Benzene-1,2-, 1,3-, and 1,4-di-*N*-Substituted Carbamates as Conformationally Constrained Inhibitors of Acetylcholinesterase

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ABSTRACT: Benzene-1,2-, 1,3-, and 1,4-di-*N*-substituted carbamates (1–15) are synthesized as the conformationally constrained inhibitors of acetylcholinesterase and mimic *gauche*, *eclipsed*, and *anti*-conformations of acetylcholine, respectively. All carbamates 1–15 are characterized as the pseudo substrate inhibitors of acetylcholinesterase. For a series of geometric isomers, the inhibitory potencies are as follows: benzene-1,4-di-*N*-substituted carbamate (*para* compound) > benzene-1,3-di-*N*-substituted carbamate (*meta* compound) > benzene-1,2-di-*N*-substituted carbamate (*ortho* compound). Therefore, benzene-1,4-di-*N*-substituted carbamates (*para* compounds), with the angle of 180° between two C(benzene)—O bonds, mimic the preferable *anti* C—O/C—N conformers of acetylcholine for the choline ethylene backbone in the acetylcholinesterase catalysis. © 2007 Wiley Periodicals, Inc. J Biochem Mol Toxicol 21:348–353, 2007; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10:1002/jbt.20202

KEYWORDS: **Acetylcholinesterase; Carbamate; Conformation; Inhibitor**

INTRODUCTION

Two forms of cholinesterase coexist ubiquitously throughout the body, acetylcholinesterase (AChE, EC 3.1.1.7) [1–3] and butyrylcholinesterase (BChE, EC 3.1.1.8) [4–7], and although highly homologous, >65%, they are products of different genes on chromosomes 7 and 3 in humans, respectively. Both subtypes unselective cholinesterase and AChE-selective inhibitors have been used in Alzheimer's disease to amplify the action of acetylcholine at remaining cholinergic synapses within the Alzheimer's disease brain. The X-ray crystal structures of AChE have revealed that AChE contains a catalytic triad similar to that present in other serine hydrolases. They have also revealed that this triad is located near the bottom of a deep and narrow gorge about 20 Å in depth [2]. The X-ray crystal structures of BChE have been recently reported [4,5,7]. AChE and BChE have a common catalytic triad, Ser–His–Glu. The active sites of both enzymes are located at the bottom of a cavity and act as nucleophiles while attacking the carbonyl groups of substrates or pseudo substrate inhibitors.

Carbamate inhibitors, such as Alzheimer's disease drug Rivastigmine (Exelon) and aryl carbamates, are characterized as the pseudo substrate inhibitors of AChE, BChE, cholesterol esterase, and lipase [3,8–15]. In the presence of substrate, the kinetic scheme for pseudo substrate inhibitions of serine hydrolases by carbamate inhibitors is proposed (Figure 1) [8]. Since this inhibition follows first-order kinetics over the observed time period for steady-state kinetics, the hydrolysis rate of carbamyl enzyme EI′ must be significantly slower than the EI′ formation rate ($k_2 \gg k_3$). Therefore, values of K_i and k_2 can be calculated from Eq. (1) [8]. In Eq. (1), the k_{app} values are first-order rate constants which are obtained by Hosie's method. The bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency:

$$k_{app} = k_2[I]/(K_i(1 + [S]/K_m) + [I]) \quad (1)$$

Benzene-1,2-, 1,3-, and 1,4-di-*N*-substituted carbamates (**1–15**) are synthesized as the conformationally constrained analogs [16,17] of acetylcholine since

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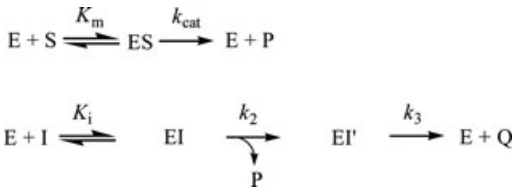

FIGURE 1. Kinetic scheme for the pseudo substrate inhibition of carbamates in the presence of substrate. E, enzyme; S, substrate; ES, acylenzyme intermediate; I, carbamate, EI, enzyme-inhibitor tetrahedral intermediate; EI′, carbamyl enzyme intermediate; P, the first product; Q, the second product.

these compounds mimic *gauche*, *eclipsed*, and *anti*-conformations of acetylcholine at the choline-ethylene backbone), respectively (Figure 2). In other words, with the angles of 60°, 120°, and 180° between two C(benzene)—O bonds, benzene-1,2-di-*N*-substituted carbamates (**1–5**), benzene-1,3-di-*N*-substituted carbamates (**6–10**), and benzene-1,4-di-*N*-substituted carbamates (**11–15**) mimic *gauche*, *eclipsed*, and *anti*-C—O/C—N conformers of acetylcholine in the acetylcholinesterase catalysis, respectively.

MATERIALS AND METHODS

Materials

All chemicals were of the highest grade available. Silica gel used in liquid chromatography and thin-layer chromatography plates were obtained from Merk. *Electrophorus electricus* AChE, acetylthiocholine, and 5,5′-dithio-bis-2- nitrobenzoate were obtained from Sigma.

Chemistry

Benzene-1,2-di-*N*-substituted carbamates (**1–5**), benzene-1,3-di-*N*-substituted carbamates (**6–10**), and benzene-1,4-di-*N*-substituted carbamates (**11–15**) (Figure 2) were synthesized from the condensation of catechol, resorcinol, and hydroquinone, respectively, with three equivalents of the corresponding isocyanate in triethylamine at 25°C for 24 h (65–90% yield). The products were purified by liquid chromatography (silica gel, hexane-ethyl acetate) and characterized by ^{1}H and ^{13}C NMR spectra.

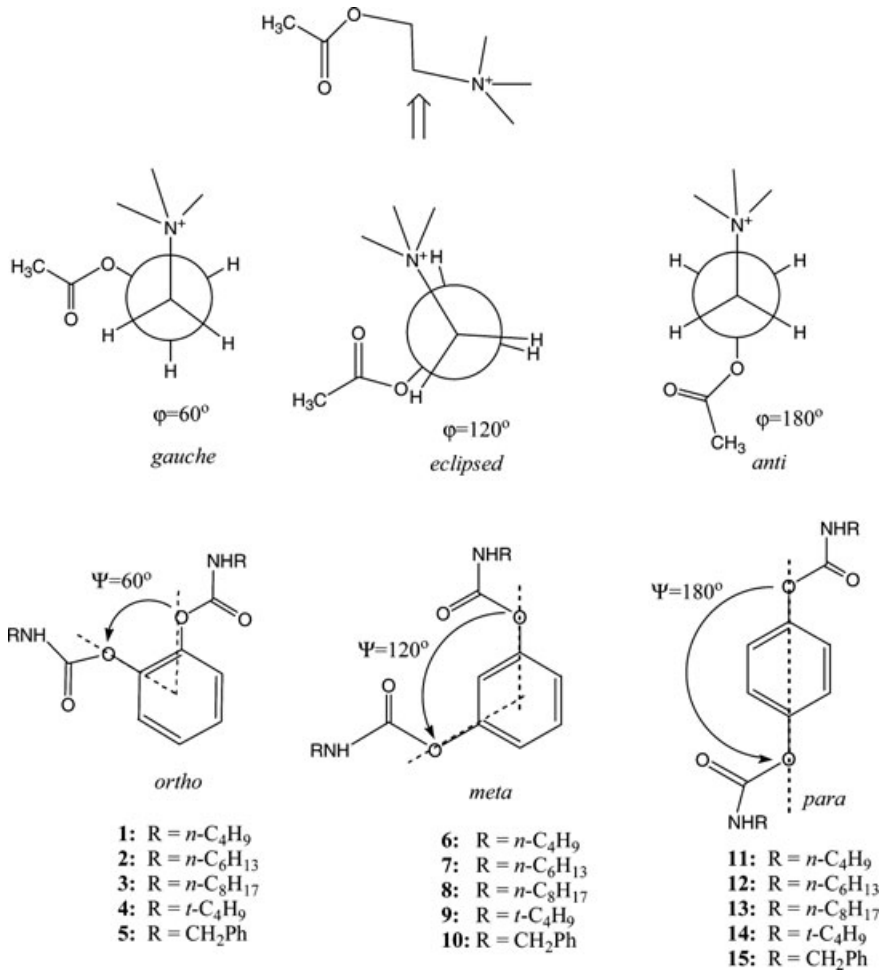

FIGURE 2. Possible conformations of acetylcholine and chemical structures of inhibitors **1–15**.

Benzene-1,2-di-*N*-*n*-butylcarbamate (**1**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.93 (t, J = 7 Hz, 6H, ω-CH_3), 1.35 (sextet, J = 7 Hz, 4H, γ-CH_2), 1.52 (quintet, J = 7 Hz, 4H, β-CH_2), 3.24 (q, J = 7 Hz, 4H, α-CH_2), 5.09 (s, 2H, NH), and 7.15–7.24 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 13.5 (ω-CH$_3$), 19.6 (γ-CH$_2$), 31.6 (β-CH$_2$), 40.8 (α-CH$_2$), 123.4 (C-4 and C-5 of benzene ring), 125.8 (C-3 and C-6 of benzene ring), 143.0 (C-1 and C-2 of benzene ring), and 153.9 (carbamate C=O).

Benzene-1,2-di-*N*-*n*-hexylcarbamate (**2**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, J = 7 Hz, 6H, ω-CH_3), 1.26–1.35 (m, 12H, γ- to ω-1-CH_2), 1.51–1.55 (m, 4H, β-CH_2), 3.23 (q, J = 7 Hz, 4H, α-CH_2), 5.08 (s, 2H, NH), and 7.15–7.24 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 13.9 (ω-CH$_3$), 22.4, 26.3, 29.6 (γ-, δ-, and ε-CH$_2$), 31.3 (β-CH$_2$), 41.2 (α-CH$_2$), 123.4 (C-4 and C-5 of benzene ring), 125.9 (C-3 and C-6 of benzene ring), 143.0 (C-1 and C-2 of benzene ring), and 153.9 (carbamate C=O).

Benzene-1,2-di-*N*-*n*-octylcarbamate (**3**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.89 (t, J = 7 Hz, 6H, ω-CH_3), 1.26–1.30 (m, 20H, γ- to ω-1-CH_2), 1.51–1.57 (m, 4H, β-CH_2), 3.23 (q, J = 7 Hz, 4H, α-CH_2), 5.06 (s, 2H, NH), and 7.15–7.24 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 14.0 (ω-CH$_3$), 22.5, 26.2, 26.3, 29.1, 29.7 (γ- to ω-1-CH$_2$), 31.7 (β-CH$_2$), 41.2 (α-CH$_2$), 123.4 (C-4 and C-5 of benzene ring), 126.0 (C-3 and C-6 of benzene ring), 143.0 (C-1 and C-2 of benzene ring), and 153.9 (carbamate C=O).

Benzene-1,2-di-*N*-*t*-butylcarbamate (**4**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.36 (s, 18H, CH_3), 5.09 (s, 2H, NH), and 7.15–7.24 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 28.7 (CH$_3$), 50.8 (C(CH$_3$)$_3$), 123.5 (C-4 and C-5 of benzene ring), 125.9 (C-3 and C-6 of benzene ring), 142.9 (C-1 and C-2 of benzene ring), and 151.8 (carbamate C=O). Benzene-1,2-di-*N*-benzylcarbamate (**5**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 4.33 (d, J = 6 Hz, 4H, CH$_2$Ph), 5.09 (s, 2H, NH), and 7.15–7.24 (m, 4H, 1,2-benzene-H), 7.27–7.30 (m, 10H, phenyl-H of benzyl group). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 45.3 (benzyl CH$_2$), 123.5, 126.2, 127.4, 127.7, 128.6, 138.1, 142.9 (aromatic C's), and 153.9 (carbamate C=O).

Benzene-1,3-di-*N*-*n*-butylcarbamate (**6**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.93 (t, J = 7 Hz, 6H, ω-CH_3), 1.37 (sextet, J = 7 Hz, 4H, γ-CH_2), 1.52 (quintet, J = 7 Hz, 4H, β-CH_2), 3.24 (q, J = 7 Hz, 4H, α-CH_2), 4.99 (s, 2H, NH), and 6.90–7.40 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 13.5 (ω-CH$_3$), 19.7 (γ-CH$_2$), 31.6 (β-CH$_2$), 40.7 (α-CH$_2$), 115.3 (C-5 of benzene ring), 118.0 (C-4 and C-6 of benzene ring), 129.0 (C-2 of benzene ring), 151.4 (C-1 and C-3 of benzene ring), and 154.2 (carbamate C=O).

Benzene-1,3-di-*N*-*n*-hexylcarbamate (**7**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, J = 7 Hz, 6H, ω-CH_3), 1.30–1.35 (m, 12H, γ- to ω-1-CH_2), 1.52–1.57 (m, 4H, β-CH_2), 3.23 (q, J = 7 Hz, 4H, α-CH_2), 4.98 (s, 2H, NH), and 6.90–7.35 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 13.9 (ω-CH$_3$), 22.4, 26.3, 29.6 (γ-, δ-, and ε-CH$_2$), 31.3 (β-CH$_2$), 41.2 (α-CH$_2$), 115.3 (C-5 of benzene ring), 118.1 (C-4 and C-6 of benzene ring), 129.1 (C-2 of benzene ring), 151.4 (C-1 and C-3 of benzene ring), and 154.2 (carbamate C=O).

Benzene-1,3-di-*N*-*n*-octylcarbamate (**8**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, J = 7 Hz, 6H, ω-CH_3), 1.26–1.33 (m, 20H, γ- to ω-1-CH_2), 1.52–1.57 (m, 4H, β-CH_2), 3.23 (q, J = 7 Hz, 4H, α-CH_2), 4.97 (s, 2H, NH), and 6.90–7.35 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 14.0 (ω-CH$_3$), 22.6, 26.7, 29.1, 29.2, 29.7 (γ- to ω-1-CH$_2$), 31.7 (β-CH$_2$), 41.2 (α-CH$_2$), 115.3 (C-5 of benzene ring), 118.2 (C-4 and C-6 of benzene ring), 129.2 (C-2 of benzene ring), 151.5 (C-1 and C-3 of benzene ring), and 154.2 (carbamate C=O).

Benzene-1,3-di-*N*-*t*-butylcarbamate (**9**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.36 (s, 18H, CH_3), 5.09 (s, 2H, NH), and 6.90–7.35 (m, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 28.7 (CH$_3$), 50.8 (C(CH$_3$)$_3$), 115.3 (C-5 of benzene ring), 118.3 (C-4 and C-6 of benzene ring), 129.2 (C-2 of benzene ring), 151.3 (C-1 and C-3 of benzene ring), and 152.3 (carbamate C=O).

Benzene-1,3-di-*N*-benzylcarbamate (**10**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 4.43 (d, J = 2 Hz, 4H, CH$_2$Ph), 5.09 (s, 2H, NH), 6.90–7.35 (m, 4H, benzene-H), and 7.27–7.37 (m, 10H, phenyl-H of benzyl group). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 45.3 (benzyl CH$_2$), 115.3, 118.4, 127.7, 127.8, 128.8, 129.4, 137.9, 151.4 (aromatic C's), and 154.2 (carbamate C=O).

Benzene-1,4-di-*N*-*n*-butylcarbamate (**11**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.93 (t, J = 7 Hz, 6H, ω-CH_3), 1.37 (sextet, J = 7 Hz, 4H, γ-CH_2), 1.53 (quintet, J = 7 Hz, 4H, β-CH_2), 3.24 (q, J = 7 Hz, 4H, α-CH_2), 4.98 (s, 2H, NH), and 7.07 (s, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 13.7 (ω-CH$_3$), 19.9 (γ-CH$_2$), 31.8 (β-CH$_2$), 40.9 (α-CH$_2$), 122.2 (C-2, C-3, C-5, and C-6 of benzene ring), 148.0 (C-1 and C-4 of benzene ring), and 154.5 (carbamate C=O).

Benzene-1,4-di-*N*-*n*-hexylcarbamate (**12**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, J = 7 Hz, 6H, ω-CH_3), 1.30–1.35 (m, 12H, γ- to ω-1-CH_2), 1.52–1.55 (m,

4H, β-CH_2), 3.24 (q, J = 7 Hz, 4H, α-CH_2), 4.97 (s, 2H, NH), and 7.07 (s, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 14.0 (ω-CH_3), 22.5, 26.4, 29.7 (γ-, δ-, and ε-CH_2), 31.4 (β-CH_2), 41.3 (α-CH_2), 122.2 (C-2, C-3, C-5 and C-6 of benzene ring), 148.0 (C-1 and C-4 of benzene ring), and 154.5 (carbamate C=O).

Benzene-1,4-di-*N*-*n*-octylcarbamate (**13**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.87 (t, J = 7 Hz, 6H, ω-CH_3), 1.26–1.30 (m, 20H, γ- to ω-1-CH_2), 1.52–1.55 (m, 4H, β-CH_2), 3.24 (q, J = 7 Hz, 4H, α-CH_2), 4.97 (s, 2H, NH), and 7.07 (s, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 14.0 (ω-CH_3), 22.6, 26.7, 29.1, 29.2, 29.8 (γ- to ω-1-CH_2), 31.7 (β-CH_2), 41.3 (α-CH_2), 122.2 (C-2, C-3, C-5 and C-6 of benzene ring), 148.0 (C-1 and C-4 of benzene ring), and 154.5 (carbamate C=O).

Benzene-1,4-di-*N*-*t*-butylcarbamate (**14**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.36 (s, 18H, CH_3), 5.09 (s, 2H, NH), and 7.06 (s, 4H, benzene-H). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 29.0 (CH_3), 51.1 (C(CH_3)$_3$), 122.6 (C-2, C-3, C-5 and C-6 of benzene ring), 148.1 (C-1 and C-4 of benzene ring), and 152.9 (carbamate C=O).

Benzene-1,4-di-*N*-benzylcarbamate (**15**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 4.34 (d, J = 6 Hz, 4H, CH_2Ph), 5.09 (s, 2H, NH), and 7.09 (s, 4H, 1,2-benzene-H), 7.28–7.35 (m, 10H, phenyl-H of benzyl group). ^{13}C NMR ($CDCl_3$, 100 MHz, assignment from DEPT experiments) δ/ppm 45.3 (benzyl CH_2), 122.1 (C-2, C-3, C-5 and C-6 of benzene ring), 127.4, 127.9, 128.7, 140.0 (phenyl C's of benzyl group), 149.0 (C-1 and C-4 of benzene ring), and 154.9 (carbamate C=O).

Instrumental Methods

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian-Gemini 400 spectrometer. All steady state kinetic data were obtained from a UV–visible spectrometer (Agilent 8453) with a cell holder circulated with a water bath.

Data Reduction

Origin (version 6.0) was used for the linear and nonlinear least-squares curve fittings.

AChE Inhibition

The inhibition reactions of AChE were determined by the Ellman assay [18] as described before [12]. The AChE-catalyzed hydrolysis of acetylthiocholine (0.1 mM) in the presence of 5,5′-dithio-bis-2-nitrobenzoate (0.1 mM) and inhibitors **1–15** were followed continuously at 410 nm on a UV–visible spectrometer at 25°C, pH 7.1. The K_i and k_2 values were obtained from the nonlinear least-squares of curve fittings of the k_{app} values vs inhibition concentration ([I]) plot against Eq. (1) (Figure 3 and Table 1).

TABLE 1. The Inhibition Constants of AChE by Inhibitors **1–15**

Inhibitors	K_i (nM)[a]	k_2 (10^{-3} s^{-1})[a]	k_i (10^5 M^{-1} s^{-1})[a]
1	23 ± 6	9.6 ± 0.4	4 ± 1
2	13 ± 5	6.1 ± 0.1	5 ± 2
3	44 ± 8	5.3 ± 0.1	1.2 ± 0.2
4	29 ± 6	7.8 ± 0.2	2.7 ± 0.6
5	70 ± 20	6.1 ± 0.1	0.8 ± 0.3
6	2 ± 1	6.9 ± 0.3	30 ± 10
7	7 ± 2	7.5 ± 0.3	11 ± 3
8	39 ± 5	6.5 ± 0.1	1.7 ± 0.2
9	9 ± 1	5.47 ± 0.03	6.0 ± 0.7
10	930 ± 80	5.91 ± 0.08	0.06 ± 0.01
11	1.7 ± 0.3	8.2 ± 0.2	48 ± 7
12	5 ± 1	7.2 ± 0.2	15 ± 4
13	16 ± 4	6.8 ± 0.1	4 ± 1
14	3.6 ± 0.6	5.90 ± 0.08	16 ± 3
15	7 ± 3	2.06 ± 0.01	3 ± 1

[a] Obtained from the nonlinear least-squares curve fittings of k_p vs [I] plot against Eq. (1) [8].

RESULTS

In order to mimic different conformations of acetylcholine at the choline ethylene backbone (Figure 2), benzene-1,2-di-*N*-substituted carbamates (**1–5**), benzene-1,3-di-*N*-substituted carbamates (**6–10**), and benzene-1,4-di-*N*-substituted carbamates (**11–15**) (Figure 2) were synthesized from condensation of catechol, resorcinol, hydroquinone, respectively, with the corresponding isocyanate in the presence of excess triethylamine.

Inhibitors **1–15** were all characterized as the pseudo substrate inhibitors (Figure 1) of AChE (Figure 3 and Table 1). For a series of geometric isomers, the inhibitory potencies are as follows: benzene-1,4-di-*N*-substituted carbamate (*para* compound) > benzene-1,3-di-*N*-substituted carbamate (*meta* compound) > benzene-1,2-di-*N*-substituted carbamate (*ortho* compound).

DISCUSSION

Inhibitory Potencies for Varied Di-Substituted Geometries of Carbamates 1–15

Benzene-1,2-di-*N*-substituted carbamates (**1–5**), benzene-1,3-di-*N*-substituted carbamates (**6–10**), and

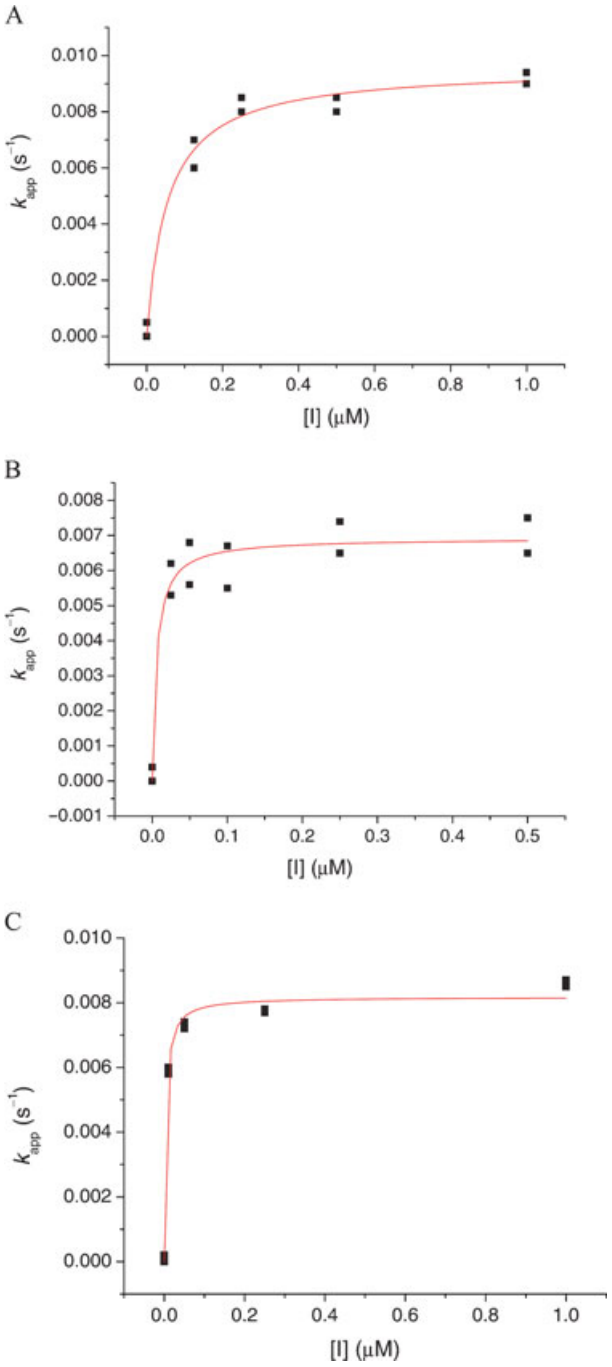

FIGURE 3. Nonlinear least-squares curve fittings of k_{app} vs inhibitor concentration ([I]) plots against Eq. (1) for the pseudo substrate inhibitions [8] of AChE by benzene-1,2-di-*N*-*n*-butylcarbamate (**1**) (A), benzene-1,3-di-*N*-*n*-butylcarbamate (**6**) (B), and benzene-1,4-di-*N*-*n*-butylcarbamate (**11**) (C). For A, the parameters of the fit were $k_2 = 0.0096 \pm 0.00004\ s^{-1}$ and $K_i = 23 \pm 6$ nM ($R^2 = 0.98337$). For B, the parameters of the fit were $k_2 = 0.0069 \pm 0.00003\ s^{-1}$ and $K_i = 2.4 \pm 1.1$ nM ($R^2 = 0.95018$). For C, the parameters of the fit were $k_2 = 0.0082 \pm 0.00002\ s^{-1}$ and $K_i = 1.7 \pm 0.3$ nM ($R^2 = 0.99085$).

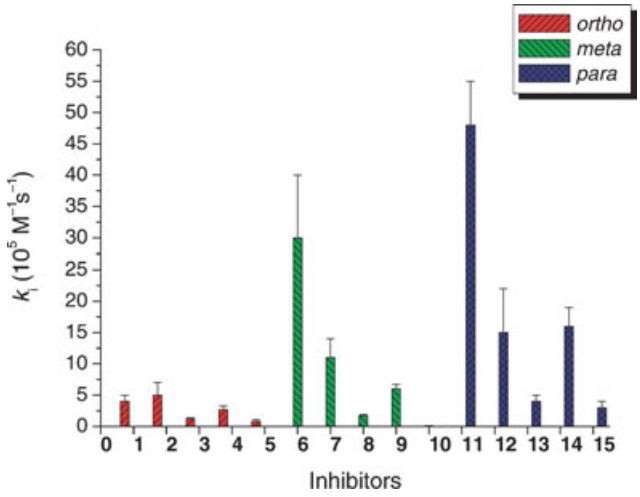

FIGURE 4. Comparisons of the k_i values of the pseudo substrate inhibitions of AChE by inhibitors **1–15**.

benzene-1,4-di-*N*-substituted carbamates (**11–15**) (Figure 2) are characterized as the pseudo substrate inhibitors (Figure 1) of AChE (Figure 3 and Table 1). For a series of geometric isomers, for example, benzene-1,2-di-*N*-*n*-butylcarbamate (**1**), benzene-1,3-di-*N*-*n*-butylcarbamate (**6**), and benzene-1,4-di-*N*-*n*-butylcarbamate (**11**), *para* compound **11** is a more potent AChE inhibitor than *meta* compound **6** and *meta* compound **6** is more potent than *ortho* compound **1** (Figure 4 and Table 1). Therefore, *para* carbamates **11–15**, with the angle of 180° between two C(benzene)—O bonds, mimic the preferable *anti* C—O/C—N conformers of acetylcholine in the acetylcholinesterase catalysis (Figure 2). In other words, the ethylene backbone conformations of acetylcholine for acetoxyl and trimethyl amine groups (Figure 2) may fully extend in space.

Inhibitory Potencies for Varied Substituents of Carbamates 1–15

When different carbamate substituents in **1–15** are compared, compounds with less bulky substituents such as *n*-butyl- and *n*-hexyl-carbamates are more potent than those with bulky substituents such as *n*-octyl-, *t*-butyl-, and benzyl-carbamates (Figure 4 and Table 1). The possible reason for this is that the acetyl binding site of AChE is relatively small when compared to other serine hydrolases and therefore is suitable for small carbamate substituents [2].

The k_2 Values

In contrast to K_i and k_i values, the k_2 values for inhibitors **1–15** are about the same (Table 1). Therefore, both sizes and geometries of the leaving groups for the AChE-carbamate complexes (benzene-1-*N*-substituted carbamate-2-, 3-, and 4-ols) (Figure 1) do not discriminate the k_2 values.

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