Homodimeric Tacrine Congeners as Acetylcholinesterase Inhibitors†

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Received July 5, 2001

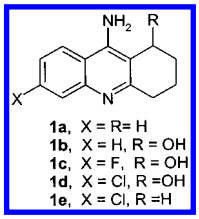
In the search for highly selective and potent derivatives of tacrine (**1a**), a number of homodimeric tacrine congeners were synthesized and conducted for their effects on rat acetylcholinesterase (AChE) and human butyrylcholinesterase (BChE) inhibitions. Heptylene-linked bis-(6-chloro)-tacrine (**3h**) was found up to 3000- and 3-fold more potent in inhibiting rat AChE than tacrine and the unsubstituted bis-tacrine **3b**, respectively. Changes in the size of the carbocyclic ring of the dimeric tacrine reduced both the selectivity and the potency of AChE inhibition as compared to **3b**. Inserting an aza into the tacrine nucleus as the desired isosteres **3j**−**m** resulted in moderate potency but tended to be detrimental to selectivity. The pronounced enhancement of AChE inhibition potency and AChE/BChE selectivity was achieved with incorporation of a halogen at the 6-position of homodimeric tacrines. The assay results of **3a**−**m** also provided evidence that the 7-methylene tether tended to be optimal to AChE inhibition potency.

Introduction

Alzheimer's disease (AD) has been recognized as one of the most severe conditions affecting the aged and is life-threatening for this group of people.[1] The disease is characterized by neuronal loss, synaptic damage, and neutritic and vascular plaques. At the cellular level, AD is associated mainly with reduced levels of synaptic acetylcholine (ACh) and other related neurotransmitters.[2] Tacrine (**1a**, tetrahydroaminoacridine or THA), a reversible acetylcholinesterase inhibitor (AChEI), has been one of the major approved drugs for use in AD (Chart 1).[3,4] The rationale for its use was related to the elevation of ACh levels that can compensate for the cholinergic deficiency associated with the brain lesions in AD. Nevertheless, the deficiency of tacrine in clinic has been related mainly to elevated liver transaminase levels and dose-related, low-selective peripheral cholinergic effects.[5,6] The search for tacrine analogues or related new candidates is still of interest to medicinal chemists involved in AD research.[7,8]

Recent contributions to the development of tacrine-related agents disclosed that tacrin-1-ol (**1b**, velnacrine),[9] an active metabolite of tacrine, has been chosen for clinical trial. 6-Fluoro-tacrin-1-ol (**1c**) was reported to be slightly more potent than tacrine, and 6-chlorotacrin-1-ol (**1d**) was found to be 30 times more potent than tacrine.[10] In particular 6-chlorotacrine (**1e**) has been found to be more potent than other substituted analogues.[11a,b] This could be due to favorable orientation and electron effects that contribute to more efficient inhibition. On the basis of theoretical calculations, **1e** was also shown to exhibit improved binding strength toward AChE.[11c] These suggested that the reduced electron density on the tacrine aromatic rings could favor π-interactions with nearby residues in active sites of the enzyme and strongly increase the inhibitory potency of tacrine. In addition to the structure−activity approach toward potent tacrine derivatives, an automated docking program was applied to simulate the binding possibilities for tacrine and an extra peripheral site was identified at the binding pocket of AChE.[12] On the basis of these studies, heptylene-linked bis-tacrine (**3b**, see Table 1) was found to be a potent and selective inhibitor of AChE[13−15] and the simultaneous binding to the active and peripheral sites of AChE was proposed to be responsible for the enhanced inhibition potency of **3b**.[16]

Chart 1. Tacrine (**1a**) and Its Derivatives **1b**−**e**



All of the above contributions suggest that the selectivity and potency of AChEIs for AD may be improved with manipulation of tacrine. In the present paper, we took a further step to integrate these findings on both monomeric and dimeric tacrine derivatives by carrying out our work on a series of homodimeric tacrine congeners. These were focused on the 6-position substitution, changes in carbocyclic ring size, and isosteric modification of the tacrine nucleus to optimize AChE inhibition potency and AChE/butyrylcholinesterase (BChE) selectivity.

Chemistry

The synthesis of dimeric tacrines is illustrated in Schemes 1 and 2. Although recent reports for the synthesis of bis-tacrines began with tacrine itself,[17] this

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† Dedicated to Professor Daniel H. Rich on the occasion of his 58th birthday.

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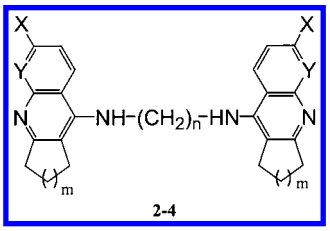
10.1021/jm010308g CCC: $22.00
Published on Web 04/26/2002

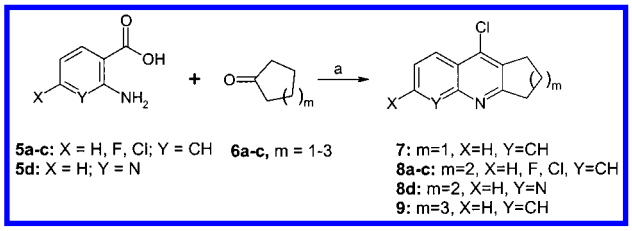
Table 1. AChE and BChE Inhibition Potency and Selectivity of Bis-tacrine Congeners



product	X	Y	*m*	*n*	IC_{50} (nM) AChE[a]	IC_{50} (nM) BChE[b]	selectivity for AChE[c]
2a	H	CH	1	6	115 ± 34	273 ± 38	2.4
2b	H	CH	1	7	75 ± 11	328 ± 40	4.4
2c	H	CH	1	8	22 ± 4	165 ± 21	7.5
3a	H	CH	2	6	1.4 ± 0.1	83 ± 19	59
3b	H	CH	2	7	0.2 ± 0.1	54 ± 14	221
3c	H	CH	2	8	1.6 ± 0.7	53 ± 17	33.1
3d	F	CH	2	6	0.9 ± 0.3	45 ± 21	50
3e	F	CH	2	7	0.6 ± 0.1	257 ± 35	428
3f	F	CH	2	8	0.7 ± 0.2	164 ± 32	234
3g	Cl	CH	2	6	0.6 ± 0.2	312 ± 72	520
3h	Cl	CH	2	7	0.07 ± 0.01	26 ± 1	371
3i	Cl	CH	2	8	0.3 ± 0.2	194 ± 64	647
3j	H	N	2	6	4.8 ± 1.3	93 ± 20	19.4
3k	H	N	2	7	1.3 ± 0.2	59 ± 14	45.4
3m	H	N	2	8	1.9 ± 0.2	23 ± 4	12.1
4a	H	CH	3	6	2.5 ± 0.7	3.3 ± 1.4	1.2
4b	H	CH	3	7	2.7 ± 0.4	2.6 ± 1.3	1.6
4c	H	CH	3	8	1.6 ± 0.4	3.8 ± 0.3	1.5
tacrine					333 ± 39	89 ± 7	0.3

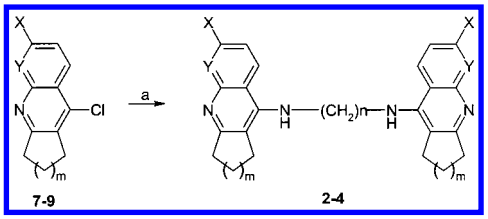
[a] Assay performed using rat cortex homogenate and ethopropazine as a specific BChE inhibitor. [b] Assay performed using human serum and BW284c51 as a specific AChE inhibitor. [c] Apparent selectivity for AChE is calculated as IC_{50}(BChE)/IC_{50}(AChE).

Scheme 1[a]



[a] Reagents: (a) $POCl_3$, heat, 2 h.

Scheme 2[a]



[a] Reagents: (a) 1,*n*-Diaminoalkane (**10a**−**c**, *n* = 6−8), PhOH, NaI, 180 °C, 2 h.

method proved to be a lot less replicable after numerous attempts by variation of the base and reaction temperature.[18] Therefore, the related chloride **8a** and its congeners **7**, **8b**−**d**, and **9** were chosen as critical intermediates for the synthesis of homodimeric tacrines (Scheme 1). The $POCl_3$-mediated cyclodehydration reaction between a variety of ortho-amino aromatic acids **5a**−**d** and cycloketones **6a**−**c** was adapted to efficiently produce in situ the corresponding chlorides **7**−**9** with moderate yields (54−94%). Treatment of the chlorides **7**−**9** with a half equivalent of 1,*n*-diaminoalkanes **10a**−**c** in heated phenol provided alkylene-linked homodimeric tacrine congeners **2**−**4** (Scheme 2).[19] This more strategy efficient method could be applied to the preparation of analogous bis-tacrine derivatives.

Biological Results and Discussion

These homodimeric tacrine congeners were assayed for AChE (rat cortex) and BChE (human plasma) inhibition potency by the Ellman method.[20] According to the previous suggestions,[18] the optimal methylene length bridging tacrine nucleus with simultaneous binding to the dual sites of AChE is possible with tether of at least five methylenes. Heptylene-linked bis-tacrine was found to be a potent and selective inhibitor of AChE in the series of alkylene-bridged analogues.[13] We, therefore, observed congeners of tacrine−tacrine homodimers spanning with 6−8 methylene units, which might display the same trends. The biological results for these congeners are given in Table 1. The IC_{50} values against rat brain AChE obtained for tacrine (**1**, 333 nM) and reported **3b** (0.2 nM) showed somewhat similar inclination to that observed by Pang (590 and 0.40 nM, respectively).[13] In comparison with **3a**−**c** (1.6−0.2 nM for AChE; 83−53 nM for BChE), carbocyclic-shrinked congeners **2a**−**c** resulted in almost 100-fold (115−22 nM) and 6-fold (328−165 nM) less potency at AChE and BChE, respectively. In contrast, ring-expanded **4a**−**c** had moderate potency (2.7−1.6 nM) on both enzymes. This suggested that the binding pockets of AChE might accommodate a little more bulky moiety.[18]

It was already known that the chlorine atom at the 6- or 8-position increases the inhibitory potency of tacrine.[11] Intriguingly, Savini and co-workers recently reported that 6,8-dichlorotacrine appeared to be more potent than the monosubstituted 6- and 8-chlorotacrine tacrines,[21] while its homodimeric tacrine showed decreased AChE inhibition. These data suggested that the peripheral site of AChE does not tolerate the simultaneous substitution at position 6 and 8 of the bis-tacrine skeleton. In our studies among these homodimeric congeners, heptylene-linked bis-(6-chloro)tacrine (**3h**) showed much improved potency with an IC_{50} value of 0.07 nM. This is three times as potent as **3b** and over 3000 times more potent than tacrine against rat AChE. Together, these results indicated that the binding pockets of AChE might fit to a limited bulky moiety as suggested by Carlier et al.[18] Moreover, bis-(6-fluoro)-tacrines **3d**−**f** exhibited a little amelioration of potency as compared to **3b**,**c**. Viewing from the data of **3d**−**i**, the AChE enzyme tends to tolerate and fit a certain variation at the 6-position of bis-tacrines. The congeners with an aza inserted in the tacrine nucleus (**3j**−**m**) still showed moderate AChEI potency (4.8−1.3 nM). These results might indicate that the AChE enzyme tends to tolerate certain changes in aromaticity of the tacrine nucleus. By summarizing the data of **3a**−**m**, the optimal potency is reserved by a 7-methylene tether among these homodimeric congeners (Figure 1). This is consistent with the trend on tacrine dimers as recently observed by Pang and co-workers.[13,16]

Inspection of the BChE data for these dimeric congeners indicated no clear trend in inhibition potency (Figure 2). Interestingly, cycloheptyl-fused congeners

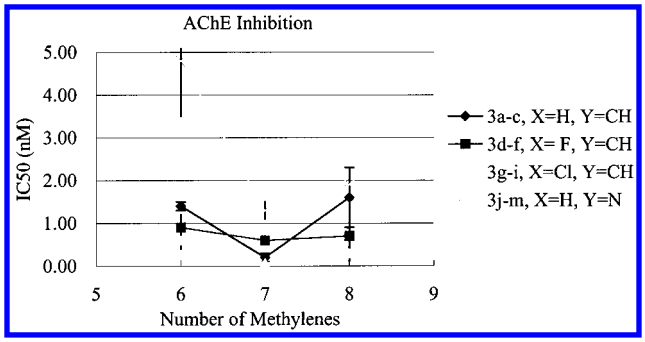


Figure 1. AChE inhibition by homodimeric tacrine congeners as a function of number of methylene units.

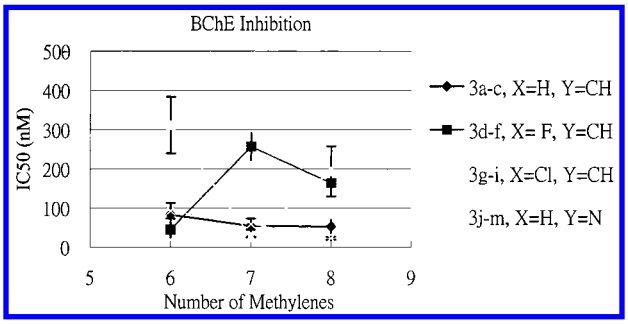


Figure 2. BChE inhibition by homodimeric tacrine congener as a function of number of methylene units.

4a−**c** showed similar accommodation to both AChE and BChE with IC_{50} values in the range of 4−1 nM, while those (e.g., **2a**−**c**) with cyclopentyl nucleus showed the least fit to both enzymes (114−22 nM and 328−165 nM). These results disclosed that BChE seems to be better able than AChE to accommodate steric bulk around the catalytic site.[18] Most 6-substituted bis-tacrines are considerably potent in inhibiting AChE and still less active toward BChE (e.g., **3d**−**i**, except **3h**, IC_{50} values, 312−45 nM). These observations come up with retaining high AChE/BChE selectivity. The marked AChE/BChE selectivity strongly implied the existence of the peripheral site at binding pockets, which was suggested as a strategy for the development of highly selective AChE inhibitors.[13]

Detailed Pharmacological Procedure

Materials and Methods. All commercial chemicals were obtained from Sigma Co. (St. Louis, MO) and were used without further purification. All of the instruments (e.g., scissors, forceps, spoons, clips, and homogenizers) were kept clean and cool in an ice bath before use.

Preparation of Rat Brain Homogenate. Rats were decapitated, and the brain was dissected on ice. Rat brain homogenate was obtained by centrifugation (2530 rpm, 10 min) of homogenized frontal cortex in 10 mM Tris buffer (3 mL, pH 7.4) at 4 °C (Eppendorf, Centrifuge 5402). It was kept in several vials at −80 °C and used as a source of AChE.

Preparation of Human Serum. Human serum was obtained by centrifugation of 10 mL of heparinized whole blood (3500 rpm, 10 min) at 4 °C. It was kept in several vials at −25 °C and was the source of BChE.

Determination of AChE Inhibition. Cortex homogenate was preincubated for 5 min with ethopropazine (20 mM). To a 1 mL UV cell were added 880 μL of Tris buffer (0.1 mM, pH 7.4), 5 μL of the tested compound (0.1 mM), 10 μL of homogenate, and followed by 50 μL of acetylthiocholine iodide (4.8 mM) after 2 min. The reaction was terminated by the addition of 5,5′-dithiobis-(2-nitrobenzoic acid) (0.2% w/v, 50 μL). Enzyme activity was determined by measuring the absorbance at 420 nm (Shimadzu UV-160A) after 7 min, relative to the drug-free control.

Determination of BChE Inhibition. Plasma was preincubated for 5 min with BW284c51 (2 mM). To a 1 mL UV cell were added 880 μL of Tris buffer (0.1 mM, pH 7.4), 5 μL of the tested compound (0.1 mM), 10 μL of plasma, and followed by 50 μL of butyrylthiocholine iodide (6.4 mM) after 2 min. The reaction was terminated by the addition of 5,5′-dithiobis(2-nitrobenzoic acid) (0.2% w/v, 50 μL). Enzyme activity was determined by measuring the absorbance at 420 nm after 7 min, relative to the drug-free control.

Triplicate measurements were performed at typically a total of six concentrations for all above enzyme studies. The IC_{50} values were determined from a plot of percentage of inhibition vs −log [drug], which was processed by a software of Sigma Plot 4.0.

Conclusions

In these studies, we integrated previous findings to carry out on the 6-position substitution, changes in carbocyclic ring size, and isosteric modification toward enhanced optimization for AChE inhibition potency. We discovered **3h** to be a highly potent and selective inhibitor of AChE. The data from these studies fit the putative model of dual site binding on AChE as previously suggested. Moreover, most homodimeric tacrines with potent AChE inhibition (e.g., **3a**−**i**, except **3h**) are still less active toward BChE, consistent with the tentative suggestion of the absence of a peripheral site on the enzyme. These studies also provided the evidence that a tether of 7-methylene units is optimal for AChE inhibition and AChE/BChE selectivity among these homodimeric bis-tacrine congeners. The pronounced enhancement of AChE inhibition potency and AChE/BChE selectivity was achieved with incorporation of a halogen at the 6-position of homodimeric tacrines.

Experimental Section

Chemistry. All ortho-amino aromatic acids and cycloketones were obtained from Aldrich (Milwaukee, WI). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini-300 instrument. High-resolution mass spectra were obtained on a JEOL J. M. S.-300 spectrometer. Elemental analysis was performed by the Taipei Instrumental Center, National Science Council (Taipei, Taiwan). Reactions were followed by thin-layer chromatography (TLC) on Merck (0.2 mm) aluminum-packed precoated silica gel plates (60 F_{254}) that were visualized by phosphomolybdic acid alcoholic solution under a heating plate.

8-Chloro-2,3-dihydro-1H-cyclopenta[1,2-*b*]quinoline (7). To a mixture of acid **5a** (4.11 g, 30.0 mmol) and ketone **6a** (2.65 mL, 30.0 mmol) was carefully added 25 mL of $POCl_3$ at ice bath. The mixture was heated under reflux for 2 h, then cooled at room temperature, and concentrated to give a slurry. The residue was diluted with EtOAc, neutralized with aqueous K_2CO_3, and washed with brine. The organic layer was dried over anhydrous K_2CO_3 and concentrated in vacuo to furnish a pale brown solid. It was recrystallized from acetone to give **7** (5.50 g, 90%); mp 85−87 °C. ^{1}H NMR ($CDCl_3$): δ 8.09 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.63 (t, J = 6.9 Hz, 1H), 7.52 (t, J = 7.1 Hz, 1H), 3.18 (t, J = 7.9 Hz, 2H), 3.10 (t,

J = 7.5 Hz, 2H), 2.19 (qint, J = 7.4 Hz, 2H). FABMS: m/z [M + H]$^+$ 204.0. HR-FABMS: exact mass calcd for $C_{12}H_{11}NCl$ [M + H]$^+$, 204.0580; found, 204.0579.

9-Chloro-1,2,3,4-tetrahydroacridine (8a).[19a] Compounds **5a** (7.4 g, 53.9 mmol) and **7b** (5.36 mL, 51.7 mmol) were condensed as above to afford **8a** (11.4 g, 94%); mp 68−70 °C (literature mp 66−68 °C). ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H), 7.63 (dd, J = 9.2, 7.5 Hz, 1H), 7.51 (dd, J = 9.2, 8.3 Hz, 1H), 3.10 (t, J = 6.3 Hz, 2H), 2.97 (t, J = 4.8 Hz, 2H), 1.91 (s br, 4H). EIMS: 217 (M$^+$, 100), 219 (M + 2$^+$, 33). HR-EIMS: exact mass calcd for $C_{13}H_{12}NCl$ [M]$^+$, 217.0659; found, 217.0648.

9-Chloro-6-fluoro-1,2,3,4-tetrahydroacridine (8b). Compounds **5b** (3.0 g, 19.6 mmol) and **6b** (2.05 mL, 19.6 mmol) were condensed as above to afford **8b** (2.49 g, 54%) as a light brown solid; mp 75−77 °C. ^{1}H NMR ($CDCl_3$): δ 8.11 (dd, J = 9.3, 6.0 Hz, 1H), 7.57 (dd, J = 9.1, 2.5 Hz, 1H), 7.29−7.26 (m, 1H), 3.06 (s br, 2H), 2.96 (s br, 2H), 1.92 (t, J = 3.3 Hz, 4H). FABMS: m/z [M + H]$^+$ 236.0. HR-FABMS: exact mass calcd for $C_{13}H_{12}NFCl$ [M + H]$^+$, 236.0642; found, 236.0644.

6,9-Dichloro-1,2,3,4-tetrahydroacridine (8c). Compounds **5c** (8.58 g, 50.0 mmol) and **6b** (5.18 mL, 50.0 mmol) were condensed as above to afford **8c** (11.67 g, 93%) as a brown solid; mp 81−83 °C. ^{1}H NMR ($CDCl_3$): δ 8.88 (s 1H), 8.27 (dd, J = 7.08, 1.38 Hz, 1H), 7.74 (dd, J = 7.10, 1.98 Hz, 1H), 3.64 (s br, 2H), 3.09 (s br, 2H), 2.02 (s br, 4H). EIMS: m/z [M]$^+$ 251 (100%). HR-EIMS: exact mass calcd for $C_{13}H_{11}NCl_2$ [M]$^+$, 251.0271; found, 251.0277.

9-Chloro-1,2,3,4-tetrahydro-cyclohexa[1,2,-*b*]pyrido-[2,3-*b*]pyridine (8d). Compounds **5d** (4.14 g, 30 mmol) and **6b** (3.11 mL, 30 mol) were condensed as above to afford **8d** (3.92 g, 86%) as a brown solid; mp 146−149 °C. ^{1}H NMR ($CDCl_3$): δ 9.02 (dd, J = 1.7, 4.2 Hz, 1H), 8.49 (dd, J = 1.8, 8.4 Hz, 1H), 7.46 (dd, J = 4.2, 8.3 Hz, 1H), 3.19 (s br, 2H), 3.00 (t, J = 6.1 Hz, 2H), 2.03−1.90 (m, 4H). EIMS: m/z 220 [M + 2$^+$, 33], 218 [M$^+$, 100].

10-Chloro-2,3,4,5-tetrahydro-1*H*-cyclohepta[1,2,-*b*]quinoline (9). Compounds **5a** (2.05 g, 14.9 mmol) and **6c** (1.27 mL, 14.9 mmol) were condensed as above to afford **9** (1.55 g, 45%) as a pale brown solid; mp 87−89 °C. ^{1}H NMR ($CDCl_3$): δ 8.56 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.2 Hz, 1H), 7.77 (t, J = 6.7 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 3.54 (t, J = 4.5 Hz, 2H), 3.24−3.16 (m, 2H), 1.90−1.70 (m, 6H). FABMS: m/z [M + H]$^+$ 232.0. HR-FABMS: exact mass calcd for $C_{14}H_{15}NCl$ [M + H]$^+$, 232.0869; found, 232.0888.

***N*,*N*′-Bis-(2,3-dihydro-1*H*-cyclopenta[1,2-*b*]quinolin-8-yl)-1,6-diaminohexane (2a).** A mixture of **7** (0.60 g, 3.00 mmol), **10a** (0.18 g, 1.50 mmol), phenol (1.5 g), and NaI (0.07 g) was carefully heated at 180 °C under an inert system for 2 h and then cooled at room temperature. The mixture was diluted with EtOAc and made basic with 10% KOH solution. The organic layer was washed with water and brine and dried over anhydrous $MgSO_4$. After concentration, the resulting residue was purified on silica gel chromatography (CH_2Cl_2:MeOH:NH_4OH = 10:1:1) to give **2a** (0.23 g, 34%) as amber glass foam; mp 64−66 °C. ^{1}H NMR ($CDCl_3$): δ 7.91 (d, J = 8.3 Hz, 2H), 7.71 (d, J = 8.7 Hz, 2H), 7.55 (t, J = 7.7 Hz, 2H), 7.35 (t, J = 7.7 Hz, 2H), 4.68 (s br, 2H, 2 NH), 3.59 (q, J = 6.6 Hz, 2H), 3.17 (t, J = 6.9 Hz, 4H), 3.05 (t, J = 7.6 Hz, 4H), 2.20−2.00 (m, 4H), 1.70−1.55 (m, 4H), 1.55−1.40 (m, 4H). FABMS: m/z [M + H]$^+$ 451.2. HR-FABMS: exact mass calcd for $C_{30}H_{35}N_4$ [M + H]$^+$, 451.2860; found, 451.2860. Anal. ($C_{30}H_{34}N_4$·1.5H_2O) C, H, N.

***N*,*N*′-Bis-(2,3-dihydro-1*H*-cyclopenta[1,2-*b*]quinolin-8-yl)-1,7-diaminoheptane (2b).** Compounds **7** (0.82 g, 4.00 mmol) and **10b** (0.26 g, 2.00 mmol) were combined as above to afford **2b** (0.33 g, 36%) as a brown solid; mp 57−59 °C. ^{1}H NMR ($CDCl_3$): δ 7.93 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.55 (t, J = 7.9 Hz, 2H), 7.35 (t, J = 7.2 Hz, 2H), 4.70 (s br, 2H, 2 NH), 3.58 (q, J = 6.8 Hz, 4H), 3.19 (t, J = 7.1 Hz, 4H), 3.05 (t, J = 7.8 Hz, 4H), 2.13 (quint, J = 7.4 Hz, 4H), 1.70−1.55 (m, 4H), 1.55−1.40 (m, 6H). FABMS: m/z [M + H]$^+$ 465.2. HR-FABMS: exact mass calcd for $C_{31}H_{37}N_4$ [M + H]$^+$, 465.3017; found, 465.3012. Anal. ($C_{31}H_{36}N_4$·H_2O) H, N. C: calcd, 77.13; found, 77.82.

***N*,*N*′-Bis-(2,3-dihydro-1*H*-cyclopenta[1,2-*b*]quinolin-8-yl)-1,8-diaminooctane (2c).** Compounds **7** (0.82 g, 4.00 mmol) and **10c** (0.29 g, 2.00 mmol) were combined as above to afford **2c** (0.30 g, 31%) as a brown solid; mp 50−52 °C. ^{1}H NMR ($CDCl_3$): δ 7.91 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.55 (t, J = 7.1 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 4.72 (s br, 2H, 2 NH), 3.57 (q, J = 6.3 Hz, 2H), 3.19 (t, J = 7.4 Hz, 4H), 3.05 (t, J = 7.7 Hz, 4H), 2.14 (quint, J = 7.4 Hz, 4H), 1.70−1.55 (m, 4H), 1.50−1.30 (m, 8H). FABMS: m/z [M + H]$^+$ 479.2. HR-FABMS: exact mass calcd for $C_{32}H_{39}N_4$ [M + H]$^+$, 479.3174; found, 479.3175. Anal. ($C_{32}H_{38}N_4$·2H_2O) C, H, N.

Hexylene-Linked Bis-tacrine (3a). Compounds **8a** (0.75 g, 3.5 mmol) and **10a** (0.2 g, 1.75 mmol) were combined as above to afford **3a** (0.47 g, 56%) as an amber glass foam; mp 94−96 °C. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, J = 4.1 Hz, 2H), 7.91 (d, J = 4.1 Hz, 2H), 7.54 (t, J = 7.0 Hz, 2H), 7.32 (t, J = 7.0 Hz, 2H), 4.00 (s br, 2H), 3.47 (t, J = 7.1 Hz, 4H), 3.06 (s br, 4H), 2.68 (s br, 4H), 1.89 (t, J = 2.4 Hz, 8H), 1.65 (s br, 4H), 1.41 (s br, 4H). FABMS (NBA as matrix): m/z [M + H]$^+$ 479.2. HR-FABMS: exact mass calcd for $C_{32}H_{39}N_4$ [M + H]$^+$, 479.3173; found, 479.3187. Anal. ($C_{32}H_{38}N_4$·H_2O) C, H, N.

Heptylene-Linked Bis-tacrine (3b).[17] Compounds **8a** (0.75 g, 3.5 mmol) and **10b** (0.23 g, 1.75 mmol) were combined as above to afford **3b** (0.40 g, 47%) as an amber oil. ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 3.2 Hz, 2H), 7.94 (d, J = 3.3 Hz, 2H), 7.55 (t, J = 6.8 Hz, 2H), 7.34 (t, J = 7.1 Hz, 2H), 4.10 (s br, 2H), 3.50 (t, J = 7.4 Hz, 4H), 3.07 (s br, 4H), 2.69 (s br, 4H), 1.90 (s br, 8H), 1.65 (s br, 4H), 1.37 (s br, 6H). FABMS: m/z [M + H]$^+$ 493.2. HR-FABMS: exact mass calcd for $C_{33}H_{41}N_4$ [M + H]$^+$, 493.3330; found, 493.3346. Anal. ($C_{33}H_{40}N_4$·1.5H_2O) C, H, N.

Octylene-Linked Bis-tacrine (3c).[17] A mixture of **8a** (0.75 g, 3.5 mmol), **10c** (0.25 g, 1.75 mmol), phenol (2.0 g), and NaI (0.1 g) was treated as above to afford **3c** (0.46 g, 51%) as an amber oil. ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 8.5 Hz, 2H), 7.55 (t, J = 7.1 Hz, 2H), 7.34 (t, J = 6.0 Hz, 2H), 3.49 (t, J = 7.4 Hz, 4H), 3.07 (s br, 4H), 2.70 (s br, 4H), 1.91 (s br, 8H), 1.65 (s br, 4H), 1.45−1.20 (m, 8H). FABMS: m/z [M + H]$^+$ 507.2. HR-FABMS: exact mass calcd for $C_{34}H_{43}N_4$ [M + H]$^+$, 507.3487; found, 507.3470. Anal. ($C_{34}H_{42}N_4$·H_2O) C, H, N.

Hexylene-Linked Bis-(6-fluoro)tacrine (3d). Compounds **8b** (0.5 g, 2.13 mmol) and **10a** (0.18 g, 1.07 mmol) were combined as above to afford **3d** (0.31 g, 56%) as brown glass foam; mp 83−85 °C. ^{1}H NMR ($CDCl_3$): δ 7.95 (dd, J = 9.3, 6.1 Hz, 2H), 7.50 (dd, J = 10.2, 2.4 Hz, 2H), 7.09 (td, J = 7.2, 2.4 Hz, 2H), 3.47 (t, J = 2.6 Hz, 4H), 3.02 (s br, 4H), 2.75−2.65 (m, 4H), 1.90−1.80 (m, 8H), 1.78−1.60 (m, 4H), 1.50−1.25 (m, 4H). FABMS: m/z [M + H]$^+$ 515.2. HR-FABMS: calcd for $C_{32}H_{37}N_4F_2$ [M + H]$^+$, 515.2985; found, 515.2996. Anal. ($C_{32}H_{36}N_4F_2$·2H_2O) C, H. N: calcd, 10.19; found, 9.34.

Heptylene-Linked Bis-(6-fluoro)tacrine (3e). Compounds **8b** (0.5 g, 2.13 mmol) and **10b** (0.14 g, 1.07 mmol) were combined as above to afford **3e** (0.26 g, 47%) as brown glass foam; mp 92−94 °C. ^{1}H NMR ($CDCl_3$): δ 7.99 (dd, J = 9.6, 6.2 Hz, 2H), 7.58 (dd, J = 9.9, 2.2 Hz, 2H), 7.10 (td, J = 8.0, 2.4 Hz, 2H), 3.53 (t, J = 7.1 Hz, 4H), 3.05 (s br, 4H), 2.66 (s br, 4H), 1.90 (t, J = 3.1 Hz, 8H), 1.80−1.60 (m, 4H), 1.50−1.20 (m, 6H). FABMS: m/z [M + H]$^+$ 529.2. HR-FABMS: exact mass calcd for $C_{33}H_{39}N_4F_2$ [M + H]$^+$, 529.3142; found, 529.3161. Anal. ($C_{33}H_{38}N_4F_2$·2H_2O) C, H, N.

Octylene-Linked Bis-(6-fluoro)tacrine (3f). Compounds **8b** (0.4 g, 1.70 mmol) and **10c** (0.13 g, 0.85 mmol) were combined as above to afford **3f** (0.24 g, 51%) as brown glass foam; mp 78−80 °C. ^{1}H NMR ($CDCl_3$): δ 7.97 (dd, J = 9.1, 6.1 Hz, 2H), 7.55 (dd, J = 10.4, 2.5 Hz, 2H), 7.09 (td, J = 7.3, 2.5 Hz, 2H), 3.50 (t, J = 6.9 Hz, 4H), 3.02 (s br, 4H), 2.65 (s br, 4H), 1.89 (s br, 8H), 1.65 (t, J = 6.9 Hz, 4H), 1.50−1.20 (m, 8H). FABMS: m/z [M + H]$^+$ 543.2. HR-FABMS: exact mass calcd for $C_{34}H_{41}N_4F_2$ [M + H]$^+$, 543.3298; found, 543. 3310. Anal. ($C_{32}H_{36}N_4F_2$·2H_2O) H, N. C: calcd, 70.55; found, 70.07.

Hexylene-Linked Bis-(6-chloro)tacrine (3g). Compounds **8c** (0.75 g, 3.00 mmol) and **10a** (0.17 g, 1.50 mmol) were combined as above to afford **3g** (0.25 g, 31%) as amber glass foam; mp 73−75 °C. ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 2H), 7.86 (d, J = 9.0 Hz, 2H), 7.25 (d, J = 9.0 Hz, 2H), 4.00 (s br, 2H), 3.47 (t, J = 7.0 Hz, 4H), 3.01 (s br, 4H), 2.63 (s be, 4H), 1.88 (s br, 8H), 1.65 (s br, 4H), 1.41 (s br, 4H). FABMS: m/z $[M + H]^+$ 547.1. HR-FABMS: exact mass calcd for $C_{32}H_{37}N_4Cl_2$ $[M + H]^+$, 547.2395; found, 547.2365. Anal. ($C_{32}H_{36}N_4Cl_2 \cdot H_2O$) C, H, N.

Heptylene-Linked Bis-(6-chloro)tacrine (3h). Compounds **8c** (0.75 g, 3.00 mmol) and **10b** (0.19 g, 1.50 mmol) were combined as above to afford **3h** (0.47 g, 56%) as brown glass foam; mp 67−69 °C. ^{1}H NMR ($CDCl_3$): δ 7.92 (s, 2H), 7.89 (d, J = 9.0 Hz, 2H), 7.25 (d, J = 9.0 Hz, 2H), 3.47 (t, J = 7.1 Hz, 4H), 3.02 (s br, 4H), 2.64 (s br, 4H), 1.90 (s br, 8H), 1.64 (s br, 4H), 1.36 (s br, 6H). FABMS: m/z $[M + H]^+$ 561.2. HR-FABMS: exact mass calcd for $C_{33}H_{39}N_4Cl_2$ $[M + H]^+$, 561.2556; found, 561.2541. Anal. ($C_{33}H_{38}N_4Cl_2 \cdot H_2O$) C, H, N.

Octylene-Linked Bis-(6-chloro)tacrine (3i). Compounds **8c** (0.75 g, 3.00 mmol) and **10c** (0.22 g, 1.50 mmol) were combined as above to afford **3i** (0.40 g, 46%) as brown glass foam; mp 62−63 °C. ^{1}H NMR ($CDCl_3$): δ 7.91 (s, 2H), 7.88 (d, J = 8.8 Hz, 2H), 24 (d, J = 8.8 Hz, 2H), 4.12 (s br, 2H), 3.51 (s br, 4H), 3.03 (s br, 4H), 2.65 (s br, 4H), 1.89 (s br, 8H), 1.65 (s br, 4H), 1.30 (s br, 8H). FABMS: m/z $[M + H]^+$ 575.2. HR-FABMS: exact mass calcd for $C_{34}H_{41}N_4Cl_2$ $[M + H]^+$, 575.2713; found, 575.2691. Anal. ($C_{34}H_{40}N_4Cl_2 \cdot H_2O$) C, H, N.

***N,N′*-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2,-*b*]pyrido-[2,3-*b*]pyrid-9-yl)-1,6-diaminohexane (3j).** Compounds **8d** (0.43 g, 2.0 mmol) and **10a** (0.12 g, 1.0 mmol) were combined as above to afford **3j** (0.12 g, 25%) as amber glass foam; mp 121−124 °C. ^{1}H NMR ($CDCl_3$): δ 8.86 (d, J = 2.7 Hz, 2H), 8.38 (d, J = 8.2 Hz, 2H), 7.26−7.21 (m, 2H), 4.50 (s br, 2H, 2 NH), 3.60−3.50 (m, 4H), 3.07 (s br, 4H), 2.70−2.60 (m, 4H), 1.95−1.80 (m, 8H), 1.70−1.60 (m, 4H), 1.45−1.35 (m, 4H). FABMS: m/z $[M + H]^+$ 481.4. HR-FABMS: exact mass calcd for $C_{30}H_{37}N_6$ $[M + H]^+$, 481.3080; found, 481.3057. Anal. ($C_{30}H_{36}N_6 \cdot 2H_2O$) H, N. C: calcd, 69.72; found, 69.30.

***N,N′*-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2,-*b*]pyrido-[2,3-*b*]pyrid-9-yl)-1,7-di-aminoheptane (3k).** Compounds **8d** (0.46 g, 2.1 mmol) and **10b** (0.14 g, 1.1 mmol) were combined as above to afford **3k** (0.18 g, 36%) as amber glass foam; mp 109−111 °C. ^{1}H NMR ($CDCl_3$): δ 8.84 (d, J = 2.5 Hz, 2), 8.41 (d, J = 8.4 Hz, 2H), 7.22 (t, J = 4.0 Hz, 2H), 4.70 (s br, 2H, 2 NH), 3.53 (s br, 4H), 3.08 (s br, 4H), 2.64 (s br, 4H), 1.86 (s br, 8H), 1.80−1.50 (m, 4H, 4H), 0.150−1.20 (m, 6H). FABMS: m/z $[M + H]^+$ 495.4. HR-FABMS: exact mass calcd for $C_{31}H_{39}N_6$ $[M + H]^+$, 495.3236; found, 495.3232. Anal. ($C_{31}H_{38}N_6 \cdot 2H_2O$) C, H, N.

***N,N′*-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2,-*b*]pyrido-[2,3-*b*]pyrid-9-yl)-1,8-di-aminooctane (3m).** Compounds **8d** (0.57 g, 2.6 mmol) and **10c** (0.19 g, 1.3 mmol) were combined as above to afford **3m** (0.15 g, 23%) as an amber solid; mp 89−91 °C. ^{1}H NMR ($CDCl_3$): δ 8.84 (d, J = 4.0 Hz, 2H), 8.37 (d, J = 8.3 Hz, 2H), 7.26−7.16 (m, 2H), 4.44 (s br, 2H, 2 NH), 3.50 (s br, 4H), 3.06 (s br, 4H), 2.62 (s br, 4H), 1.85 (s br, 8H), 1.70−1.50 (m, 4H), 1.45−1.20 (m, 8H). FABMS: m/z $[M + H]^+$ 509.3. HR-FABMS: exact mass calcd for $C_{32}H_{41}N_6$ $[M + H]^+$, 509.3393; found, 509.3392. Anal. ($C_{32}H_{40}N_6 \cdot 2H_2O$) C, H, N.

***N,N′*-Bis-(2,3,4,5-tetrahydro-1*H*-cyclohepta[1,2,-*b*]quinolin-10-yl)-1,6-diaminohexane (4a).** Compounds **9** (0.25 g, 1.08 mmol) and **10a** (63 mg, 0.54 mmol) were combined as above to afford **4a** (75 mg, 22%) as an amber glass form; mp 95−97 °C. ^{1}H NMR ($CDCl_3$): δ 7.98 (d, J = 7.8 Hz, 2H), 7.88 (d, J = 7.9 Hz, 2H), 7.55 (t, J = 6.9 Hz, 2H), 7.41 (t, J = 7.0 Hz, 2H), 3.30 (t, J = 4.6 Hz, 2H), 3.20−3.10 (m, 4H), 2.90−2.80 (m, 4H), 1.95−1.60 (m, 16H), 1.50−1.30 (m, 4H). FABMS: m/z $[M + H]^+$ 507.2. HR-FABMS: exact mass calcd for $C_{34}H_{43}N_4$ $[M + H]^+$, 507.3488; found, 507.3483. Anal. ($C_{34}H_{42}N_4 \cdot 2H_2O$) C, H, N.

***N,N′*-Bis-(2,3,4,5-tetrahydro-1*H*-cyclohepta[1,2,-*b*]quinolin-10-yl)-1,7-diaminoheptane (4b).** Compounds **9** (0.26 g, 1.12 mmol) and **10b** (73 mg, 0.56 mmol) were combined above to afford **4b** (64 mg, 22%) as a glass foam; mp 64−66 °C. ^{1}H NMR ($CDCl_3$): δ 7.91 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 7.51 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 3.88 (s br, 2H, 2 NH), 3.21 (s br, 4H), 3.15−3.10 (m, 4H), 2.91 (s br, 4H), 1.95−1.70 (m, 16H), 1.50−1.25 (m, 6H). FABMS: m/z $[M + H]^+$ 521.2. HR-FABMS: exact mass calcd for $C_{35}H_{45}N_4$ $[M + H]^+$, 521.4644; found, 521.3640. Anal. ($C_{35}H_{44}N_4 \cdot 2H_2O$) C, H, N.

***N,N′*-Bis-(2,3,4,5-tetrahydro-1*H*-cyclohepta[1,2,-*b*]quinolin-10-yl)-1,8-diaminooctane (4c).** Compounds **9** (0.29 g, 1.25 mmol) and **10c** (90 mg, 0.63 mmol) were combined as above to afford **4c** (94 mg, 28%) as an amber oil. ^{1}H NMR ($CDCl_3$): δ 8.05 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.5 Hz, 2H), 7.54 (t, J = 7.3 Hz, 2H), 7.41 (t, J = 8.8 Hz, 2H), 3.37 (t, J = 4.4 Hz, 2H), 3.20 (s br, 4H), 2.90 (s br, 4H), 1.95−1.65 (m, 16H), 1.45−1.20 (m, 8H). FABMS: m/z $[M + H]^+$ 535.2. HR-FABMS: exact mass calcd for $C_{36}H_{47}N_4$ $[M + H]^+$, 535.3801; found, 535.3777. Anal. ($C_{36}H_{46}N_4 \cdot 2H_2O$) H, N. C: calcd, 78.21; found, 77.79.

Enzyme Preparations and Inhibition Studies on AChE and BChE. AChE and BChE enzyme preparations were prepared from cortex of decapitated rats and human plasma, respectively. Rat brain homogenate was obtained by centrifugation (2530 rpm, 10 min) of homogenized frontal cortex in Tris buffer (10 mM, pH 7.4) at 4 °C and kept at −80 °C, which was used as a source of AChE. Human plasma was the source of BChE and obtained by centrifugation of whole blood (3500 rpm, 10 min) at 4 °C and kept at −25 °C. The cholinesterase assays were performed using colorimetric method reported by Ellman.[20] For the determination of AChE inhibition, cortex homogenate was preincubated for 5 min with ethopropazine (20 mM), a selective inhibitor of BChE. Similarly, for the determination of BChE inhibition, plasma was preincubated with BW284c51 (2 mM), a selective inhibitor of AChE. A 1 mL mixture containing acetylthiocholine iodide (4.8 mM) or butyrylthiocholine iodide (6.4 mM), 880 μL of Tris buffer (0.1 mM, pH 7.4), a solution of the tested compound (0.1 mM), and homogenate or plasma (10 μL) was incubated at 37 °C for 5 min. The reaction was terminated by the addition of 5,5′-dithiobis(2-nitrobenzoic acid) (0.2% w/v, 50 μL). Enzyme activity was determined by measuring the absorbance at 420 nm after 7 min, relative to the drug-free control. Triplicate measurements were carried out at typically a total of six drug concentrations. The IC_{50} values were determined from a plot of percentage of inhibition vs −log [drug], which was processed by a software of Sigma Plot 4.0.

Acknowledgment. This study was in part supported by a grant from the National Science Council, Taiwan (NSC 89-2320-B-016-101). We thank National Taiwan University and National Tsing Hua University for fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HR-MS) analyses.

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JM010308G