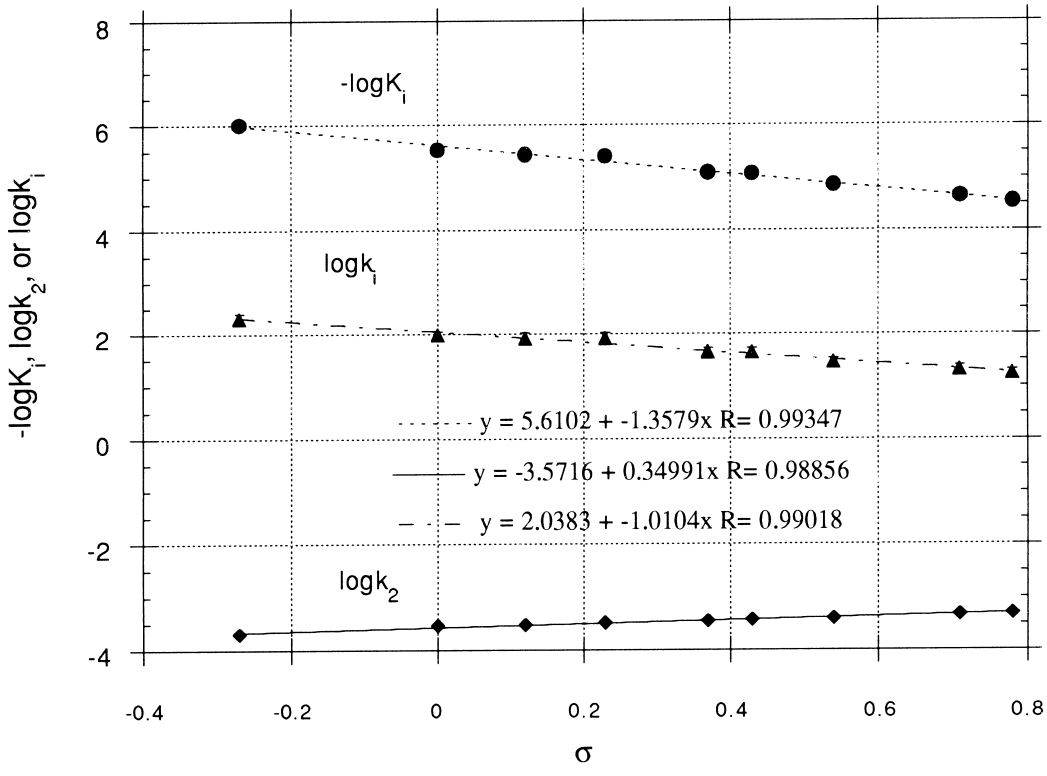


Figure 3. Plot of $-\log K_i$, $\log k_2$, and $\log k_i$ for the inhibition of AChE by carbamates **1–9** against σ.

value for $-\log K_i$-σ-correlation, the positive ρ values for $-\log k_2$-σ-correlation (Table 2), the PAS-directed inhibitors, and X-ray structural data for the PAS.[3,5,18,41] The first step of this mechanism (Fig. 4) is to form the non-covalent EI adducts through the donation of electrons from the inhibitor molecules to the aromatic rings of PAS (Trp279, Tyr70, Tyr121 and Phe290). Therefore, the PAS of AChE acts as the Lewis base to accept electrons from the inhibitor molecules. Moreover, the EI adduct is more positive than both enzyme and inhibitors due to a negative ρ value (−1.36). The second step of this mechanism is less sensitive to the σ values of substituents than the first step. Therefore, the second step of this mechanism is probably the conformational change of the enzyme, which results in a small ρ value (0.35). The second step of this mechanism (Fig. 4), therefore, is proposed to be the conformational change at Phe330 (the residue of AS near the entrance) of AChE, which results in the partial blockade for the entrance of substrate.[18,42] The rotation of Phe330 also results in the donation of electrons from Phe330 to PAS. Therefore, the character of the Lewis base, PAS, is weakened by this conformational change, and electrons partially feed back to the inhibitor molecules, which results in a slightly positive ρ value for this step. The overall inhibition results in the donation of electrons from the

Table 2. Structure–activity correlation results for the inhibition of AChE by carbamates **1**–**9**[a]

	$-\log K_i$	$\log k_2$	$\log k_i$
ρ	−1.36 ± 0.06	0.35 ± 0.02	−1.01 ± 0.05
Constant[b]	5.61 ± 0.03	−3.57 ± 0.01	2.04 ± 0.02
R[c]	0.99	0.99	0.99

[a] Hammett equation (eq (1)) is used in correlations.
[b] Calculated $-\log K_i$, $\log K_2$, and $\log K_i$ values for carbamate **2** (X = H) from eq (1).
[c] The correlation coefficient.

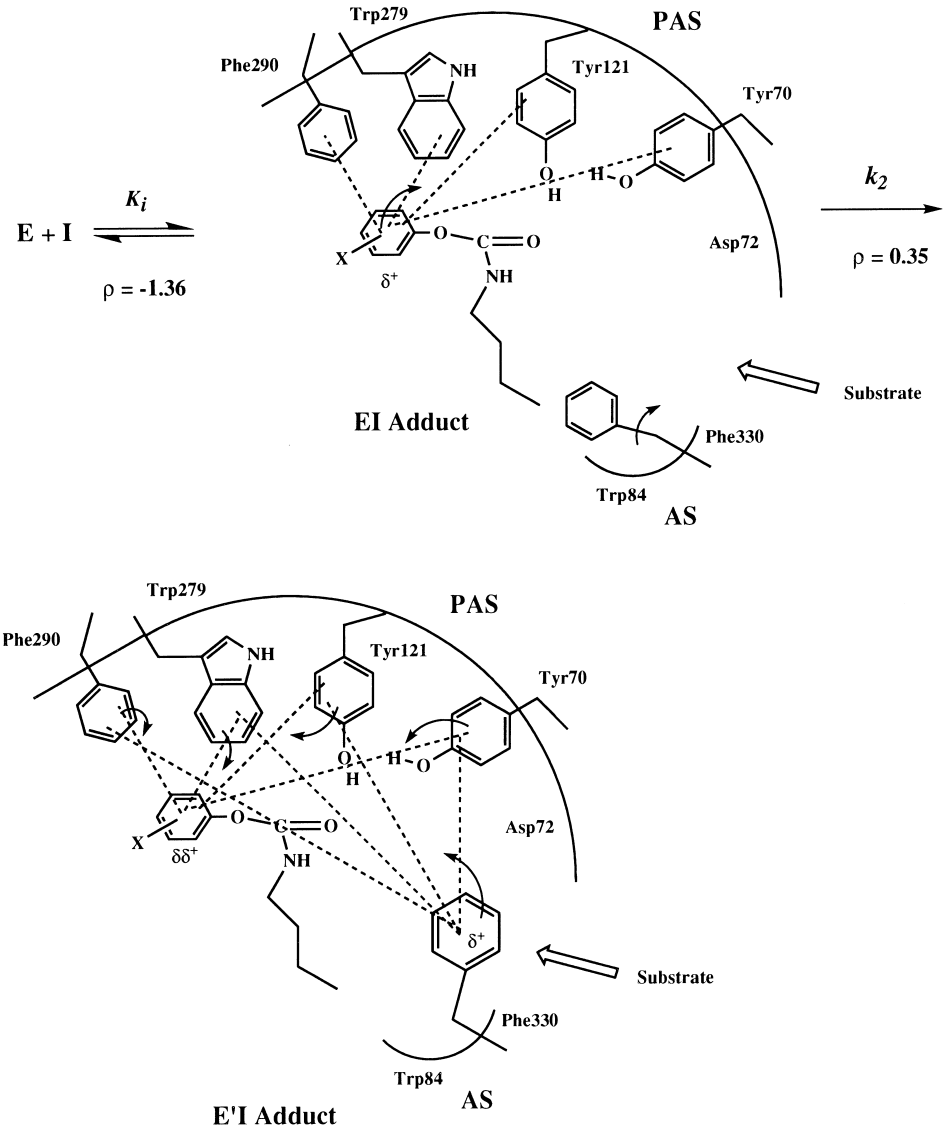


Figure 4. The proposed mechanism for the inhibition of AChE by carbamates **1**–**9** at the PAS of the enzyme. Numbers refer to residue positions in *Torpedo californica* ACh.[3]

inhibitor molecules to the PAS of the enzyme due to the negative ρ value (−1.01) for the logarithms of k_i.

Conclusion

Carbamates **1**–**9** are the PAS-directed irreversible inhibitors of *Electrophorus electricus* AChE. The structure–activity relationships for the inhibition of AChE by carbamates **1**–**9** allow us to solve, partially, the inhibition mechanism at the PAS of the enzyme. Linear correlations between the logarithms of K_i, k_2, and k_i values and the σ values of *m*- and *p*-substituents of carbamates **1**–**9** are observed, and the ρ values are −1.36, 0.35 and −1.01, respectively. The inhibition reaction may form a positive charged enzyme–inhibitor intermediate at the peripheral anionic site of the enzyme and may follow the irreversible inactivation by a conformational change of the enzyme. Overall, the structure–activity relationships for this study allow us to solve, partially, the inhibition mechanism at the PAS of the enzyme.

Experimental

Materials. All chemicals were of the highest grade available. 5,5′-Dithio-bis-2-nitrobenzoate (DTNB) and acetylthiocholine (ATCh) were obtained from Sigma; other chemicals were obtained from Aldrich; silica gel used in liquid chromatography (Licorpre Silica 60, 200–400 mesh) and thin layer chromatography plates (60 F254) were obtained from Merck; other chemicals and biochemicals were of the highest quality available commercially.

Instrumental methods. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz (Varian-XL 300 spectrometer), respectively. The ^{1}H chemical shift was referred to internal Me_4Si. All steady state kinetic data were obtained from an UV–visible spectrophotometer (HP 8452 or Beckman DU-650) with a cell holder circulated with a water bath.

Synthesis of carbamates 1–9. Carbamates **1**–**9** (Fig. 2) were prepared from the condensation of the corresponding phenol with 1.2 mol equivalents of *n*-butyl isocyanate in the presence of a catalytic amount of pyridine in dichloromethane at 25°C for 48 h (76–85% yield). All compounds were purified by liquid chromatography on silica gel (hexane–ethyl acetate) and characterized by ^{1}H (300 MHz) NMR, ^{13}C (75.4 MHz) NMR, mass spectrum, and elemental analysis.

Phenyl-*N*-*n*-butyl carbamate (1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, J=7 Hz, 3H, ω-CH_3), 1.42 (sextet, J=7 Hz, 2H, γ-CH_2), 1.56 (quintet, J=7 Hz, 2H, β-CH_2), 3.22 and 3.27 (ABq, J=7 Hz, 2H, α-CH_2), 5.02 (s, 1H, N*H*), 7.11–7.37 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.60 (ω-*C*), 19.76 (γ-*C*), 31.76 (β-*C*), 40.81 (α-*C*), 121.57, 125.13 and 129.20 (phenyl *C*-2,6), 151.01 (phenyl *C*-1), 154.66 (*C*=O). High resolution mass spectrum: exact mass: 193.1103. Found: 193.1107. Anal. calcd for $C_{11}H_{15}O_2N$: C, 68.35; H, 7.83; N, 7.25. Found: C, 68.27; H, 7.96, N, 7.15.

4-Methoxyphenyl-*N*-*n*-butyl carbamate (2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, J=7 Hz, 3H, ω-CH_3), 1.38 (sextet, J=7.2 Hz, 2H, γ-CH_2), 1.54 (quintet, J=7.2 Hz, 2H, β-CH_2), 3.22 and 3.28 (ABq, J=6.6 Hz, 2H, α-CH_2), 3.78 (s, 3H, OCH_3), 5.04 (s, 1H, N*H*), 6.84–7.06 (m, 4H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.51 (ω-*C*), 16.79 (γ-*C*), 31.67 (β-*C*), 40.75 (α-*C*), 55.37 (O*C*H$_3$), 114.14 (phenyl *C*-3,5), 122.37 (phenyl *C*-2,6), 144.59 (phenyl *C*-4), 155.06 (phenyl *C*-1), 156.74 (*C*=O). High resolution mass spectrum: exact mass: 223.1208. Found: 223.1201. Anal. calcd for $C_{12}H_{17}O_3N$: C, 64.54; H, 7.68; N, 6.28. Found: C, 64.47; H, 7.76; N, 6.19.

3-Methoxyphenyl-*N*-*n*-butyl carbamate (3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, J=7.5 Hz, 3H, ω-CH_3), 1.39 (sextet, J=7.5 Hz, 2H, γ-CH_2), 1.55 (quintet, J=7.5 Hz, 2H, β-CH_2), 3.24 and 3.30 (ABq, J=7.2 Hz, 2H, α-CH_2), 3.79 (s, 3H, OCH_3), 5.01 (s, 1H, N*H*), 6.69–7.27 (m, 4H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.66 (ω-*C*), 19.83 (γ-*C*), 31.82 (β-*C*), 40.91 (α-*C*), 55.53 (O*C*H$_3$), 17.55, 111.22, 113.85 and 129.65 (phenyl *C*-2,4,5,6), 152.09 (phenyl *C*-3), 154.52 (phenyl *C*-1), 160.40 (phenyl *C*-1), 156.74 (*C*=O); High resolution mass spectrum: exact mass: 223.1208. Found: 223.1196. Anal. calcd for $C_{12}H_{17}O_3N$: C, 64.54; H, 7.68; N, 6.28. Found: C, 64.44; H, 7.77; N, 6.18.

4-Chlorophenyl-*N*-*n*-butyl carbamate (4). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (t, J=7.5 Hz, 3H, ω-CH_3), 1.40 (sextet, J=6.9 Hz, 2H, γ-CH_2), 1.56 (quintet, J=7.2 Hz, 2H, β-CH_2), 3.24 and 3.30 (ABq, J=6.9 Hz, 2H, α-CH_2), 5.00 (s, 1H, N*H*), 7.05–7.32 (m, 4H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.62 (ω-*C*), 19.80 (γ-*C*), 31.77 (β-*C*), 40.93 (α-*C*), 122.95 (phenyl *C*-3,5), 129.27 (phenyl *C*-2,6), 130.48 (phenyl *C*-4), 149.63 (phenyl *C*-1), 154.25 (*C*=O). High resolution mass spectrum: exact mass: 227.7013. Found: 227.7026. Anal. calcd for $C_{11}H_{14}O_2NCl$: C, 58.13; H, 6.21; N, 6.17. Found: C, 58.03; H, 6.33; N, 6.07.

3-Chlorophenyl-*N*-*n*-butyl carbamate (5). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (t, J=7.5 Hz, 3H, ω-CH_3), 1.40 (sextet, J=6.9 Hz, 2H, γ-CH_2), 1.55 (quintet, J=7.2 Hz, 2H, β-CH_2), 3.24 and 3.30 (ABq, J=6.9 Hz, 2H, α-CH_2), 5.00 (s, 1H, N*H*), 7.02–7.30 (m, 4H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.64 (ω-*C*), 19.82 (γ-*C*), 31.78 (β-*C*), 40.97 (α-*C*), 119.97, 122.26, 125.48 and 129.98 (phenyl *C*-2,4,5,6), 134.49 (phenyl *C*-3), 151.64 (phenyl *C*-1), 154.05 (*C*=O). High resolution mass spectrum: exact mass: 227.7013. Found: 227.7026. Anal. calcd for $C_{11}H_{14}O_2NCl$: C, 58.13; H, 6.21; N, 6.17. Found: C, 58.05; H, 6.30; N, 6.08.

4-Trifluorophenyl-*N*-*n*-butyl carbamate (6). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (t, J=7.5 Hz, 3H, ω-CH_3), 1.40 (sextet, J=7.5 Hz, 2H, γ-CH_2), 1.55 (quintet, J=7.0 Hz, 2H, β-CH_2), 3.26 and 3.32 (ABq, J=6.6 Hz, 2H, α-CH_2), 5.04 (s, 1H, N*H*), 7.24–7.63 (m, 4H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.61 (ω-*C*), 19.80 (γ-*C*), 31.73 (β-*C*), 40.96 (α-*C*), 121.87 (phenyl *C*-3,5), 124.24 (q, $^1J_{CF}$=300 Hz, CF_3), 126.60 (phenyl *C*-2,6), 126.63 (q, $^2J_{CF}$=20 Hz, phenyl *C*-4), 153.67 (phenyl *C*-1), 153.82 (*C*=O). High resolution mass spectrum: exact mass:

261.0976. Found: 261.0988. Anal. calcd for $C_{12}H_{14}O_2NF_3$: C, 55.15; H, 5.40; N, 5.36. Found: C, 55.09; H, 5.48; N, 5.25.

3-Trifluorophenyl-*N*-*n*-butyl carbamate (7). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (t, J = 7.5 Hz, 3H, ω-CH_3), 1.41 (sextet, J = 6.9 Hz, 2H, γ-CH_2), 1.58 (quintet, J = 7.2 Hz, 2H, β-CH_2), 3.26 and 3.32 (ABq, J = 6.6 Hz, 2H, α-CH_2), 5.06 (s, 1H, NH), 7.31–7.50 (m, 4H, phenyl-H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.56 (ω-C), 19.77 (γ-C), 31.69 (β-C), 40.94 (α-C), 118.78, 121.89, 125.17 and 129.77 (phenyl C-2,4,5,6), 123.55 (q, $^1J_{CF}$ = 230 Hz, CF$_3$), 131.24 (q, $^2J_{CF}$ = 20 Hz, phenyl C-3), 151.21 (phenyl C-1), 154.02 (C=O). High resolution mass spectrum: exact mass: 261.0976. Found: 261.0985. Anal. calcd for $C_{12}H_{14}O_2NF_3$: C, 55.15; H, 5.40; N, 5.36. Found: C, 55.06; H, 5.49; N, 5.24.

3-Nitrophenyl-*N*-*n*-butyl carbamate (8). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, J = 7.2 Hz, 3H, ω-CH_3), 1.41 (sextet, J = 6.9 Hz, 2H, γ-CH_2), 1.59 (quintet, J = 7.2 Hz, 2H, β-CH_2), 3.26 and 3.32 (ABq, J = 6.6 Hz, 2H, α-CH_2), 5.13 (s, 1H, NH), 7.48–8.09 (m, 4H, phenyl-H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 13.62 (ω-C), 19.80 (γ-C), 31.70 (β-C), 41.07 (α-C), 117.24, 120.14, 128.08 and 129.81 (phenyl C-2,4,5,6), 148.72 (phenyl C-1), 151.46 (phenyl C-3), 153.61 (C=O). High resolution mass spectrum: exact mass: 238.0953. Found: 238.0942. Anal. calcd for $C_{11}H_{14}N_2O_4$: C, 55.44; H, 5.93; N, 11.76. Found: C, 55.33; H, 6.02; N, 11.65.

4-Nitrophenyl-*N*-*n*-butyl carbamate (9). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, J = 6.9 Hz, 3H, ω-CH_3), 1.41 (sextet, J = 6.9 Hz, 2H, γ-CH_2), 1.58 (m, 2H, β-CH_2), 3.26 and 3.32 (ABq, J = 6.9 Hz, 2H, α-CH_2), 5.15 (s, 1H, NH), 7.31 (dd, J = 4.5 and 1.5 Hz, 2H, 2,6-phenyl H), 8.24 (dd, J = 4.5 and 1.5 Hz, 2H, 3,5-phenyl H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) σ 13.59 (ω-C), 19.77 (γ-C), 31.64 (β-C), 41.02 (α-C), 121.94 (phenyl C-2,6), 125.10 (phenyl C-3,5), 144.66 (phenyl C-1), 153.16 (phenyl C-4), 156.04 (C=O). High resolution mass spectrum: exact mass: 238.0953. Found: 238.0957. Anal. calcd for $C_{10}H_{14}O_4N_2$: C, 55.44; H, 5.93; N, 11.76. Found: C, 55.36; H, 6.01; N, 11.68.

Data reduction. Kaleida Graph™ (version 2.0) and Origin (version 4.0) were used for all linear and non-linear least squares curve fittings.

Steady state enzyme kinetics. The AChE inhibition was assayed by the Ellman method.[35] The temperature was maintained at 25.0 ± 0.1°C by a refrigerated circulating water bath. All reactions were performed in sodium phosphate buffer (0.1 M, pH 7.0) containing NaCl (0.1 M), acetonitrile (2% by volume), triton X-100 (0.5% by weight), substrate ATCh (50 μM), a chromogenic reagent DTNB (50 μM), and varying concentration of inhibitors. Requisite volumes of stock solution of substrate and inhibitors in acetonitrile were injected into reaction buffers via a pipet. *Electrophorus electricus* AChE was dissolved in sodium phosphate buffer (0.1 M, pH 7.0). Reactions were initiated by injecting enzyme and monitored at 405 nm on the UV–visible spectrophotometer. First-order rate constants (k_{app} values) for inhibition of AChE were calculated as described by Hosie et al.[36] Values of K_i and k_2 can be obtained by fitting the data of k_{app} and [I] to eq (1) by nonlinear least squares regression analyses. Duplicate or triplicate sets of data were collected for each inhibitor concentration.

Return of activity and protection by edrophonium. For the return of activity study, AChE was incubated with each carbamate **1**–**9** (1 μM) in the absence and presence of edrophonium chloride (2 μM), a known competitive inhibitor of the enzyme,[2] before the inhibition reaction. All the other procedures followed those of Hosie et al.[17,36]

Acknowledgement

This work was supported by the National Science Council of Taiwan.

References

1. Rosenberry, T. L. *Adv. Enzymol. Real. Areas Mol. Biol* **1975**, *43*, 103.
2. Quinn, D. M. *Chem. Rev*. **1987**, *87*, 955.
3. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
4. Harel, M.; Quinn, D. M.; Nair, H. K.; Silman, I.; Sussman, J. L. *J. Am. Chem. Soc*. **1996**, *118*, 2340.
5. Radic, Z.; Pickering, N. A.; Vellom, D. C.; Camp, S.; Taylor, P. *Biochemistry* **1993**, *32*, 12074.
6. Taylor, P.; Mayer, R. T.; Himel, C. M. *Mol. Pharmacol*. **1994**, *45*, 74.
7. Pang, Y.-P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimjoin, S. *J. Biol. Chem*. **1996**, *271*, 23646.
8. Marchbanks, R. M. *J. Neurochem*. **1982**, *39*, 9.
9. Taylor, P. In *Pharmacological Basis of Therapeutics*; Gilman, A. G.; Goodman, L. S.; Murad, F., Eds.; MacMillan: New York, 1985.
10. Hart, G. J.; O'Brien, R. D. *Biochemistry* **1973**, *12*, 2940.
11. Debord, J.; Penicaut, B.; Labadie, M. *Phosphorus and Sulfur* **1986**, *29*, 57.
12. Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, *28*, 8466.
13. Nair, H. K.; Quinn, D. M. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2619.
14. Davis, K. L.; Mohs, R. C.; Trinklenberg, J. R.; Pfefferbaum, A.; Hollister, L. E.; Kopell, B. S. *Science* **1978**, *201*, 272.
15. Brufani, M.; Marta, M.; Pomponi, M. *Eur. J. Biochem*. **1986**, *157*, 115.
16. Yu, Q.-S.; Pei, X.-F.; Holloway, H. W.; Greig, N. H. *J. Med. Chem*. **1997**, *40*, 2895.
17. Lin, G.; Tsai, Y.-C.; Liu, H.-C.; Liao, W.-C.; Chang, C.-H. *Biochim. Biophys. Acta* **1998**, *1388*, 161.
18. Saxena, A.; Redman, A. M. G.; Jiang, X.; Lockridge, O.; Doctor, B. P. *Biochemistry* **1997**, *36*, 14642.
19. Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. *N. Engl. J. Med*. **1986**, *315*, 1241.
20. March, J. *Advanced Organic Chemistry*; 4th Ed.; John Wiley and Sons: New York, 1992.
21. Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; John Wiley and Sons: New York.
22. Isaaks, N. *Physical Organic Chemistry*; 2nd Ed.; Longman: England, 1995.

23. Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; 3rd Ed.; Harper and Row: New York, 1987.
24. Ikeda, K.; Kunugi, S.; Ise, N. *Arch. Biochem. Biophys.* **1982**, *217*, 37.
25. Nakatani, H.; Morita, T.; Hiromi, K. *Arch. Biochem. Biophys.* **1978**, *525*, 423.
26. Kanerva, L. T.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 6864.
27. Shimamoto, N.; Fukutome, H. *J. Biochem.* **1975**, *78*, 663.
28. Hubbard, C. D.; Shoupe, T. S. *J. Biol. Chem.* **1977**, *252*, 1633.
29. Bender, M. L.; Nakamura, K. *J. Am. Chem. Soc.* **1962**, *84*, 2577.
30. Järv, J.; Kesvatera, T.; Aaviksaar, A. *Eur. J. Biochem.* **1976**, *67*, 315.
31. Nakatani, H.; Fujiwake, H.; Hiromi, K. *J. Biochem.* **1977**, *81*, 1269.
32. Lin, G.; Lai, C.-Y. *Tetrahedron Lett.* **1995**, *36*, 6117.
33. Lin, G.; Lai, C.-Y. *Tetrahedron Lett.* **1996**, *37*, 193.
34. Feaster, S. R.; Lee, K.; Baker, N.; Hui, D. Y.; Quinn, D. M. *Biochemistry* **1996**, *35*, 16723.
35. Ellman, G. L.; Courtney, K. D.; Andres, V. Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
36. Hosie, L.; Sutton, L. D.; Quinn, D. M. *J. Biol. Chem.* **1987**, *262*, 260.
37. Lin, G.; Liu, H.-C.; Tsai, Y.-C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 43.
38. Lin, G.; Shieh, C.-T.; Tsai, Y.-C.; Hwang, C.-I.; Lu, C.-P.; Chen, G.-H. *Biochim. Biophys. Acta* **1999**, *1431*, 500.
39. Lin, G.; Shieh, C.-T.; Ho, H.-C.; Chouhwang, J.-Y.; Lin, W.-Y.; Lu, C.-P. *Biochemistry* **1999**, *38*, 9971.
40. Abeles, R. H.; Maycock, A. L. *Acc. Chem. Res.* **1976**, 313.
41. Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9031.
42. Shafferman, A.; Velan, B.; Ordentlich, A.; Kronman, C.; Grosfeld, H.; Leitner, M.; Flashner, Y.; Cohen, S.; Barak, D.; Ariel, N. *EMBO J.* **1992**, *11*, 3561.