Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 1394–1408
Bioorganic & Medicinal Chemistry

Study on dual-site inhibitors of acetylcholinesterase: Highly potent derivatives of bis- and bifunctional huperzine B

Xu-Chang He,* Song Feng, Zhi-Fei Wang, Yufang Shi, Suxin Zheng, Yu Xia, Hualiang Jiang, Xi-can Tang and Donglu Bai

State Key Laboratory of Drug Research, Department of Synthetic Drugs, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

Received 24 August 2006; revised 6 November 2006; accepted 6 November 2006
Available online 9 November 2006

Abstract—Natural (−)-huperzine B (HupB), isolated from Chinese medicinal herb, displayed moderate inhibitory activity of acetylcholinesterase (AChE). Based on the active dual-site of AChE, a series of novel derivatives of bis- and bifunctional HupB were designed and synthesized. The AChE inhibition potency of most derivatives of HupB was enhanced about 2–3 orders of magnitude as compared with the parental HupB. Among bis-HupB derivatives, **12h** exhibited the most potent in the AChE inhibition and has been evaluated for its pharmacological actions in vivo on ChE inhibition, cognitive enhancement, and neuroprotection. The docking study on the bis-HupB derivatives **12** series with *Tc*AChE has demonstrated that the ligands bound to the dual-site of the enzyme in different level.

1. Introduction

To date, the cholinergic hypothesis is still the practical approach for treating Alzheimer's disease (AD), therefore, the clinical use of acetylcholinesterase (AChE) inhibitors is wide to alleviate symptoms of moderate AD patients.[1–3] Several AChE inhibitors, such as tacrine, rivastigmine, donepezil, and galanthamine, have been approved by FDA for the treatment of AD.[4] However, the clinical use of the AChE inhibitors is sometimes limited mainly due to their some adverse effects and modest benefits to AD patients. Therefore, novel more effective therapy, including AChE inhibitors, needs to be developed for AD therapy.[5–7]

In 1991, X-ray crystallographic structural analysis of the AChE from *Torpedo californica* (*Tc*AChE) has demonstrated that active site laid near the bottom of a deep and narrow gorge, that reaches halfway into the protein, and 14 aromatic residues lined a substantial portion of the surface of the gorge.[8] This cavity was named as the 'active site gorge' and, further, the peripheral sites existed at the gorge mouth.[9]

Recently, scientists have reported that AChE was also responsible for the non-cholinergic actions. It has been demonstrated that AChE might function to accelerate β-amyloid peptide (Aβ) formation and could play a role during amyloid deposition in AD brain.[10] The peculiar feature of AChE was affected by peripheral site binding ligands, such as decamethonium and propidium, and was not acted by active site inhibitors, such as edrophonium. Moreover, it has been shown that molecules that were able to interact with both active and peripheral sites of AChE (i.e. a dual-site inhibitor) could prevent the aggregating activity of AChE toward Aβ besides the inhibitory activity.[11] In addition, the butyrylcholinesterase (BuChE) did not affect amyloid formation. Therefore, inhibitors with the dual-site binding to AChE have recently presented a new therapeutic strategic option.[12,13]

Pang et al. first reported that bis(7)-tacrine (**1**), the heptylene-linked tacrine dimer, possessed both optimal AChE inhibitory potency and AChE/BuChE selectivity than tacrine itself.[14,15] The derivatives of bis-HupA[16] and bis-5-amino-5,6,7,8-tetrahydroquinolinone (**2**)[17,18] have also been reported. The bis-galanthamine linked

Keywords: Huperzine B; Acetylcholinesterase; Alzheimer's disease; Dual-site binding inhibitors.
* Corresponding author. Tel.: +86 21 50806600x3518; fax: +86 21 50807088; e-mail: hexuchan@mail.shcnc.ac.cn

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.11.009

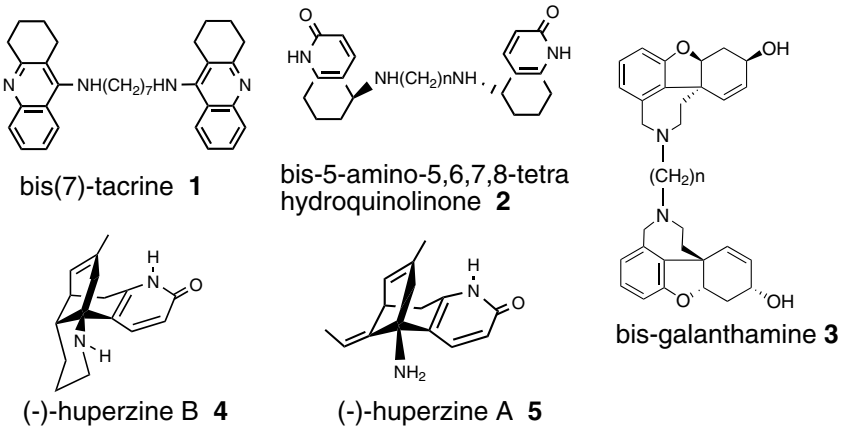


Figure 1. Several novel AChE inhibitors.

by alkylene (**3**) was more potent than galanthamine in the inhibition.[19] The crystal structure of *Tc*AChE-(bis-5-amino-5,6,7,8-tetrahydroquinolinone) complexes showed that they bound to *Tc*AChE in the bivalent binding fashion.[18] It was evident that the increased affinities of inhibitors **1**, **2**, and **3** (Fig. 1) to AChE were presented by binding to the dual-site of the enzyme.

Natural (−)-huperzine B (HupB, **4**) was a *Lycopodium* alkaloid isolated from Chinese medicinal club moss *Huperzia serrata*. HupB was less potent and selective in inhibition of AChE than (−)-huperzine A (HupA, **5**).[20,21] The latter was first isolated from the same herbs and has been approved as a new drug for treating symptoms of AD in China.[22] However, HupB exhibited a higher therapeutic index in comparison with HupA.[23,24] We have reported preliminary results of a series of derivatives of bis- and bifunctional HupB.[25,26]

In this paper, we would present our detail research results on design and synthesis of a series of derivatives of bis- and bifunctional HupB for discovering AD drug candidates from natural HupB. Besides study on the pharmacological activities of the HupB derivatives, the docking program by computational modeling was also performed.

2. Design and synthesis of bis- and bifunctional HupB derivatives

Should HupB be a lead compound for AD drug candidate, it will be necessary first to enhance inhibitory potency of the derivatives to AChE. The design of novel inhibitors derived from HupB should bind to the dual-site of the enzyme and, further, the structures of the derivatives will be optimized for improving their pharmacological properties.

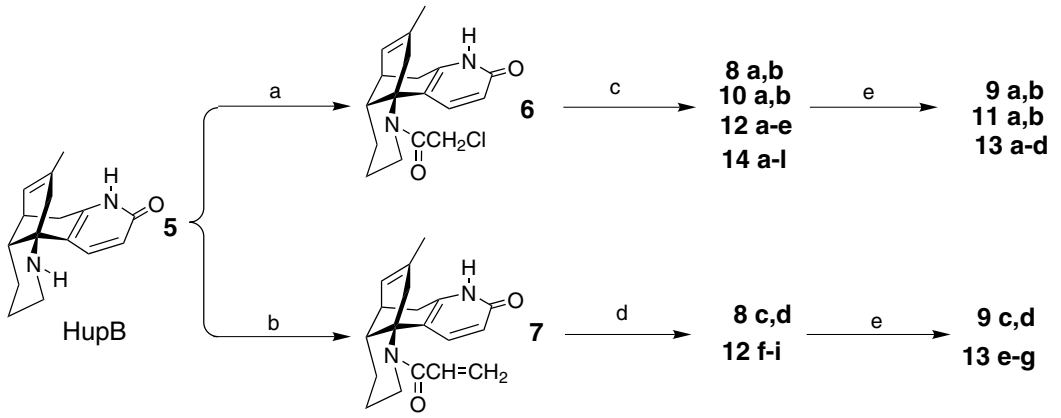
Several methods were tried during the preparation of bis-HupB derivatives. The reaction of HupB with α,ω-dihaloalkanes was carried out in the presence of triethylamine, but obtained products were very complicated. Reductive-amination of α,ω-alkanedialdehydes with HupB was implemented, which successfully delivered several homodimers of HupA,[16] and failed in preparing the bis-HupB derivatives.

Despite the steric hindrance of the secondary amino group and a few reactive places in the HupB molecule, we have successfully developed the procedures to prepare the derivatives of HupB, which was first acylated with chloroacetyl chloride or acryloyl chloride to afford chloroacetyl HupB **6** and acryloyl HupB **7** in high yield, respectively.[25,26]

In addition, after reaction of 4-chloro-butyryl chloride with HupB under the same conditions, the only product was identified to be O-acylated product on the pyridone ring of HupB molecule. Based on our experience of HupB derivatives, all derivatives from O-substituted on the pyridone ring of HupB were to decrease the inhibition. Furthermore, X-ray structure of *Tc*AChE complexed with HupB has demonstrated the pyridone moiety was responsible for its key interaction with active site via hydrogen bonding, and possibly via C–H···π interaction.[27]

Therefore, the acylated HupB derivatives **6** and **7** provided two key intermediates for preparation of these derivatives of bis- and bifunctional HupB in our research as shown in Scheme 1. As regards the spacer tether, we reasoned that a linking tether bearing N atoms might have the chance to favorably interact with some aromatic residues lining the wall of the gorge in the enzyme.[28]

At first, tethers with two N atoms were used to link two molecules of HupB together, a series of derivatives of bis-HupB were prepared. Reaction of 2 equiv of **6** and 1 equiv of piperazine or homopiperazine was carried out, in presence of potassium carbonate and potassium iodide, to afford derivatives **8a** and **8b**. The derivatives **8c** and **8d** were obtained by Michael addition, catalyzed by silica gel, of piperazine or homopiperazine to acryloyl HupB **7**. We found that silica gel played a very important role in the 1,4-Michael addition of a secondary amine to the acrylamide derivatives. The amide groups



Scheme 1. The preparation of derivatives of bis- and bifunctional HupB. Reagents and conditions: (a) chloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C, 1 h, 96.5%; (b) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, 95.1%; (c) prim-amine or sec-diamine for bis-HupB and sec-monoamine for bifunctional HupB derivatives, K_2CO_3, KI, CH_3CN or $CHCl_3$, refluxing, 28–90%; (d) sec-diamine, silica gel, CH_3CN, refluxing, 24 h, 56–95%; (e) $LiAlH_4$, THF, refluxing 2 h, 29–68%.

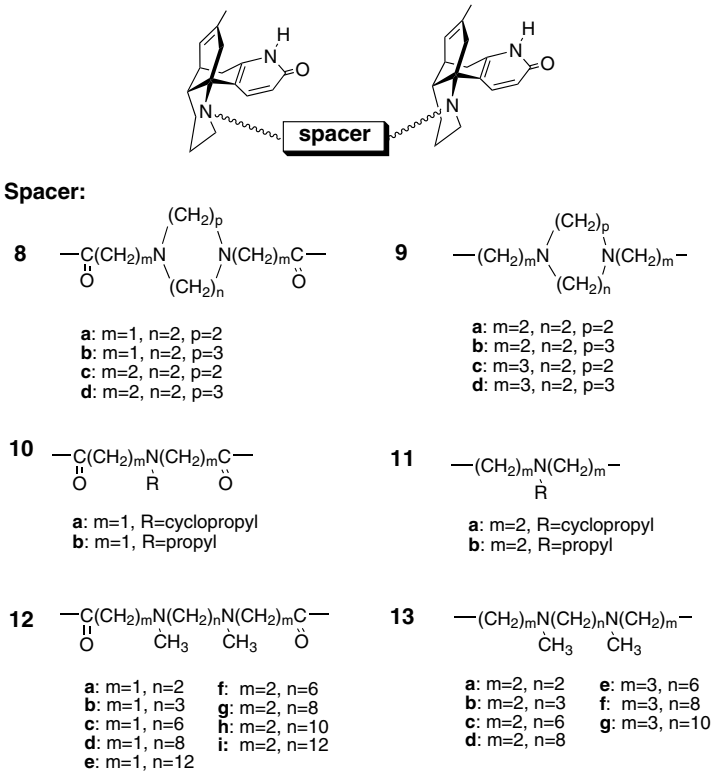


Figure 2. The structure of bis-HupB derivatives linked by various spacer.

in **8** series were reduced with LAH to produce corresponding derivatives **9a**, **9b**, **9c**, and **9d**, respectively (Fig. 2).

Under the same reaction conditions, cyclopropyl amine and propylamine were treated with **6** to produce derivatives **10a** and **10b**, providing bis-HupB derivatives with special tether with one N atom. Further, corresponding reductive products **11a** and **11b** were afforded, respectively.

The preliminary pharmacological tests of the bis-HupB **8**–**11** series showed that the inhibitory activities of AChE were evidently increased in comparison with HupB. In order to search out more effective inhibitors binding to the dual-site of AChE, we have noted that the tether length with the potency enhancement varied with different kinds of AChE inhibitors. Furthermore, we prepared bis-HupB derivatives **12** and **13** series with different tether for exploring the structure–activity relationships among the bis-HupB derivatives.[25]

Reaction of α,ω-*N*,*N*′-dimethyl alkylenediamines (1 equiv) with chloroacetyl HupB **6** (2 equiv) proceeded smoothly, a series of bis-HupB **12a**–**e** were afforded in 28–81% yield. In addition, Michael addition of

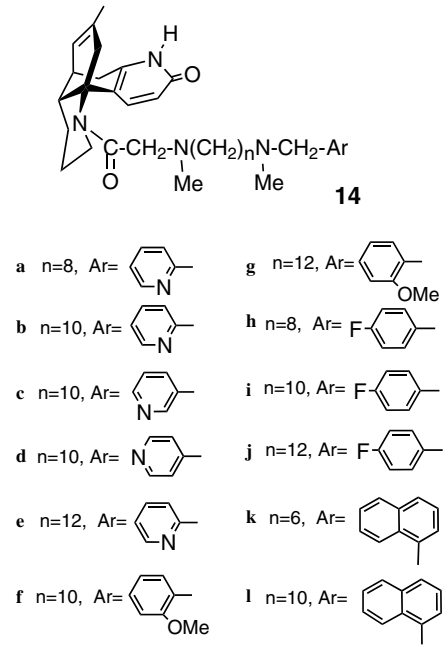


Figure 3. The structures of bifunctional HupB derivatives.

α,ω-*N*,*N*′-dimethyl-alkylenediamines (1 equiv) to acryloyl HupB **7** (2 equiv) was catalyzed by silica gel in refluxing acetonitrile to furnish bis-HupB **12f–i** in 56–95% yields. These amide groups in **12** series of bis-HupB derivatives were subsequently reduced with LAH to produce corresponding bis-HupB **13** series of derivatives, respectively. The reductive yields were about 29–59%.

Meanwhile, we also pursued another series of derivatives, namely the bifunctional HupB, which were designed more reasonably from modification of the molecular structure for optimization of the pharmacological activities.

Based on our initial work of bifunctional HupB derivatives, the novel derivatives **14a–h** with longer tether shown in Figure 3 were prepared as compared with the previous derivatives.[26] The bifunctional HupB derivatives characterized that HupB moiety connected through a tether chain with terminal aromatic ring, since a π-system moiety of the ligand had an important feature for binding to the peripheral sites of AChE.

A little different way was used to obtain bifunctional HupB from bis-HupB derivatives. At first, α,ω-*N*,*N*′-dimethyl alkylenediamines reacted with substitute arylmethyl chloride to give *N*-*N*′-dimethyl-*N*-arylmethyl alkylenediamines, which were then treated with **6**, respectively (see Scheme 1) to produce **14a–l** in 41–88% yields.

3. AChE inhibition results and SAR discussion

These HupB derivatives have been assayed for their AChE (rat cortex homogenate) and, partially, BuChE (rat serum) inhibition potency using the Ellman method with slight modification.[29] The pharmacological inhibitory results of bis-HupB are shown in Table 1.

All of the bis-HupB derivatives have displayed more potent and selective AChE inhibition than its parental HupB. The evaluation of the pharmacological results from between **8** and **9**, **10** and **11**, and particularly, **12** and **13** series revealed that there was no substantial difference of the inhibitory activities between the amide and corresponding amine functional group of the derivatives, which linked two HupB moieties in same number of atoms, respectively.

Further, the AChE inhibition potency of the HupB derivatives varied evidently with the length of the tether chain, which, however, was insensitive to BuChE inhibition. Consequently, the variation of the length of the tether chain among the HupB derivatives should efficiently affect the interaction on binding the dual-site in AChE.

Although bis-HupB derivatives **8–11** had increased inhibitory activities and selectivity in comparison with parental HupB, the tether length of those initial derivatives was too short to bind well the dual-site of the enzyme.

Among **12** and **13** series, particularly, bis-HupB derivatives with 12–20 atom tether (**12c–i** and **13c–g**) displayed significantly high potent inhibition of AChE, the inhibitory activity enhanced about two to three orders of magnitude than parental HupB. The most potent derivative **12h** exhibited about 1635-fold increase in AChE inhibition and 459-fold greater selectivity.

The spacer tether in bis-HupB derivatives was different from other bis-molecules inhibitors. Interestingly, the optimal tether length among our bis-HupB derivatives was a chain of 18 atoms, which was longer than other bis-molecule AChE inhibitors.[14–19] The heptylene-linked bis-tacrine was found to be the most potent and selective inhibitor of AChE in the series of bis-tacrine derivatives. A length of eight to ten methylenes is favored in the bis-galanthamine series. Among the bis-HupA derivatives, the most favorable tether length was 7 atoms, but the inhibitory potency of the HupA dimers was less than that of HupA itself.[16] The optimal tether length of AChE inhibitory dimer of 5-amino-2(1H)-tetrahydroquinolinones in the *S*,*S*-series configuration was 10 methylene to *Tc*AChE and 12 methylene to rat AChE.[18] An intuitive reason inferred that the endocyclic secondary amino group in the bridge-ring HupB molecule possessed the special steric hindrance and, therefore, the longer tether chain was needed for optimal binding to AChE.

The inhibition results of derivatives of bifunctional HupB **14a–l** are shown in Table 2. It is noteworthy that all derivatives displayed more potent from 62- to 914-fold enhancement in AChE inhibition than HupB itself. The novel **14a–l** bifunctional derivatives are more potent than that of our previous bifunctional HupB derivatives,[26] among latter the spacers from HupB moiety to aromatic group were too short. The AChE

Table 1. Cholinesterase inhibition and selectivity of derivatives of bis-HupB[a]

Compound	Atom numbers of tether	AChE[b] (IC_{50}, nM)	BuChE[c] (IC_{50}, nM)	Selectivity for AChE[d]
HupA	—	72.4 ± 3.8	70,200 ± 800	970
HupB	—	19,300 ± 174	228,000 ± 600	12
8a	8	1173 ± 137	>184,000	>157
8b	8	204 ± 41	171,000 ± 700	838
9a	8	407 ± 26	192,000 ± 500	472
9b	8	218 ± 24	125,000 ± 3200	573
8c	10	170 ± 32	—[e]	—
8d	10	179 ± 5.0	—	—
9c	10	202 ± 8	—	—
9d	10	332 ± 52	—	—
10a	5	1002 ± 100	—	—
10b	5	157 ± 39	—	—
11a	5	695 ± 13.4	—	—
11b	5	1005 ± 76	—	—
12a	8	1020 ±38.5	—	—
12b	9	471 ± 52	60,000 ± 3000	127
12c	12	76.2 ± 4.3	64,500 ± 4500	846
12d	14	50.8 ± 1.9	65,400 ± 2400	1287
12e	18	53.4 ± 8.0	—	—
12f	14	35.9 ± 7.5	67,900 ± 3800	1891
12g	16	18.5 ± 6.2	63,000 ± 2500	3405
12h	18	11.8 ± 1.6[f]	65,000 ± 2300	5508
12i	20	23.1 ± 4.2	88,400 ± 3500	3827
13a	8	3480 ± 135	—	—
13b	9	416 ± 28	—	—
13c	12	78.4 ± 5.4	—	—
13d	14	20.1 ± 3.2	—	—
13e	14	33.6 ± 1.1	—	—
13f	16	40.6 ± 1.7	—	—
13g	18	26.2 ± 5.0	—	—

[a] Assay performed by the modified Ellman method at pH 7.4.[29] Results are the mean ± SD.
[b] Assay performed using rat cortex homogenate in aqueous solution at pH 5.
[c] Assay performed using rat serum, values are means of three different experiments.
[d] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).
[e] Not determined.
[f] This potency of inhibition was somewhat less than previous report in Ref. 25.

Table 2. ChE inhibition and selectivity of bifunctional HupB derivatives[a]

Compound	Atom numbers of tether	AChE (IC_{50}, nM)[b]	BuChE (IC_{50}, nM)[c]	Selectivity BuChE/AChE[d]
(−)-HupA		72.4 ± 3.8	70,200 ± 800	970
(−)-HupB		19,300 ± 174	228,000 ± 600	12
14a	13	41.2 ± 2.2	—[e]	—
14b	15	42.0 ± 1.7	—	—
14c	15	38.2 ± 3.2	12,900 ± 1000	338
14d	15	50.0 ± 3.7	—	—
14e	17	65.8 ± 5.6	—	—
14f	15	21.1 ± 1.4	8650 ± 810	410
14g	17	62.2 ± 8.1	—	—
14h	13	56.5 ± 2.0	—	—
14i	15	52.0 ± 5.1	3480 ± 630	67
14j	17	309 ± 84	—	—
14k	11	124 ± 25	—	—
14l	15	121 ± 23	—	—

[a] Assay performed by the modified Ellman method at pH 7.4.[29] Results are the mean ± SD.
[b] Assay performed using rat cortex homogenate in aqueous solution at pH 5.
[c] Assay performed using rat serum, values are means of three different experiments.
[d] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).
[e] Not determined.

inhibition indicated that the optimal length of the tether among novel **14** series derivatives was 15 atoms with decamethylenediamine between HupB and the aromatic group.

Among these bifunctional HupB derivatives, the most potent and selective compound was **14f** ($n = 10$), which was 914-fold more potent and 34-fold more selective in the inhibition of AChE than parental HupB.

The inhibitory activities were almost at same level of effect between the bis-HupB and bifunctional derivatives. However, the bifunctional derivatives of HupB are possibly more reasonable and potential candidates for AD drug than Bis-HupB derivatives.

4. Pharmacological effects of the derivative 12h

The bis-HupB derivative **12h** was taken as a typical sample and has been evaluated for its pharmacological effects on AChE inhibition, cognitive enhancement, and neuroprotection in vitro and in vivo. Spectrophotometrical methods were used to determine cholinesterase (ChE) activity, cell viability, antioxidants and lipid peroxidation. Mice water maze performance was used to evaluate the effects of **12h** on the acquisition and memory impairment. Apoptosis was determined by DAPI staining under a fluorescence microscope.

Based on IC_{50} ratios, the inhibition of **12h** on serum BuChE was much less potent than donepezil, and showed more pronounced selective inhibition on AChE than donepezil and HupB. On an equimolar basis, oral administration of **12h** showed threefold more and twofold less potent than donepezil on AChE and BuChE inhibition, respectively, with longer duration of inhibition than donepezil and HupB. There was no significant difference in AChE and BuChE inhibition between po and ip administrations. A Lineweaver–Burk plot for **12h** indicated a pattern of inhibition of brain AChE of the mixed competitive type, as the intersection of the lines occurred in the second quadrant. Mice water maze studies showed that **12h** remarkably improved the spatial performance deficits induced by scopolamine and transient cerebral ischemia/reperfusion, respectively. As determined by MTT reduction and morphological observation, **12h** attenuated Aβ-induced cytotoxicity in PC12 cells. In addition, **12h** significantly ameliorated Aβ-induced redox disequilibrium, as shown by increasing glutathione peroxidase (GSH-Px) activity, and decreasing superoxide dismutase (SOD) activity and malondialdehyde (MDA) level. We found that **12h** was more potent than parental HupB on neuroprotection against oxygen-glucose deprivation in PC12 cells. As evaluated by DAPI staining, apoptosis induced by staurosporine in PC12 cells was significantly ameliorated by **12h**.

Therefore, **12h** as a novel bis-HupB derivative was a potent, reversible, and highly selective AChE inhibitor and it could remarkably improve the acquisition and memory deficits. Its cytoprotective effects may contribute to these beneficial effects. These results suggest that bis-HupB derivatives could be a promising candidate in the palliative treatment of AD. The detailed pharmacological results of the bis-HupB derivative **12h** will be submitted to a biological magazine.

5. Docking study for derivatives 12 series

To explain the interaction modes of the bis-HupB derivatives to AChE, molecular docking simulations for derivatives **12a**–**12h** to *Tc*AChE were performed employing the program DOCK4.0[30,31] based on the X-ray crystal structure of *Tc*AChE-HupB complex[27] (PDB entry 1GPN). The binding gorge of *Tc*AChE composed of the central catalytic pocket and peripheral sites was taken as the binding site for docking. There were two conformations of Phe330 in the binding sites and conformation A occupies 65% of the conformations. Consequently, we used conformation A of Phe330 during the docking simulations. Each bis-HupB derivative was docked into the binding site flexibly, meanwhile the structure of *Tc*AChE was fixed. The two sp^3 N atoms in bis-HupB derivatives were both protonized during the docking simulations. The maximum-orientations and configurations-per-cycle for docking were set to 600 and 150, respectively. For each bis-HupB inhibitor, conformation with the lowest interaction energy was taken out for further analysis. Binding free energy between each inhibitor and AChE was predicted using the scoring function encoded in the program Autodock3.0.[32]

The docking results showed that different bis-HupB derivatives could bind with the central pocket and peripheral sites simultaneously, as shown in Figure 4A. Obviously, all the HupB moieties had a nice fit in the central pocket as the same mode that HupB adopted in the crystal structure (1GPN); meanwhile, varied interaction way with the peripheral site would be taken by the bis-HupB derivatives containing the tethers of varied lengths. In the central pocket, all the HupB moieties contacted with Phe330 and Trp84 through C–H$\cdots\pi$ interaction,[27] and formed a hydrogen bond with residue Tyr130, as shown in Figure 5.

The cation–π interaction with Trp279 was a major component for the interactions of some positive bifunctional AChE inhibitors with peripheral sites. However, only if the tether contains proper atoms, the bis-HupB derivatives could adopt the cation–π interaction with Trp279. The distance between outer protonated N and the centroid of the Trp279 aromatic ring (D1) is listed in Table 3. When the tether contains less than 12 atoms, such as **12a** and **12b**, the D1 was too long to form the cation–π interaction with Trp279 and the bis-HupB contacted with the peripheral site with hydrophobic interaction, as shown in Figure 4B. While the tether contains atoms from 12 to 14, such as **12c**, **12d**, and **12f**, the bis-HupB derivatives could adopt the cation–π interaction with Trp279 for the proper D1. Meanwhile, the bis-HupBs could form a hydrogen bond with Asp285 and hydrophobic interactions with Ser286, Ile287, and Tyr334 in the peripheral sites, as shown in Figure 4C. When the tether exceeded 14 atoms, such as **12e**, **12g**, **12h**, and **12i**, the D1 became longer again. Thus, the bis-HupB could not form effective cation–π interaction with Trp279, while they could form a hydrogen bond with Asp280 and hydrophobic contact with Trp279, Leu282, and Ile279, as shown in Figure 4D.

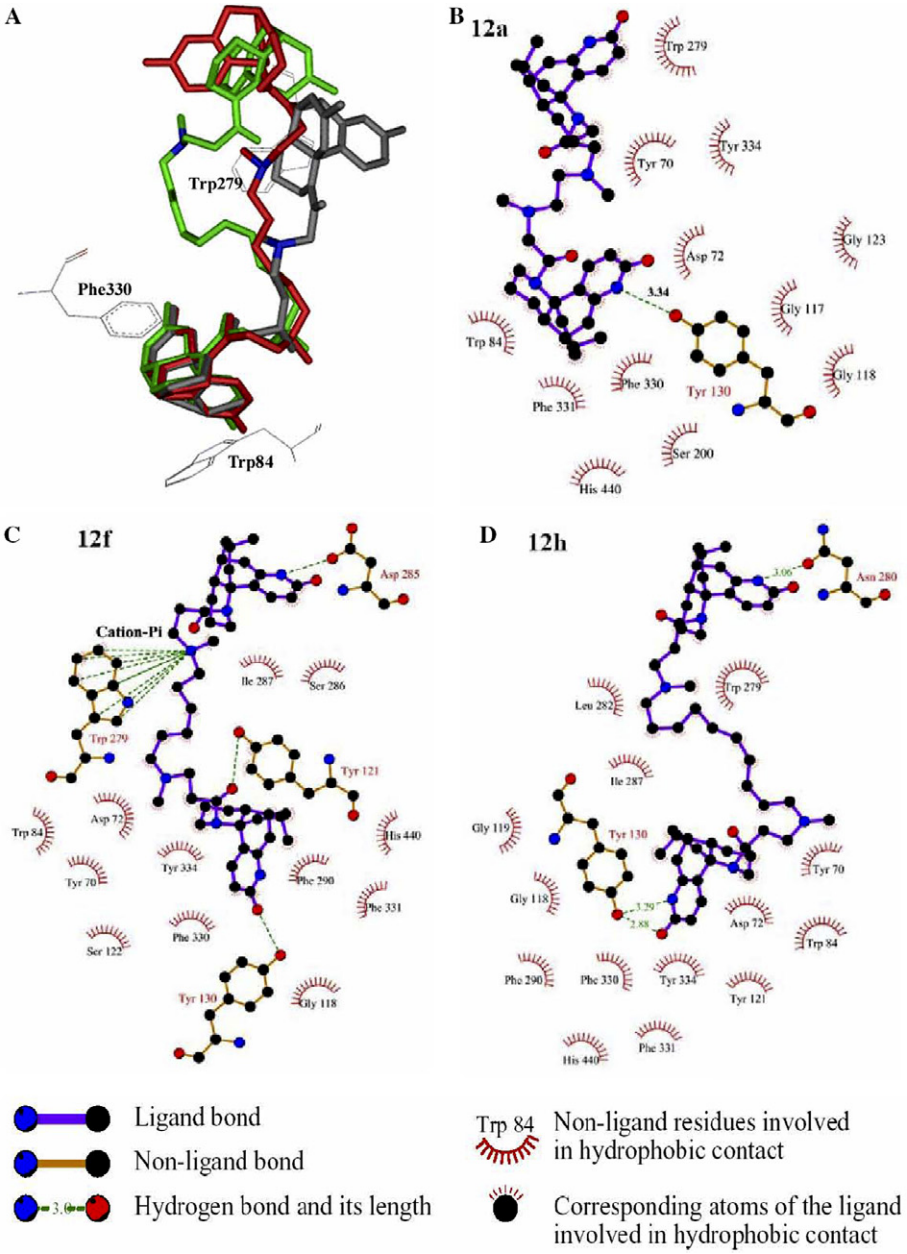


Figure 4. The binding modes of bis-HupB derivatives of **12** series in both central and peripheral sites. (A) The bis-HupBs with varied length are shown in stick model: **12a** in gray color, **12f** in red color and **12h** in green color. (B–D) was generated with Ligplos.[33]

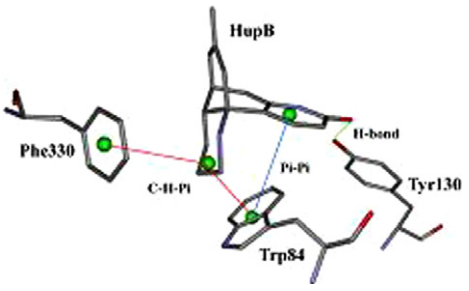


Figure 5. The interaction mode of HupB moiety of bis-HupB derivatives in central catalytic pocket.

Based on the binding mode analysis, in spite of the same interaction way in central pocket, the bis-HupB derivatives adopted different binding mode in the peripheral sites according to the varied lengths of the tethers. The simultaneous interaction with the central and peripheral sites caused the bis-HupB derivatives to have lower binding free energies, which contributed to the much more high binding potency than HupB.

The docking studies have revealed the simultaneous interactions of some bis-HupB derivatives containing favorite spacer and optimal tether in the central pocket, gorge, and peripheral sites of *Tc*AChE highly improved the inhibitory potency of AChE. Consequently, the bis-HupB with the optimal tether containing 18 atoms has the most favorable binding ability.

Table 3. The distance between outer N^+ and the centroid of Trp279

Bis-HupB	12a	12b	12c	12d	12f	12g	12e	12h	12i
Tether[a]	8	9	12	14	14	16	18	18	20
D1[b] (Å)	6.85	6.47	4.74	4.14	3.87	5.68	5.69	5.25	5.42

[a] The atom numbers of the tethers between two HupB moieties.
[b] The distance from the outer protonated N to the centroid of the aromatic ring of Trp279.

6. Conclusions

On the basis of the dual-site binding strategy in rational design of AChE inhibitors, natural HupB was chosen as a lead compound to synthesize a series of derivatives of bis- and bifunctional HupB. All bis-HupB and bifunctional HupB derivatives exhibited significantly more potent inhibition of AChE as compared with its parental HupB. The bis-HupB derivatives were able to bind simultaneously to both the catalytic and peripheral dual-site of AChE in different level, supported by the docking research. Moreover, the peripheral inhibition had potential to prevent the Aβ aggregation, which will be further proved. The important role of the dual-site suggested that properly designed AChE inhibitors, which not only palliative AD symptoms but also might be able to act as disease-modifying agents.

The preliminary pharmacological results in vitro and in vivo suggested that the bis-HupB derivative **12h** could meet the basic criteria for a promising AD drug candidate. However, the bifunctional HupB derivatives designed are more reasonable and practical, further seeking the AD candidates from fine-tuning of molecular structure of the bifunctional HupB derivatives is underway in our laboratory.

7. Experimental

7.1. Chemical preparation

The ^{1}H NMR spectra were recorded on Bruker AMX 400 MHz NMR spectrometer. The NMR data were reported in parts per million relative to TMS or referenced to the solvent in which they were run. Melting points (uncorrected) were determined on a Buchi-510 capillary apparatus. IR spectra were recorded on Nicolet Magna IR 750 spectrometers. The MS(ESI) and HRMS (ESI) spectra were obtained on an Finnigan MAT 95 mass spectrometer, EI: 70 eV. The optical rotation value $[\alpha]_D$ was determined with Perkin-Elmer 241 (589 nm). The solvent was removed by rotary evaporation under reduced pressure, and flash column chromatography was performed on silica gel (200–300 mesh). Anhydrous solvents were obtained by distilling from sodium wire. Column chromatography was performed on silica gel (200–300 mesh).

7.1.1. *N*-Chloroacetyl-HupB (6). Chloroacetyl chloride (0.20 mL, 2.5 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a stirred solution of HupB (256 mg, 1.0 mmol) and Et_3N (0.27 mL, 2.5 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was stirred at 0 °C for 1.0 h and then at rt for 10 min. A saturated solution of NH_3 in CH_3OH (1 mL) was added. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL) and then washed with brine (20× 3 mL). After drying over anhydrous Na_2SO_4, the organic layer was concentrated. The residue was purified by flash column chromatography (silica gel, $CHCl_3/CH_3OH$ = 100/9) to give the product as solid 321 mg (Y: 96.5%), mp > 204 °C (dec). $[\alpha]_D^{20}$ 150° (*c* 0.92, $CHCl_3$). ^{1}H NMR(400 MHz, $CDCl_3$) δ 13.11 (1H, br s), 7.49 (1H, d, J = 9.5 Hz), 6.42 (1H, d, J = 9.5 Hz), 5.40 (1H, d, J = 4.6 Hz), 4.19 (1H, d, J = 12.5 Hz), 4.10 (1H, d, J = 12.5 Hz), 3.64 (1H, m), 3.42 (1H, d, J = 17.7 Hz), 2.89 (1H, dd, J = 18.0, 5.2 Hz), 2.67 (1H, m), 2.58 (1H, d, J = 17.7 Hz), 2.45 (1H, d, J = 18.3 Hz), 2.38 (1H, m), 1.96 (1H, m), 1.62–1.77 (3H, m), 1.55 (3H, s), 1.32 (1H, m); EIMS (*m*/*z*) 334 (M^+, 14%), 332 (M^+, 42%), 318, 297 (100%), 255, 225, 210, 149, 109, 83, 72.

7.1.2. *N*-Acryloyl-HupB (7). Acryloyl chloride (0.24 mL, 2.5 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a stirred solution of HupB (256 mg, 1.0 mmol) and Et_3N (0.27 mL, 2.5 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then at rt for 10 min. A saturated solution of NH_3 in CH_3OH (1 mL) was added. The mixture was filtered, and the filtrate was concentrated. The residue was dissolved in $CHCl_3$ (30 mL) and treated with NH_3/CH_3OH (2 mL). The mixture was stirred for 12 h and washed with brine (20× 3 mL). After drying over anhydrous Na_2SO_4, the organic layer was concentrated. The residue was purified by flash column chromatography (silica gel, $CHCl_3/CH_3OH$ = 100:9) to give the product as yellow solid 295 mg (Y: 95.1%), mp >250 °C (dec). $[\alpha]_D^{25}$ 228.7° (*c* 0.95, $CHCl_3$). IR(KBr): 3431, 2926, 1655, 1610, 1412, 1250, 1171, 1107, 833, 534 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.09 (1H, br s), 7.64 (1H, d, J = 9.3 Hz), 6.52 (1H, dd, J = 10.4, 16.8 Hz), 6.44 (1H, d, J = 9.3 Hz), 6.36 (1H, dd, J = 1.7, 10.3 Hz), 5.67 (1H, dd, J = 1.7, 10.3 Hz), 5.42 (1H, d, J = 5.5 Hz), 3.74 (1H, d, J = 14.4 Hz), 3.38 (1H, d, J = 17.7 Hz), 2.91 (1H, dd, J = 5.5, 17.6 Hz), 2.38–2.66 (3H, m), 1.96 (2H, dd, J = 17.9, 5.5 Hz), 1.53–1.67 (7H, m); EIMS (*m*/*z*): 310 (M^+, 100%), 295, 255, 198, 173, 83.

7.1.3. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-piperazine (8a). To a solution of **6** (134 mg, 0.4 mmol) and piperazine (17.2 mg, 0.2 mmol) in $CHCl_3$ (20 mL), K_2CO_3 (138 mg) and KI (10 mg) were added. The mixture was refluxed for 24 h, then cooled to rt, filtered, and evaporated. The residue was purified by silica gel flash column chromatography with $CHCl_3/CH_3OH$ (10:1) to afford **8a**, white solid 99 mg (Y: 73%), mp

>220 °C (dec). $[\alpha]_D^{25}$ 107.3° (*c* 1.0, $CHCl_3$). IR(KBr) 3419, 2928, 1655, 1610, 1544, 1408, 1306, 1107, 839, 634 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 12.09 (2H, br s), 7.58 (2H, d, J = 9.6 Hz), 6.42 (2H, d, J = 9.3 Hz), 5.42 (2H, br s), 3.91 (2H, m), 3.37–3.50 (4H, m), 3.08 (2H, d, J = 14.0 Hz), 2.92 (2H, m), 2.32–2.72 (16H, m), 1.91 (2H, m), 1.60–1.82 (6H, m), 1.58 (6H, s) 1.35 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5 × 2, 165.4 × 2, 142.8 × 2, 142.1 × 2, 133.3 × 2, 123.8 × 2, 118.2 × 2, 117.1 × 2, 64.7 × 2, 61.4 × 2, 52.9 × 2, 45.5 × 2, 44.8 × 2, 40.7 × 2, 34.5 × 2, 29.6 × 2, 28.8 × 2, 26.2 × 2, 25.6 × 2, 22.9 × 2; ESIMS (*m*/*z*) 679.5 $(M+H)^+$, 395.3, 378.2, 338.1, 257.1.

7.1.4. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-homopiperazine (8b). The derivative **8b** was prepared from**6** and homopiperazine according to procedure **8a** as yellow solid in 90% yield. mp >220 °C (dec). $[\alpha]_D^{25}$ −3.5° (*c* 1.35, $CHCl_3$). IR(KBr) 3427, 2928, 1655, 1556, 1406, 1186, 1107, 833, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.90 (2H, br s), 75.6 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.5 Hz), 5.42 (2H, d, J = 4.3 Hz), 3.91 (2H, d, J = 11.4 Hz), 3.42 (4H, m, J = 14.7, J = 17.9 Hz), 3.28 (2H, d, J = 14.1 Hz), 2.79–2.92 (8H, m), 2.39–2.58 (8H, m), 1.91 (4H, m), 1.57–1.66 (10H, m), 1.21–1.39 (6H, m); ESIMS (*m*/*z*) 693.5 $(M+H)^+$.

7.1.5. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinocarbonylethyl)-piperazine (8c). To a solution of piperazine (27.7 mg, 0.322 mmol) and **7** (200 mg, 0.65 mmol) in acetonitrile (20 mL), silica gel (100 mg, 200–300 mesh) was added. The reaction mixture was refluxed for 24 h and then cooled to rt. After removal of the solvent, the residue was diluted with $CHCl_3$ (40 mL). The silica gel was filtered off, and the organic filtrate was washed with brine (10× 2 mL), dried with anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, $CHCl_3/CH_3OH/NH_4OH$ = 100:10:1) to afford product **8c** as pale solid 40 mg (Y: 62%), mp >250 °C (dec). $[\alpha]_D^{25}$ 147.4° (*c* 0.9, $CHCl_3$). IR(KBr) 3431, 2933, 1657, 1612, 1556, 1460, 1402, 1171, 1107, 831 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.95 (2H, br, s), 7.55 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.5 Hz), 5.41 (2H, t, J = 0.4, 4.5 Hz), 3.72 (2H, q, J = 14.0, 7.0 Hz), 3.38 (2H, d, J = 18.1 Hz), 2.37–2.93 (20H, m), 1.93 (2H, d, J = 12.5 Hz), 1.52–1.70 (14H, m), 1.22–1.35 (6H, m); EIMS (*m*/*z*) 503, 396, 310, 255 (100%), 174, 146; ESIMS (*m*/*z*) 707.4 $[M+H]^+$, 729.5 $[M+Na]^+$.

7.1.6. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinocarbonylethyl)-homopiperazine (8d). The derivative **8d** was prepared from**7** and homopiperazine according to procedure **8c** as colorless oil in 63% yield. $[\alpha]_D^{25}$ 119.1° (*c* 0.98, $CHCl_3$). IR(KBr) 3423, 2929, 1664, 1612, 1556, 1437, 1252, 1171, 1107, 833, 752, 635, 532 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.04 (2H, br s), 7.49 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.5 Hz), 5.40 (2H, d, J = 4.5 Hz), 3.70 (2H, d, J = 13.4 Hz), 3.34 (2H, d, J = 17.7 Hz), 2.76–3.09 (14H, m), 2.54–2.63 (4H, m), 2.37–2.47 (4H, m), 1.91–2.02 (4H, m), 1.56–1.65 (12H, m), 1.24–1.34 (6H, m); ESIMS (*m*/*z*) 721.5 $(M+H)^+$, 743.4 $(M+Na)^+$, 707.5.

7.1.7. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinoethyl)-piperazine (9a). A mixture of $LiAlH_4$ (23 mg, 0.60 mmol) and **8a** (99 mg, 0.15 mmol) in THF (10 mL) was refluxed for 2 h. After cooling to rt, to the mixture, in turn, H_2O (0.02 mL), 15% NaOH (0.06 m), and H_2O (0.06 mL) were added, then stirred for additional 30 min. The suspension solution was added with anhydrous Na_2SO_4 and stirred for 2 h and then filtered under reduced pressure. The filtrate was concentrated and the resulting residue was purified on preparative TLC ($CHCl_3/CH_3OH$ = 15:1) to afford **9a** as white solid 65 mg (Y: 8%). Mp >230 °C (dec). $[\alpha]_D^{25}$ 52.4 (*c* 1.0, $CHCl_3$). IR(KBr) 3419, 2925, 1658, 1604, 1552, 1457, 1299, 1112, 833, 638 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 11.95 (2H, br s), 7.63 (2H, d, J = 9.3 Hz), 6.42 (2H, d, J = 9.6 Hz), 5.47 (2H, d, J = 4.1 Hz), 2.84 (2H, dd, J = 17.6, 4.9 Hz), 2.46–2.72 (8H, m), 2.38–2.52 (8H, m), 2.28–2.36 (10H, m), 2.05 (2H, d, J = 16.5 Hz), 1.81 (2H, m), 1.58 (6H, s), 1.48–1.72 (4H, m), 1.42 (2H, m), 1.28 (2H, m). ^{13}C NMR (100 MHz,$CDCl_3$) δ 163.8 × 2, 142.5 × 2, 141.2 × 2, 131.5 × 2, 124.8 × 2, 120.4 × 2, 116.7 × 2, 59.9 × 2, 57.0 × 2, 52.6 × 4, 47.2 × 2, 46.8 × 2, 43.5 × 2, 36.9 × 2, 33.6 × 2, 28.7 × 2, 25.1 × 2, 23.5 × 2, 22.1 × 2; ESIMS (*m*/*z*) 651.4 $(M+H)^+$, 395.2, 283.2.

7.1.8. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinoethyl)-homopiperazine (9b). The derivative **9b** was prepared from**8b** according to procedure **9a** as yellow solid in 51% yield. Mp 202–204 °C. $[\alpha]_D^{25}$ −3.5° (*c* 1.35, $CHCl_3$). IR 3419, 2925, 1658, 1604, 1552, 1457, 1299, 1112, 833 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.91 (2H, br s), 7.63 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.5 Hz), 5.45 (2H, d, J = 4.5 Hz), 3.30 (2H, m), 2.86 (10H, m), 2.61 (6H, m), 2.33–2.47 (8H, m), 1.71–2.02 (10H, m), 1.50–1.65 (6H, m), 1.40 (2H, d, J = 10.1 Hz), 1.17–1.25 (4H, m). ^{13}C NMR (100MHz, $CDCl_3$) δ 165.1 × 2, 143.4 × 2, 141.1 × 2, 131.8 × 2, 125.6 × 2, 120.0 × 2, 117.4 × 2, 60.4 × 2, 57.4 × 2, 55.3 × 2, 54.7 × 2, 48.0 × 4, 44.2 × 2, 37.9 × 2, 34.0 × 2, 29.5 × 3, 25.7 × 2, 24.5 × 2, 23.1 × 2; ESIMS (*m*/*z*) 665.5 $[M+H]^+$, 409.4, 283.3.

7.1.9. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinopropyl)-piperazine (9c). The derivative **9c** was prepared from**8c** according to procedure **9a** as white solid in 31% yield. Mp >260 °C (dec). $[\alpha]_D^{25}$ −12.4° (*c* 1.35, $CHCl_3$). ^1H NMR (400 MHz,$CDCl_3$) δ 12.21 (2H, b, s), 7.66 (2H, d, J = 9.4 Hz), 6.40 (2H, d, J = 9.4 Hz), 5.43 (2H, d, J = 5.0 Hz), 3.10 (2H, m), 2.81 (18H, m), 1.94 (2H, d, J = 16.8 Hz), 1.50–1.81 (22H, m), 1.38 (2H, d, J = 12.3 Hz), 1.16–1.26 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 × 2, 143.0 × 2, 141.5 × 2, 132.0 × 2, 125.3 × 2, 120.8 × 2, 117.1 × 2, 57.4 × 2, 56.0 × 2, 52.9 × 2, 47.4 × 2, 47.2 × 2, 44.0 × 2, 37.2 × 2, 34.0 × 2, 29.5 × 2, 29.3 × 2, 28.6 × 2, 25.7 × 2, 23.8 × 2, 23.0 × 2. IR: 3427, 1657, 1606, 1554, 1462, 1437, 1375, 1109, 825, 752, 521 cm^{-1}; ESIMS (*m*/*z*) 679.7 $[M+H]^+$, 701.7 $[M+Na]^+$, 423.6, 297.4.

7.1.10. *N*,*N*′-Bis(1-oxo-8,15-didehydrolycodinopropyl)-homopiperazine (9d). The derivative **9d** was prepared from**8d** according to procedure **9a** as colorless oil in 30% yield. $[\alpha]_D^{25}$ −16.5° (*c* 0.65, $CHCl_3$). IR(KBr) 3419, 2928, 1655, 1603, 1551, 1439, 1111, 833, 750 cm^{-1}. ^1H

NMR (400 MHz, $CDCl_3$) δ 12.88 (2H, br s), 7.62 (2H, br s), 6.43 (2H, d, J = 7.5 Hz), 5.44 (2H, d, J = 3.9 Hz), 2.81–3.44 (16H, m), 2.63 (2H, m), 2.25–2.43 (8H, m), 1.22–2.04 (26H, m); ESIMS (m/z) 693.7 $(M+H)^+$, 437.7.

7.1.11. *N,N*-Bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-cyclopropylamine (10a). To a solution of **6** (30 mg, 0.09 mmol) and cyclopropylamine (3 mg, 0.05 mmol) in CH_3CN (2 mL), K_2CO_3 (25 mg, 0.09 mmol) and KI (5 mg) were added, the mixture was stirred at 45–50 °C for 20 h. After cooling to rt, the solution was evaporated. The residue was dissolved in solution of $CHCl_3$ (5 mL) and CH_3OH (0.5 mL), then the mixture was filtered. The filtrate was washed with brine (2× 3 mL), dried over anhydrous Na_2SO_4, and concentrated. The resulting residue was purified on preparative TLC (CH_3Cl/CH_3OH = 10:1) to give **10a** as pale oil 15 mg (Y: 52%). $[\alpha]_D^{25}$ 123.5° (c 0.75, $CHCl_3$). IR(KBr) 3427, 2926, 1660, 1610, 1404, 1107, 833, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.03 (2H, br s), 7.56 (2H, d, J = 8.0 Hz), 6.41 (2H, d, J = 9.4 Hz), 5.41 (2H, d, J = 4.4 Hz), 3.36–3.74 (6H, m), 2.51–2.65 (2H, m), 2.37–2.49 (7H, m), 1.56–1.95 (14H, m), 1.24–1.42 (6H, m), 0.50–0.58 (4H, m); ESIMS (m/z) 650.4 $(M+H)^+$, 367.3.

7.1.12. *N,N*-Bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-propylamine (10b). The derivative **10b** was prepared from **6** and *n*-propylamine according to procedure **10a** as white solid in 48% yield. mp >200 °C. $[\alpha]_D^{25}$ 112.9° (c 0.90, $CHCl_3$). IR(KBr) 3419, 2926, 1655, 1608, 1556, 1442, 1306, 1173, 1107, 835, 636 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.04 (2H, br s), 7.55 (2H, s), 6.41 (2H, d, J = 9.3 Hz), 5.41 (2H, d, J = 3.4 Hz), 3.36–3.86 (8H, m), 2.38–2.92 (12H, m), 0.87–1.92 (21H, m); ESIMS (m/z) 652.4 $(M+H)^+$, 674.5 $(M+Na)^+$, 368.3, 269.3

7.1.13. *N,N*-Bis(1-oxo-8,15-didehydrolycodinoethyl)-cyclopropylamine (11a). The derivative **11a** was prepared from **10a** according to procedure **9a** as pale solid in 38% yield. Mp >180 °C dec. $[\alpha]_D^{25}$ −3.5° (c 0. 80, $CHCl_3$). IR(KBr) 3427, 2926, 1659, 1605, 1552, 1458, 1309, 1113, 831, 752 cm^{-1}. ^1H NMR(400 MHz, $CDCl_3$) δ 12.90 (2H, br s), 7.62 (2H, s), 6.42 (2H, d, J = 8.8 Hz), 5.45 (2H, d, J = 4.7 Hz), 3.33–3.48 (4H, m), 2.33–2.89 (13H, m), 1.43–2.14 (20H, m), 1.25 (6H, s); ESIMS (m/z) 1243.5 $(2M+H)^+$, 622.4 $(M+H)^+$, 397.3, 283.3.

7.1.14. *N,N*-Bis(1-oxo-8,15-didehydrolycodinoethyl)-propylamine (11b). The derivative **11b** was prepared from **10b** according to procedure **9a** as white solid in 36% yield. Mp >170 °C (dec). $[\alpha]_D^{25}$ −10.3° (c 1.05, $CHCl_3$). IR(KBr) 3406, 2928, 1655, 1603, 1552, 1456, 1261, 1103, 1024, 802 cm^{-1};1H NMR (400 MHz, $CDCl_3$) δ 12.92 (2H, br s), 7.52 (2H, s), 6.42 (2 × 1H, d, J = 9.4 Hz), 5.46 (2H, d, J = 4.4 Hz), 3.70 (4H, m), 3.15–3.30 (5H, m), 2.85–2.88 (2H, d, J = 12.5 Hz), 2.27–2.65 (8H, m), 1.77–2.02 (5H, m), 1.43–1.70 (13H, m), 1.07–1.24 (8H, m); ESIMS (m/z) 1447.4 $(2M+H)^+$, 624.4 $(M+H)^+$, 368.3, 283.3.

7.1.15. *N,N′*-Dimethyl-*N,N′*-bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-ethylenediamine (12a). The derivative **12a** was prepared from **6** and *N,N′*-dimethyl ethylenediamine according to procedure **8a** as yellow solid in 81% yield. Mp 195–198 °C. $[\alpha]_D^{25}$ 53.2° (c 1.70, $CHCl_3$). IR(KBr) 3423, 2928, 1655, 1610, 1556, 1456, 1408, 1107, 833 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.75 (2H, br s), 7.23 (2H, d, J = 9.3 Hz), 5.36 (2H, d, J = 5.2 Hz), 6.18 (2H, d, J = 9.5 Hz), 4.25 (2H, d, J = 11.4 Hz), 3.21 (2H, d, J = 16.7 Hz), 3.05 (4H, m), 2.73 (2H, d, J = 7.8 Hz), 2.59 (2H, d, J = 17.9 Hz), 2.02–2.45 (10H, m), 1.89–1.92 (2H, m), 1.58–1.69 (12H, m), 1.37–1.48 (6H, m), 1.20–1.28 (2H, m); ESIMS (m/z) 681.5 $(M+1)^+$, 703.5 $(M+Na)^+$.

7.1.16. *N,N′*-Dimethyl-*N,N′*-bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,3-propanediamine (12b). The derivative **12b** was prepared from **6** and *N,N′*-dimethyl trimethylenediamine according to procedure **8a** as yellow solid in 28% yield. Mp 202–204 °C. $[\alpha]_D^{25}$ 124.2° (c 1.35, $CHCl_3$). IR 3439, 2929, 1655, 1610, 1556, 1406, 1107, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.30 (2H, br s), 7.54 (2H, t, J = 9.2, 4.7 Hz), 6.38 (2H, J = 9.3 Hz), 5.40 (2H, J = 4.8 Hz), 3.89 (2H, m), 3.37–3.50 (4H, dd, J = 12.2, 17.7 Hz), 2.90 (4H, J = 17.9, 5.4 Hz), 2.28–2.55 (14H, m), 1.92 (2H, d, J = 12.8 Hz), 1.25–1.43 (4H, m), 1.56–1.69 (16H, m); ESIMS (m/z) 695.5 $(M+H)^+$.

7.1.17. *N,N′*-Dimethyl-*N,N′*-bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,6-hexanediamine (12c). The derivative **12c** was prepared from **6** and *N,N′*-dimethyl-hexane-1,6-diamine according to procedure **8a** as yellow solid in 57% yield. Mp 175–178 °C. $[\alpha]_D^{20}$ 105.4° (c 1.0, $CHCl_3$). IR(KBr) 3431, 2928, 1655, 1610, 1404, 1107, 833 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.02 (2H, br s), 7.52 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.3 Hz), 5.40 (2H, d, J = 4.8 Hz), 3.36 (2H, d, J = 17.6 Hz), 3.60–3.70 (4H, br, d), 2.37–2.63 (20H, m), 1.88 (2 × 1H, m), 1.25–1.62 (12 × 2H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 175.6 × 2, 166.2 × 2, 144.8 × 2, 144.1 × 2, 134.7 × 2, 125.9 × 2, 119.5 × 2, 119.2 × 2, 65.4 × 2, 63.4 × 2, 59.2 × 2, 47.4 × 2, 46.5 × 2, 43.1 × 2, 42.6 × 2, 36.4 × 2, 30.3 × 2, 28.8 × 2, 28.6 × 2, 27.9 × 2, 27.1 × 2, 23.7 × 2; ESIMS (m/z) 737.5 $(M+H^+)$, 759.5 $(M+Na^+)$.

7.1.18. *N,N′*-Dimethyl-*N,N′*-bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,8-octanediamine (12d). The derivative **12d** was prepared from **6** and *N,N′*-dimethyl-octane-1,8-diamine according to procedure **8a** as yellow solid in 66% yield. Mp 165–168 °C. $[\alpha]_D^{25}$ 90.1° (c 0.85, $CHCl_3$). IR(KBr) 3408, 2928, 1653, 1605, 1556, 1444, 1306, 1107, 839, 638 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.10 (2 × 1H, br s), 7.54 (2 × 1H, d, J = 9.5 Hz), 6.37 (2H, d, J = 9.3 Hz), 5.39 (2H, d, J = 5.7 Hz), 3.92 (2H, d, J = 9.6 Hz), 3.32–3.51 (4H, m), 2.86 (2H, dd, J = 5.4, 17.9 Hz), 2.13–2.70 (14H, m), 1.81–1.94 (12H, m), 1.70–1.54 (12H, m), 1.29–1.16 (10H, m); ESIMS (m/z) 765.5 $(M+H^+)$, 751.6.

7.1.19. *N,N′*-Dimethyl-*N,N′*-bis(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,12-dodecanediamine (12e). The derivative **12e** was prepared from **6** and *N,N′*-dimethyl-dodecane-1,12-diamine according to procedure **8a** as yellow solid in 73% yield. Mp 178–180 °C. $[\alpha]_D^{25}$ 117.6°

(c 0.79, $CHCl_3$). IR(KBr) 3419, 2926, 2852, 1655, 1556, 1456, 1406, 1185, 1107, 833, 638 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (2H, d, J = 9.6 Hz), 6.40 (2H, d, J = 9.3 Hz), 5.40 (2H, d, J = 5.1 Hz), 3.70 (2H, m), 3.31 (2H, d, J = 17.8 Hz), 2.87 (4H, m), 2.54–2.62 (6H, m), 2.39–2.43 (4H, m), 1.96 (2H, m), 1.53–1.64 (26H, m), 1.22–1.30 (18H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 175.7 × 2, 166.2 × 2, 144.9 × 2, 143.7 × 2, 134.6 × 2, 125.8 × 2, 119.6 × 2, 118.8 × 2, 64.0 × 2, 63.5 × 2, 59.4 × 2, 47.4 × 2, 46.0 × 2, 43.1 × 2, 42.4 × 2, 36.4 × 2, 31.2 × 2, 31.1 × 2, 30.9 × 2, 30.3 × 2, 28.5 × 2, 27.7 × 2, 27.5 × 2, 26.8 × 2, 23.7 × 2; ESIMS (m/z) 821.8 $(M+H)^+$.

7.1.20. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodinocarbonyl)ethyl]-1,6-hexanediamine (12f). The derivative **12f** was prepared from **7** and *N,N′*-dimethylhexane-1,6-diamine according to procedure **8c** as pale oil in 78% yield. $[\alpha]_D^{25}$ 92.2° (c 0.84, $CHCl_3$). IR(KBr) 3423, 2928, 2856, 1659, 1610, 1556, 1458, 1398, 1169, 1107, 833 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.12 (2H, br s), 7.51 (2 × 1H, d, J = 9.5 Hz), 6.42 (2H, d, J = 9.5 Hz), 5.40 (2H, d, J = 4.9 Hz), 3.72 (2H, d, J = 12.8 Hz), 3.35 (2H, d, J = 17.7 Hz), 2.38–2.93 (28H, m), 1.87–1.95 (2H, m), 1.56–1.64 (12H, m), 1.24–1.28 (6H, m); ESIMS (m/z) 765.7 $(M+H)^+$, 787.7 $(M+Na)^+$.

7.1.21. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodinocarbonyl)ethyl]-1,8-octanediamine (12g). The derivative **12g** was prepared from **7** and *N,N′*-dimethyl-octane-1,8-diamine according to procedure **8c** as pale oil in 95% yield. $[\alpha]_D^{25}$ 122.2° (c 1.04, $CHCl_3$). IR(KBr) 3439, 2928, 2854, 1659, 1612, 1462, 1398, 1252, 1169, 1107, 833, 752, 534 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 13.17 (2H, br s), 7.53 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.3 Hz), 5.40 (2H, d, J = 5.2 Hz), 3.68–3.74 (4H, m), 3.36 (2H, d, J = 17.9), 2.32–2.93 (26H, m), 1.93 (2H, m), 1.51–1.66 (16H, m), 1.22–1.34 (10H, m); ESIMS (m/z) 793.7 $(M+H)^+$.

7.1.22. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodinocarbonyl)ethyl]-1,10-decanediamine (12h). The derivative **12h** was prepared from **7** and *N,N′*-dimethyl-decane-1,10-diamine according to procedure **8c** as solid in 89% yield. Mp 157–160 °C. $[\alpha]_D^{25}$ 130.5° (c 0.81, $CHCl_3$). IR(KBr) 3439, 2928, 2854, 1659, 1612, 1462, 1398, 1252, 1169, 1107, 833, 752, 534 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.06 (2H, br s), 7.53 (2H, d, J = 9.3 Hz), 6.40 (2H, d, J = 9.6 Hz), 5.38 (2H, d, J = 5.4 Hz), 3.70 (2H, d, J = 12.9 Hz), 3.36 (2H, d, J = 18.2 Hz), 2.88 (2H, dd, J = 5.5, 17.5 Hz), 2.74–2.78 (2H, m), 2.52–2.71 (8H, m), 2.43 (2H, d, J = 18.1 Hz), 2.36 (6H, t, J = 7.6 Hz), 2.25 (2 × 3H, s), 1.96 (2H, m), 1.75 (4H, m), 1.62 (4H, m), 1.54 (2 × 3H, s), 1.45 (4H, m), 1.22–1.33 (14H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3 × 2, 165.3 × 2, 142.7 × 2, 142.1 × 2, 133.5 × 2, 123.6 × 2, 118.3 × 2, 117.3 × 2, 61.5 × 2, 58.0 × 2, 53.9 × 2, 45.6 × 2, 44.4 × 2, 42.3 × 2, 40.5 × 2, 35.3 × 2, 34.5 × 2, 29.6 × 4, 28.9 × 2, 27.5 × 2, 27.3 × 2, 26.4 × 2, 25.4 × 2, 23.1 × 2; HRMS (ESI, m/z): $(M+H)^+$ calcd for $C_{50}H_{73}N_6O_4$, 821.5693; found, 821.5717.

7.1.23. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodinocarbonyl)ethyl]-1,12-dodecanediamine (12i). The derivative **12i** was prepared from **7** and N,N′-dimethyl-dodecane-1,12-diamine according to procedure **8c** as yellowish oil in 56% yield. $[\alpha]_D^{25}$ 115° (c 0.81, $CHCl_3$). IR(KBr) 3419, 2926, 2852, 1655, 1608, 1556, 1460, 1402, 1254, 1169, 1107, 833, 633 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.90 (2 × 1H, br s), 7.48 (2 × 1H, d, J = 9.6 Hz), 6.41 (2 × 1H, d, J = 9.4 Hz), 5.40 (2H, d, J = 5.5 Hz), 3.72 (2H, m), 3.33 (2H, d, J = 17.8 Hz), 2.87–3.09 (8H, m), 2.37–2.69 (18H, m), 1.93 (2H, m), 1.55–1.65 (16H, m), 1.22–1.31 (20H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9 × 2, 164.9 × 2, 142.5 × 2, 141,7 × 2, 133.2 × 2, 123.6 × 2, 118.3 × 2, 116.9 × 2, 61.8 × 2, 57.8 × 2, 53.6 × 2, 45.7 × 2, 44.5 × 2, 41.7 × 2, 40.5 × 2, 34.6 × 2, 34.4 × 2, 29.8 × 2, 29.6 × 2, 29.0 × 2, 27.4 × 2, 26.5 × 2, 26.3 × 2, 25.4 × 2, 23.2 × 2; ESIMS (m/z) 849.9 $(M+H)^+$.

7.1.24. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodino)ethyl]-ethylenediamine (13a). The derivative **13a** was prepared from **12a** according to procedure **9a** as yellowish solid in 42% yield. Mp >220 °C dec. $[\alpha]_D^{25}$ −20.8° (c 0.60, $CHCl_3$). IR(KBr) 3415, 2927, 1655, 1605, 1552, 1458, 1300, 1113, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.62 (2H, br s), 7.61 (2H, d, J = 9.5 Hz), 6.38 (2H, d, J = 9.5 Hz), 5.43 (2H, d, J = 5.4 Hz), 3.25 (2H, m), 2.83 (4H, J = 5.3, 17.7 Hz), 2.24–2.62 (14H, m), 1.97 (2H, J = 16.6 Hz), 1.48–1.78 (16H, m), 1.37 (2H, m), 1.16–1.25 (6H, m); ESIMS (m/z) 653.4 $(M+H)^+$, 673.5 $(M+Na)^+$, 397.3.

7.1.25. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodino)ethyl]-1,3-propanediamine (13b). The derivative **13b** was prepared from **12b** according to procedure **9a** as yellowish solid in 41% yield. Mp >230 °C (dec). $[\alpha]_D^{25}$ −14.7° (c 0.55, $CHCl_3$). ^1H NMR(400 MHz, $CDCl_3$) δ 12.63 (2H, br s), 7.62 (2H, d, J = 9.5 Hz), 6.39 (2H, d, J = 9.3 Hz), 5.45 (2H, d, J = 5.1 Hz), 3.28 (2H, m), 2.84 (4H, dd, J = 5.4, 17.4 Hz), 2.62 (2H, J = 12.4 Hz), 2.26–2.47 (16H, m), 1.99 (2H, d, J = 16.35 Hz), 1.65–1.79 (12H, m), 1.49–1.58 (8H, m), 1.39 (2H, d, J = 12.1 Hz), 1.17–1.25 (4H, m). IR(KBr) 3423, 2928, 2794, 1657, 1605, 1552, 1458, 1113, 752 cm^{-1}; ESIMS (m/z) 667.5 $(M+H)^+$, 689.6 $(M+Na)^+$, 411.4(5), 354.3(2).

7.1.26. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodino)ethyl]-1,6-hexanediamine (13c). The derivative **13c** was prepared from **12c** according to procedure **9a** as yellowish solid in 40% yield. Mp 143–145 °C. $[\alpha]_D^{25}$ −0.6° (c 0.90, $CHCl_3$). IR(KBr) 3415, 2924, 1655, 1603, 1552, 1458, 1113 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.60 (2H, br s), 7.56 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.3 Hz), 5.45 (2H, d, J = 5.2 Hz), 3.62 (2H, br s), 2.88–2.17 (24H, m), 2.00 (2H, d, J = 16.8 Hz), 1.41–1.88 (12H, m), 1.25–1.33 (24H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 166.1 × 2, 144.9 × 2, 144.0 × 2, 134.6 × 2, 125.9 × 2, 119.5 × 2, 119.1 × 2, 64.3 × 2, 63.7 × 2, 59.3 × 2, 47.4 × 2, 46.2 × 2, 43.2 × 2, 42.4 × 2, 36.3 × 2, 31.0 × 2, 30.3 × 2, 28.7 × 2, 28.0 × 2, 27.8 × 2, 26.9 × 2, 23.7 × 2; ESIMS (m/z) 709.6 $(M+H)^+$.

7.1.27. *N,N′*-Dimethyl-*N,N′*-bis[2-(1-oxo-8,15-didehydrolycodino)ethyl]-1,8-octanediamine (13d). The derivative **13d** was prepared from **12d** according to procedure **9a** as pale solid in 45% yield. Mp 140–143 °C. $[\alpha]_D^{25}$ −6.0° (*c* 0.80, $CHCl_3$). IR(KBr) 3423, 2928, 1659, 1606, 1552, 1462, 1302, 1103, 831 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.94 (2H, br s), 7.61 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.3 Hz), 5.44 (2H, d, J = 5.1 Hz), 3.51 (2H, br s), 2.81–2.94 (4H, m), 2.32–2.63 (22H, m), 1.98 (2H, d, J = 17.6 Hz), 1.78 (2H, dt, J = 3.6, 12.5 Hz), 1.50–1.61 (12H, m), 1.18–1.42 (16H, m); HRMS (ESI, *m/z*): $(M+H)^+$ calcd for $C_{46}H_{69}N_6O_2$, 737.5484; found, 737.5480.

7.1.28. *N,N′*-Dimethyl-*N,N′*-bis[3-(1-oxo-8,15-didehydrolycodino)propyl]-1,6-hexanediamine (13e). The derivative **13e** was prepared from **12f** according to procedure **9a** as white oil in 29% yield. $[\alpha]_D^{25}$ −13.0° (*c* 0.88, $CHCl_3$). IR(KBr) 3415, 2929, 1657, 1605, 1552, 1460, 1375, 1103, 831, 644, 513 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.07 (2H, br s), 7.66 (2H, d, J = 9.6 Hz), 6.40 (2 × 1H, d, J = 9.3 Hz), 5.42 (2H, d, J = 5.1 Hz), 3.08 (2H, m), 2.76–2.89 (4H, m), 2.29–2.63 (24H, m), 1.76–1.97 (4H, m), 1.54–1.66 (16H, m), 1.18–1.38 (10H, m); ESIMS (*m/z*) 737.7 $(M+H)^+$, 735.9 $(M-H)^+$.

7.1.29. *N,N′*-Dimethyl-*N,N′*-bis[3-(1-oxo-8,15-didehydrolycodino)propyl]-1,8-octanediamine (13f). The derivative **13f** was prepared from **12g** according to procedure **9a** as white oil in 59% yield. $[\alpha]_D^{25}$ 8.8° (*c* 1.0, $CHCl_3$). IR(KBr) 3417, 2926, 1653, 1601, 1551, 1458, 1302, 1113, 835 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.78 (2H, br s), 7.56 (2H, d, J = 9.5 Hz), 6.41 (2H, d, J = 9.3 Hz), 5.44 (2H, d, J = 4.7 Hz), 2.63–3.13 (12H, m), 2.18–2.63 (6H, m), 1.22–2.00 (40H, m), 0.83–0.91 (6H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2 × 2, 142.8 × 2, 141.3 × 2, 131.7 × 2, 125.1 × 2, 120.3 × 2, 117.3 × 2, 57.5 × 2, 55.9 × 2, 54.1 × 2, 46.9 × 2, 46.3 × 2, 43.7 × 2, 39.8 × 2, 36.9 × 2, 33.8 × 2, 29.5 × 2, 29.2 × 2, 28.3 × 2, 26.1 × 2, 25.4 × 2, 23.8 × 2, 23.5 × 2, 22.8 × 2; ESIMS (*m/z*) 765.7 $(M+H)^+$.

7.1.30. *N,N′*-Dimethyl-*N,N′*-bis[3-(1-oxo-8,15-didehydrolycodino)propyl]-1,10-decanediamine (13g). The derivative **13g** was prepared from **12h** according to procedure **9a** as white oil in 30% yield. $[\alpha]_D^{25}$ −10.5° (*c* 0.78, $CHCl_3$). IR(KBr) 3417, 2926, 1653, 1601, 1551, 1458, 1302, 1113, 835, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 12.72 (2H, br s), 7.62 (2H, d, J = 9.6 Hz), 6.40 (2H, d, J = 9.3 Hz), 5.44 (2H, d, J = 5.2 Hz), 3.10 (2H, m), 2.78–2.88 (5H, m), 2.60–2.63 (6H, m), 2.48 (4H, s), 2.30–2.41 (10H, m), 1.94 (3H, d, J = 16.2 Hz), 1.78 (6H, m), 1.51–1.62 (12H, m), 1.22–1.39 (20H, m); ESIMS (*m/z*) 793.6 $(M+H)^+$, 537.4, 397.5.

7.1.31. *N,N′*-Dimethyl-*N*-(2-pyridylmethyl)-*N′*-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,8-octanediamine (14a). To the solution of *N,N′*-dimethyl *N*-(2-pyridinylmethyl)-octamethylenediamine(16 mg, 0.055 mmol) and **6** (18 mg, 0.055 mmol) in acetonitrile (3.0 mL), K_2CO_3 (15 mg, 0.11 mmol) and KI (5 mg) were added, the reaction mixture was stirred at 65–70 °C for 24 h, and then cooled to rt. After removal of the solvent, the residue was diluted with $CHCl_3$ (5 mL) and CH_3OH (0.5 mL). After filtration, the organic filtrate was washed with brine (3× 2 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by preparative TLC ($CHCl_3/CH_3OH/NH_4OH$ = 100:12:0.6) to afford product **14a** as a yellowish oil 13 mg (Y: 41%). $[\alpha]_D^{20}$ 92.2° (*c* 1.32, $CHCl_3$). IR(KBr): 3427, 2926, 1666, 1556, 1464, 1405, 1306, 1256, 1186, 1107. ^1H NMR (400 MHz, $CDCl_3$): δ 13.06 (1H, br s), 8.53 (1H, d, J = 4.6 Hz), 7.65 (1H, dt, J = 1.9, 7.7 Hz), 7.56 (1H, d, J = 9.3 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.16 (1H, m), 6.40 (1H, d, J = 9.3 Hz), 3.96 (1H, d, J = 12.9 Hz), 3.68 (2H, s), 3.42 (2H, m), 3.00 (1H, d, J = 14.3 Hz), 2.89 (1H, dd, J = 5.5, 18.2 Hz), 2.32–2.54 (8H, m), 2.27–2.31 (6H, m), 1.91 (1H, m), 1.45–1.68 (10H, m), 1.24–1.35 (10H, m); EIMS (*m/z*): 559 (M^+, 5), 467(20), 276(100), 256(5), 199(7), 183(9), 135(12); HRMS: calcd for $C_{34}H_{49}O_2N_5$ $(M)^+$, 559.3886; found, 559.3881.

7.1.32. *N,N′*-Dimethyl-*N*-(2-pyridylmethyl)-*N′*-(1-oxo-8, 15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14b). The derivative **14b** was prepared from **6** and *N,N′*-dimethyl-*N*-(2-pyridinylmethyl)-decamethylene diamine according to procedure **14a** as yellowish oil in 88% yield. $[\alpha]_D^{20}$ 96.0 (*c* 0.455, $CHCl_3$). IR(KBr): 3427, 2926, 1666, 1556, 1464, 1405, 1306, 1256, 1186, 1107, 1045. ^1H NMR (400 MHz, $CDCl_3$): δ 12.89 (1H, br s), 8.55 (1H, m), 7.67 (1H, dt, J = 1.8, 7.6 Hz), 7.57 (1H, d, J = 9.5 Hz), 7.49 (1H, d, J = 7.5 Hz), 7.18 (1H, m), 6.40 (1H, d, J = 9.4 Hz), 5.41 (1H, d, J = 5.1 Hz), 3.97 (1H, d, J = 12.9 Hz), 3.75 (2H, s), 3.45 (2H, m), 3.00 (1H, d, J = 14.3 Hz), 2.90 (1H, dd, J = 5.7, 18.0 Hz), 2.48–2.55 (4H, m), 2.28–2.39 (9H, m), 1.92 (1H, dt, J = 3.4, 12.9 Hz), 1.46–1.69 (10H, m), 1.26–1.36 (14H, m); HRMS (*m/z*): calcd for $C_{36}H_{53}N_5O_2$(M), 587.4199 (M^+); found, 587.4205.

7.1.33. *N,N′*-Dimethyl-*N*-(3-pyridylmethyl)-*N′*-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14c). The derivative **14c** was prepared from **6** and *N,N′*-dimethyl-*N*-(3-pyridinylmethyl)-decamethylene diamine according to procedure **14a** as yellowish oil in 44% yield. $[\alpha]_D^{23}$ 85.2 (*c* 0.575, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 12.78 (1H, br s), 8.53 (1H, s), 8.51 (1H, d, J = 1.2 Hz), 7.77 (1H, s), 7.56 (1H, d, J = 9.4 Hz), 7.28 (1H, m), 6.41 (1H, d, J = 9.5 Hz), 5.16 (1H, d, J = 5.3 Hz), 3.95 (1H, d, J = 13.7 Hz), 3.59 (2H, s), 3.44 (2H, m), 3.07 (1H, d, J = 14.3 Hz), 2.90 (1H, m), 2.38–2.56 (7H, m), 2.25–2.32 (6H, m), 1.93 (1H, m), 1.48–1.69 (10H, m), 1.26–1.36 (14H, m); ESIMS (*m/z*): 588.4 $(M+H)^+$.

7.1.34. *N,N′*-Dimethyl-*N*-(4-pyridylmethyl)-*N′*-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14d). The derivative **14d** was prepared from **6** and *N,N′*-dimethyl-*N*-(4-pyridinylmethyl)-decamethylene diamine according to procedure **14a** as yellowish oil in 43% yield. $[\alpha]_D^{23}$ 68.5 (*c* 0.390, $CHCl_3$). IR (KBr): 3427, 2926, 1666, 1556, 1464, 1405, 1306, 1256, 1186, 1107, 1045. ^1H NMR (400 MHz, $CDCl_3$): δ 12.78 (1H, br s), 8.54 (2H, d, J = 4.3 Hz), 7.55 (1H, d, J = 9.6 Hz), 7.29 (2H, d, J = 4.9 Hz), 6.40 (1H, d, J = 9.4 Hz), 5.41 (1H, d, J = 5.3 Hz), 3.92 (1H, d,

J = 13.2 Hz), 3.50 (2H, d, J = 14.7 Hz), 3.40 (1H, d, J = 18.0 Hz), 3.11 (1H, d, J = 13.3 Hz), 2.90 (1H,dd, J = 6.2, 17.8 Hz), 2.35–2.57 (10H, m), 2.22 (3H, s), 1.93 (2H, m), 1.50–1.68 (10H, m), 1.26–1.33 (14H, m); ESIMS (m/z): 588.4 $(M+H)^+$.

7.1.35. *N*,*N*′-Dimethyl-*N*-(2-pyridylmethyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,12-dodecanediamine (14e). The derivative **14e** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(2-pyridylmethyl)-1,12-dodecane diamine according to procedure **14a** as yellowish oil in 66% yield. $[\alpha]_D^{25}$ 82.6° (c 0.66, $CHCl_3$). IR (KBr): 3427, 2926, 1666, 1556, 1464, 1405, 1306, 1256, 1186, 1107, 1045, 833, 758, 640, 538 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 12.78 (1H, br s), 8.56 (1H, m), 7.69 (1H, dt, J = 1.8, 7.6 Hz), 7.57 (1H, d, J = 9.5 Hz), 7.53 (1H, m), 7.20 (1H, m), 6.40 (1H, d, J = 9.5 Hz), 5.41 (1H, d, J = 5.4 Hz), 3.98 (1H, d, J = 12.2 Hz), 3.81 (2H, s), 3.43 (2H, m), 3.02 (1H, d, J = 14.3 Hz), 2.90 (1H, dd, J = 5.6, 18.1 Hz), 2.38–2.56 (10H, m), 2.29 (3H, s), 1.92 (1H, m), 1.46–1.68 (10H, m), 1.22–1.68 (18H, m); HRMS (m/z): calcd for $C_{38}H_{57}O_2N_5$, 615.4512; found, 615.4524.

7.1.36. *N*,*N*′-Dimethyl-*N*-(2-methoxybenzyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14f). The derivative **14f** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(2-methoxybenzyl)-1,10-decanediamine according to procedure **14a** as yellowish oil in 59% yield. $[\alpha]_D^{20}$ 76.1° (c 0.615, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 12.88 (1H, br s), 7.58 (1H, d, J = 9.4 Hz), 7.41 (1H, d, J = 6.7 Hz), 7.30 (1H, d, J = 8.3 Hz), 6.95 (1H, t, J = 7.4 Hz), 6.89 (1H, d, J = 8.1 Hz), 6.40 (1H, d, J = 9.4 Hz), 5.41 (1H, d, J = 5.4 Hz), 3.98 (1H, d, J = 12.1 Hz), 3.83 (3H, s), 3.76 (1H, s), 3.42 (2H, m), 2.99 (1H, d, J = 14.1 Hz), 2.90 (1H, dd, J = 5.7, 18.2 Hz), 2.36–2.58 (10H, m), 2.27 (3H, s), 1.92 (1H, m), 1.56–1.65 (8H, m), 1.45–1.48 (3H, m), 1.24–1.34 (14H, m); HRMS (m/z): calcd for $C_{38}H_{56}O_3N_4$, 616.4352; found, 616.4345.

7.1.37. *N*,*N*′-Dimethyl-*N*-(2-methoxybenzyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,12-dodecanediamine (14g). The derivative **14g** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(2-methoxybenzyl)-1,12-dodecane diamine according to procedure **14a** as yellowish oil in 53% yield. $[\alpha]_D^{20}$ 80.6° (c 0.67, $CHCl_3$). IR (film): 3390, 2926, 2852, 2793, 1659, 1606, 1456, 1404, 1250, 1107, 1032 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 12.88 (1H, bs), 7.58 (1H, d, J = 9.4 Hz), 7.41 (1H, d, J = 6.7 Hz), 7.30 (1H, d, J = 8.3 Hz), 6.95 (1H, t, J = 7.4 Hz), 6.89 (1H, d, J = 8.1 Hz), 6.40 (1H, d, J = 9.4 Hz), 5.41 (1H, d, J = 5.4 Hz), 3.98 (1H, d, J = 12.1 Hz), 3.83 (3H, s), 3.76 (1H, s), 3.42 (2H, m), 2.99 (1H, d, J = 14.1 Hz), 2.90 (1H, dd, J = 5.7, 18.2 Hz), 2.36–2.58 (10H, m), 2.27 (3H, s), 1.92 (1H, m), 1.56–1.65 (8H, m), 1.45–1.48 (3H, m), 1.24–1.34 (16H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 166.0, 157.2, 146.7, 145.3, 143.5, 141.5, 140.1, 133.6, 133.0, 127.6, 127.1, 124.7, 121.7, 118.9, 61.4, 59.9, 58.6, 58.0, 46.8, 46.3, 46.1, 45.2, 41.9, 41.8, 38.9, 38.8, 36.2, 35.9, 34.5, 31.2, 31.0, 30.3, 27.9, 27.5, 27.2, 25.7, 25.6 25.4, 23.7, 23.6; HRMS (m/z): calcd for $C_{40}H_{60}O_3N_4$, 644.4665; found, 644.4662.

7.1.38. *N*,*N*′-Dimethyl-*N*-(4-fluorobenzyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,8-octanediamine (14h). The derivative **14h** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(4-fluoro-benzyl)-octamethylene diamine according to procedure **14a** as oil in 72% yield. $[\alpha]_D^{20}$ 26.9° (c 1.50, $CHCl_3$). IR (film): 2926, 2852, 2793, 1653, 1614, 1506, 1464, 1404, 1221. ^1H NMR (400 MHz, $CDCl_3$): δ 12.65 (1H, br s), 7.54 (1H, d, J = 9.4 Hz), 7.35 (2H, m), 7.00 (2H, t, J = 8.5 Hz), 5.38 (1H, d, J = 4.2 Hz), 3.94 (1H, d, J = 12.6 Hz), 3.62 (2H, br), 3.41 (2H, m), 3.02 (1H, d, J = 14.3 Hz), 2.85 (1H, dd, J = 5.5 Hz, 17.6 Hz), 2.35–2.53 (8H, m), 2.27 (6H, m), 1.90 (1H, m), 1.45–1.66 (10H, m), 1.23–1.31 (10H, m); EIMS (m/z): 576(M^+, 17), 467(19), 453(12), 293(100), 152(7), 109(18); HRMS: calcd for $C_{35}H_{49}FN_4O_2$, 576.3840 (M^+); found, 576.3855.

7.1.39. *N*,*N*′-Dimethyl-*N*-(4-fluorobenzyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14i). The derivative **14i** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(4-fluoro-benzyl)-decamethylene diamine according to procedure **14a** as oil in 66% yield. $[\alpha]_D^{20}$ 27.1° (c 1.25, $CHCl_3$). IR (film): 2926, 2852, 2793, 1653, 1614, 1506, 1464, 1404, 1221, 1107. ^1H NMR (400 MHz, $CDCl_3$): δ 12.68 (1H, br s), 7.57 (1H, d, J = 9.4 Hz), 7.35 (2H, t, J = 7.8 Hz), 7.01 (2H, t, J = 8.6 Hz), 6.41 (1H, d, J = 9.4 Hz), 5.41 (1H, d, J = 4.9 Hz), 3.98 (1H, d, J = 12.7 Hz), 3.56 (2H, s), 3.43 (2H, t, J = 14.2 Hz), 3.02 (1H, d, J = 14.1 Hz), 2.90 (1H, dd, J = 5.3, 17.8 Hz), 2.38–2.56 (7H, m), 2.25–2.28 (6H, m), 1.92 (1H, m), 1.46–1.75 (10H, m), 1.26–1.36 (14H, m); ESIMS (m/z): 604.4 $(M+H)^+$.

7.1.40. *N*,*N*′-Dimethyl-*N*-(4-fluorobenzyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,12-dodecanediamine (14j). The derivative **14j** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(4-fluoro-benzyl)-dodecamethylene diamine according to procedure **14a** as oil in 46% yield. $[\alpha]_D^{20}$ 85.6° (c 1.08, $CHCl_3$);IR (film): 2926, 2852, 2793, 1653, 1614, 1506, 1464, 1404, 1221, 1107, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 12.80 (1H, br s), 7.58 (1H, d, J = 9.5 Hz), 7.34 (2H, m), 7.02 (2H, t, J = 8.5 Hz), 6.41 (1H, d, J = 9.5 Hz), 5.41 (1H, d, J = 4.9 Hz), 3.98 (1H, d, J = 12.9 Hz), 3.62 (2H, s), 3.43 (2H, d, J = 17.5 Hz), 2.88–3.03 (3H, m), 2.28–2.56 (14H, m), 1.93 (2H, m), 1.47–1.69(12H, m), 1.25–1.27 (15H, m); HRMS (m/z): calcd for $C_{39}H_{57}FN_4O_2$, 632.4466 (M^+); found, 632.4450.

7.1.41. *N*,*N*′-Dimethyl-*N*-(1-naphthylmethyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,6-hexanediamine (14k). The derivative **14k** was prepared from **6** and *N*,*N*′-dimethyl-*N*-(1-naphthylmethyl)-1,6-hexanediamine according to procedure **14a** as yellowish oil in 67% yield. $[\alpha]_D^{25}$ 73.8° (c 0.75, $CHCl_3$). IR (film): 3420, 2928, 2854, 2791, 1653, 1614, 1556, 1464, 1404, 1107, 754 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 13.72 (1H, bs), 8.27 (1H, d, J = 8.0 Hz), 7.84 (1H, dd, J = 1.5, 7.6 Hz), 7.77 (1H, d, J = 8.0 Hz), 7.57 (1H, d,

J = 9.4 Hz), 7.37–7.52 (4H, m), 6.41 (1H, d, J = 9.4 Hz), 5.40 (1H, d, J = 5.2 Hz), 3.96 (3H, m), 3.43 (2H, t, J = 14.6 Hz), 3.01 (1H, d, J = 14.4 Hz), 2.89 (1H, dd, J = 5.9, 17.8 Hz), 2.35–2.55 (7H, m), 2.28 (3H, s), 2.22 (2H, s), 1.57–1.84 (12H, m), 1.24–1.48 (6H, m); HRMS (*m*/*z*): calcd for $C_{37}H_{48}N_4O_2$, 580.3777; found, 580.3777.

7.1.42. *N*,*N*′-Dimethyl-*N*-(1-naphthylmethyl)-*N*′-(1-oxo-8,15-didehydrolycodinocarbonylmethyl)-1,10-decanediamine (14l). The derivative **14l** was prepared from 6 and *N*,*N*′-dimethyl-*N*-(1-naphthylmethyl)-decamethylene diamine according to procedure **14a** as yellowish oil in 61% yield. $[\alpha]_D^{25}$ 77.2° (*c* 0.83, $CHCl_3$). IR (film): 3420, 2928, 2854, 2791, 1653, 1614, 1556, 1464, 1404, 1107, 793 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.72 (1H, br s), 8.27 (1H, d, J = 8.0 Hz), 7.84 (1H, dd, J = 1.5, 7.6 Hz), 7.77 (1H, d, J = 8.0 Hz), 7.57 (1H, d, J = 9.4 Hz), 7.37–7.52 (4H, m), 6.41 (1H, d, J = 9.4 Hz), 5.40 (1H, d, J = 5.2 Hz), 3.96 (3H, m), 3.43 (2H, t, J = 14.6 Hz), 3.01 (1H, d, J = 14.4 Hz), 2.89 (1H, dd, J = 5.9, 17.8 Hz), 2.35–2.55 (7H, m), 2.28 (3H, s), 2.22 (2H, s), 1.57–1.84 (12H, m), 1.24–1.48 (14H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 168.1, 166.0, 145.1, 143.3, 136.1, 134.5, 133.9, 132.7, 132.6, 130.8, 129.2, 128.2, 128.0, 127.0, 125.9, 124.6, 119.8, 118.4, 64.7, 60.9, 59.5, 58.4, 58.3, 47.3, 45.5, 43.1, 42.1, 41.3, 36.3, 31.2, 30.9, 30.7, 30.6, 30.3, 28.2, 28.0, 27.5, 26.5, 26.1, 26.0, 23.6; EIMS (*m*/*z*): 636 (M^+, 3), 495(7), 353(100), 256(10), 141(97); HRMS (*m*/*z*): calcd for $C_{41}H_{56}N_4O_2$, 636.4707 (M^+); found, 636.4772.

7.2. Pharmacology

7.2.1. Materials. Acetylthiocholine iodide (*S*-ACh), *S*-butyrylthiocholine iodide (*S*-BuCh), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) and tetraisopropyl pyrophosphoramide (iso-OMPA), amyloid β-peptide$_{25–35}$, scopolamine, and stauroporine were purchased from Sigma Chemical Co. Natural HupB and donepezil were prepared and purified by the Department of Medicinal Chemistry of this Institute.

7.2.2. Enzyme source. Rat cortex homogenate was used as the brain AChE source. To prepare brain AChE, rat was decapitated and the cortex was dissected on ice and homogenized in 9 (wt/vol) volumes of ice-cold sodium phosphate buffer (75 mmol/L).

Rat serum was used as the sources of BuChE. The serum was obtained after centrifugation (3500*g*, 15 min). All enzyme source was kept in −20 °C until used.

7.2.3. ChE activity assay. AChE and BuChE activities were measured by the spectrophotometric method,[29] *S*-ACh 2 mmol/mL or *S*-BuCh 2 mmol/mL was used as substrates for assay of AChE and BuChE, respectively. The mixture, including substrates, sodium phosphate buffer (0.1 mmol/L) 1 mL, and enzyme 0.1 mL, was incubated in a total volume of 4 mL at 37 °C for 8 min. The reaction was terminated by adding 3% SDS 1 mL, then 0.2% DTNB 1 mL was added to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production was measured spectrophotometrically at 440 nm. The enzyme activity was expressed as a percentage of the activity observed in the absence of inhibitor. The IC_{50} was defined as the concentration of inhibitor necessary to yield 50% inhibition of enzyme activity. The K_i value was determined using Lineweaver–Burk plot. A plot of 1/OD versus the inverse of substrate concentration was also made to distinguish modes of inhibition of AChE. All the samples were assayed in duplicate.

Acknowledgments

This study was financially supported by Shanghai SK Foundation for Research and Development (Project-2004010-h) and the National Natural Science Foundation of China (No. 30472067).

References and notes

1. Giacobini, E. In *Cholinesterases and Cholinesterase Inhibitors*; Giacobini, E., Ed.; Martin Dunitz: London, 2000; pp 181–226.
2. Barril, X.; Orozco, M.; Luque, F. J. *Mini-Rev. Med. Chem.* **2001**, *1*, 255.
3. Palmer, A. M. *TIPS* **2002**, *23*, 426.
4. Ibach, B.; Haen, E. *Curr. Pharm. Des.* **2004**, *10*, 231.
5. Francotte, P.; Graindorge, E.; Boverie, S.; de Tullio, P.; Pirotte, B. *Curr. Med. Chem.* **2004**, *11*, 1757.
6. Robichaud, A. J. *Curr. Top. Med. Chem.* **2006**, *6*, 553.
7. Martinez, A.; Castro, A. *Expert Opin. Investig. Drugs* **2006**, *15*, 1.
8. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
9. 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. U.S.A.* **1993**, *90*, 9031.
10. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. *Neuron* **1996**, *16*, 881.
11. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407.
12. Castro, A.; Martinez, A. *Mini-Rev. Med. Chem* **2001**, *1*, 267.
13. Dorronsoro, I.; Castro, A.; Martinez, A. *Expert Opin. Ther. Patents* **2003**, *13*, 1725.
14. Pang, Y. P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.* **1996**, *271*, 23646.
15. Carlier, P. R.; Han, Y. F.; Chow, E. S. H.; Li, C. P. L.; Wang, H.; Lieu, T. X.; Wong, H. S.; Pang, Y. P. *Bioorg. Med. Chem.* **1999**, *7*, 351.
16. Jin, G. Y.; Luo, X. M.; He, X. C.; Jiang, H. L.; Zhang, H. Y.; Bai, D. L. *Arzneim.-Forsch.* **2003**, *53*, 753.
17. Carlier, P. R.; Du, D. M.; Han, Y. F.; Liu, J.; Perola, E.; Williams, I. D.; Pang, Y. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 1775.
18. Wong, D. M.; Greenblatt, H. M.; Dvir, H.; Carlier, P. R.; Han, Y. F.; Pang, Y. P.; Silman, I.; Sussman, J. L. *J. Am. Chem. Soc.* **2003**, *125*, 363.
19. Catherine, G.; Mary, A.; Renko, D. Z.; Gras, E.; Thal, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 637.
20. Zhu, X. D.; Tang, X. C. *Acta Pharmacol. Sin.* **1988**, *9*, 492.
21. Xu, H.; Tang, X. C. *Acta Pharmacol. Sin.* **1987**, *8*, 18.
22. Bai, D. L.; Tang, X. C.; He, X. C. *Curr. Med. Chem.* **2000**, *7*, 355.

23. Yan, X. F.; Lu, W. H.; Lou, J. W.; Tang, X. C. *Acta Pharmacol. Sin.* **1987**, *8*, 117.
24. Liu, J.; Zhang, H. Y.; Wang, L. M.; Tang, X. C. *Acta Pharmacol. Sin.* **1999**, *20*, 141.
25. Feng, S.; Wang, Z. F.; He, X. C.; Zheng, S. X.; Xia, Y.; Jiang, H. L.; Tang, X. C.; Bai, D. L. *J. Med. Chem.* **2005**, *48*, 655.
26. Feng, S.; Xia, Y.; Han, D.; Zheng, C.; He, X. C.; Tang, X. C.; Bai, D. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 523.
27. Dvir, H.; Jiang, H. L.; Wong, D. M.; Harel, M.; Chetrit, M.; He, X. C.; Jin, G. Y.; Yu, G. L.; Tang, X. C.; Silman, I.; Bai, D. L.; Sussman, J. L. *Biochemistry* **2002**, *41*, 10810.
28. Melchiorre, C.; Andrisano, V.; Bolognesi, M. L.; Budriesi, R.; Cavalli, A.; Cavrini, V.; Rosini, M.; Tuniatti, V.; Recanatini, M. *J. Med. Chem* **1998**, *41*, 4186.
29. Cheng, D. H.; Tang, X. C. *Pharmacol. Biochem. Behav.* **1998**, *60*, 377.
30. DOCK 4.0. Molecular Design Institute, San Francisco, CA, USA.
31. Ewing, T. J. A.; Kuntz, I. D. *J. Comput. Chem.* **1997**, *18*, 1175.
32. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E. *J. Comput. Chem.* **1998**, *19*, 1639.
33. Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. *Protein Eng.* **1995**, *8*, 127.