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Cage Amines as the Stopper Inhibitors of Cholinesterases

Gialih Lin,[a,*] Hou-Jen Tsai[b] and Yi-Hon Tsai[a]

[a]*Department of Chemistry, National Chung-Hsing University, Taichung, Taiwan*
[b]*Department of Applied Chemistry, National Chung Cheng Institute of Technology, Ta-Shi, Tao-Yuan, Taiwan*

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Abstract—Cage amines **1**–**4** are potent peripheral anionic site-bound reversible inhibitors of both acetylcholinesterase and butyrylcholinesterase. Cage amines **1**–**3** are selective butyrylcholinesterase inhibitor versus acetylcholinesterase. For both enzymes, the $-\log K_i$ values linearly correlate with the difference of substituted phenyl radius of cage amines ($-\log K_i = 5.4 + 3.4\Delta\gamma$ for acetylcholinesterase, $-\log K_i = 5.9 + 3.2\Delta\gamma$ for butyrylcholinesterase). Moreover, the relationship between the enzymes and cage amines mimics that between bottles and stoppers.
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The drugs used in Alzheimer's disease are cholinesterase inhibitors.[1] Two forms of cholinesterase coexist ubiquitously throughout the body, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), and although highly homologous, >65%, they are products of different genes on chromosomes 7 and 3 in humans, respectively.[2]

The analogues of physostigmine (Fig. 1), an alkaloid extracted from a tropical plant, have been shown to display cholinergic activity, and have been widely studied as potential drugs for Alzheimer's disease.[3,4] 2,4,6,8,10,12-Hexabenzyl-2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{5,9}.0^{3,11}]dodecane (**1**), 2,4,6,8,10,12-hexa(2-chloro phenylmethyl)-2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{5,9}.0^{3,11}]dodecane (**2**), 2,4,6,8,10,12-hexa(3,5-dimethoxyphenylmethyl)-2,4,6,8,10,12-hexaazatetracyclo [5.5.0.0^{5,9}.0^{3,11}]dodecane (**3**), and 2,4,6,8,10,12-hexa(3,4-methylenedioxyphenyl-methyl)-2,4,6,8,10,12-hexaazatetracyclo [5.5.0. 0^{5,9}.0^{3,11}] dodecane (**4**) are called as cage amines and available in one step reaction (Scheme 1).[5] Cage amines partially resemble the structure of physostigmine (Fig. 1) and are less possible to enter the narrow gorge active sites of AChE[6–11] and BchE[12,13] due to the bulkiness of cage amines.

The active site serine, S200(or 198), of both AChE and BChE is located at the bottom of a 20 Å gorge.[6–8,13] Cage amines may bind to the peripheral anionic site (PAS) of both enzymes, which is located at the entrance of active site gorge and is regarded as the recognition binding site.[13] Since an active site-bound reversible inhibitor such as edrophonium (Fig. 1)[7,8] further inhibits both enzymes in the presence of cage amines **1**–**4**, cage amines **1**–**4** do not bound to active site but PAS of both enzymes (Fig. 2).

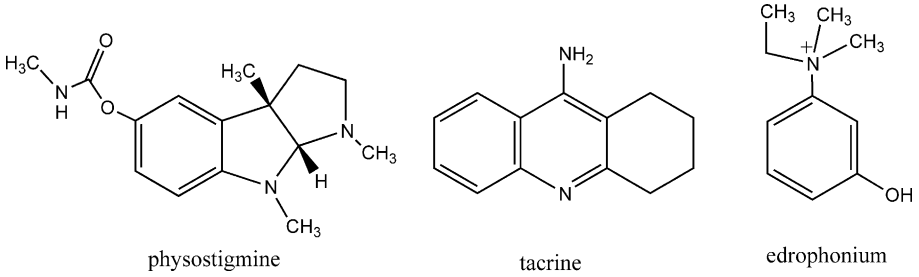

Figure 1. Structures of physostigmine, tacrine, and edrophonium.

*Corresponding author. Tel.: +886-930-383816; fax: +886-4-286-2547; e-mail: gilin@dragon.nchu.edu.tw

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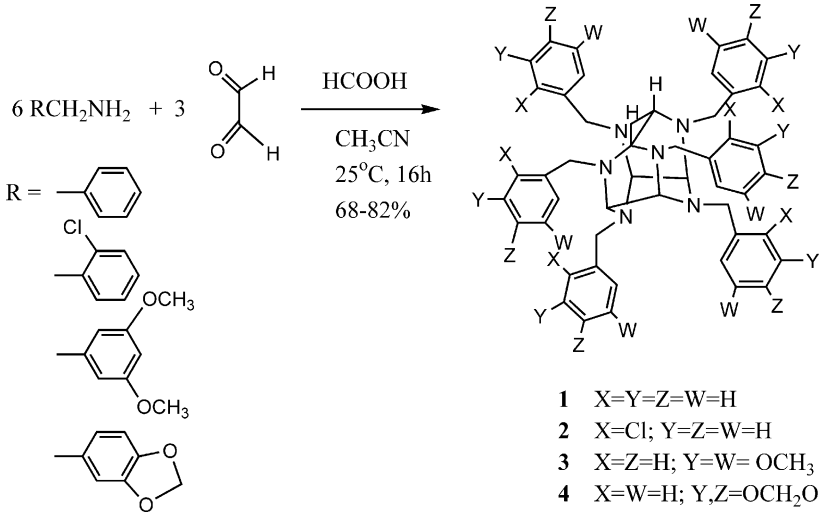

Scheme 1. Synthesis of cage amines **1–4**.

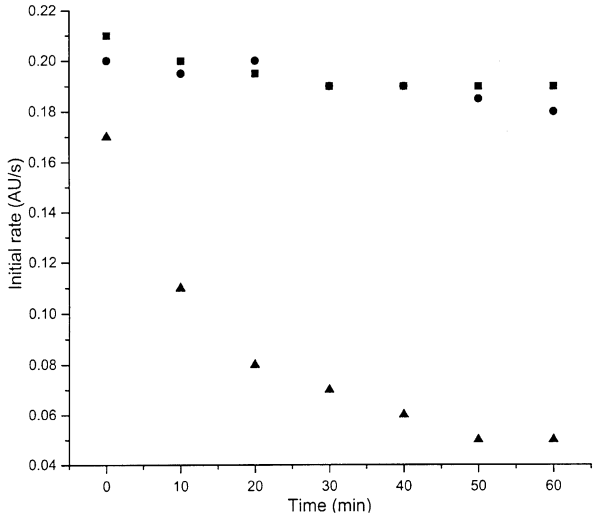

Figure 2. Stopped-time assay for AChE inhibition by cage amine **1**. Initial rates were AChE catalyzed hydrolysis of acetylthiocholine (0.1 mM) in the presence of 5,5′-dithio-bis-2- nitrobenzoate (0.1 mM) and cage amine **1** (0.1 mM) at 25 °C. The control, squares, were initial rates for incubation of AChE at 25 °C for a period of time before the reaction. Circles were incubation of AChE and cage amine **1** at 25 °C for a period of time before the reaction. Triangles were incubation of AChE, cage amine **1**, and edrophonium (0.1 mM) at 25 °C for a period of time before the reaction.

Table 1. $\Delta\gamma$ and kinetic data for AChE and BChE inhibitions by cage amines **1–4**

Inhibitors	Substituents	$\Delta\gamma$ (Å)[a]	AChE K_i (nM)[b]	BChE K_i (nM)[c]	BChE Selectivity[d]
1[e]	H	0	4000±1000	900±300	4.4
2	*o*-Cl	0.582	39±3	10±3	3.9
3	3,5-di-OCH_3	0.468	130±9	60±10	2.2
4	3,4-OCH_2O	0.279	320±50	310±80	1.0

[a]Calculation from the MM-2 minimized 3D structure by Chem 3D™.
[b]Determination by the Ellman assay[15] as described by Radić et al.[16] and Nair et al.[17] The K_i values were obtained from the Lineweaver–Burk plots. Initial rates of *Electrophorus electricus* AChE (Sigma)-catalyzed hydrolysis of acetylthiocholine (0.1 mM) in the presence of 5,5′-dithio-bis-2-nitrobenzoate (0.1 mM) and cage amines **1–4** were followed continuously at 410 nm on an UV–visible spectrometer (Agilent 8453).
[c]Horse serum BChE (Sigma)-catalyzed hydrolysis of butyrylthiocholine (0.1 mM). All the other procedures were the same as described in footnote b.
[d]K_i(AChE)/K_i(BChE).
[e]Synthesis from Scheme 1.[5] Characterization of **1**: Yellow solid; mp = 153–157 °C; ^{1}H NMR ($CDCl_3$) δ 3.62 (2H, ring C*H*), 4.08 (4H, ring C*H*), 4.11 (8H, benzyl CH_2), 4.20 (s, 4H, benzyl CH_2), 7.18–7.27 (30H, phenyl); ^{13}C NMR ($CDCl_3$) δ 56.2, 56.8 (benzyl *C*H_2), 76.7, 80.6 (ring), 126.6, 126.7, 128.0, 128.1, 128.3, 129.1 (phenyl); IR (KBr) λ 2853, 1637, 1351, 699 cm^{-1}; Mass spectrum 708 (M^+, 10), 617(100), 341(100). Anal. calcd for $C_{48}H_{48}N_6$: C, 81.32; H, 6.83; N, 11.86. Found C, 81.31; H, 6.79; N, 12.12.

Cage amines **1–4** are reversible inhibitors of both AChE and BChE (Table 1). Like physostigmine[14] and tacrine[11] (Fig. 1), cage amines **1–3** are selective inhibitors of BChE versus AChE (Table 1).

Difference in substituted phenyl radius ($\Delta\gamma$) is defined as substituted phenyl radius minus phenyl radius (Fig. 3 and Table 1). The $-\log K_i$ values for both AChE and BChE inhibitions linearly correlate with $\Delta\gamma$ (Fig. 4 and Table 2) by eq 1, where the h and s values are the calculated $-\log K_i$ values for cage amine **1** ($-\log K_{i0}$) and the substituent steric sensitivity, respectively.

$$-\log K_i = h + s\Delta\gamma \quad (1)$$

Table 2. Correlation of the $-\log K_i$ values of AChE and BChE inhibitions by cage amines **1–4**[a] with $\Delta\gamma$

Enzyme	h	s	R
AChE	5.4±0.1	3.4±0.3	0.992
BChE	5.9±0.3	3.2±0.3	0.957

[a]Correlations with eq 1, $-\log K_i = h + s\Delta\gamma$.

Straight lines are linear least-squares fits to eq 1 and give $s = 3.4\pm0.3$ and $s = 3.2\pm0.7$ for AChE and BChE inhibitions, respectively (Fig. 4 and Table 2). Since these s values are about the same, cage amines interact with

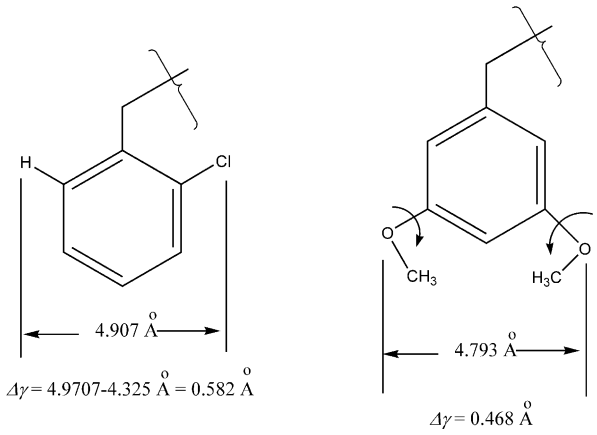

Figure 3. $\Delta\gamma$ of cage amines **2** and **3**.

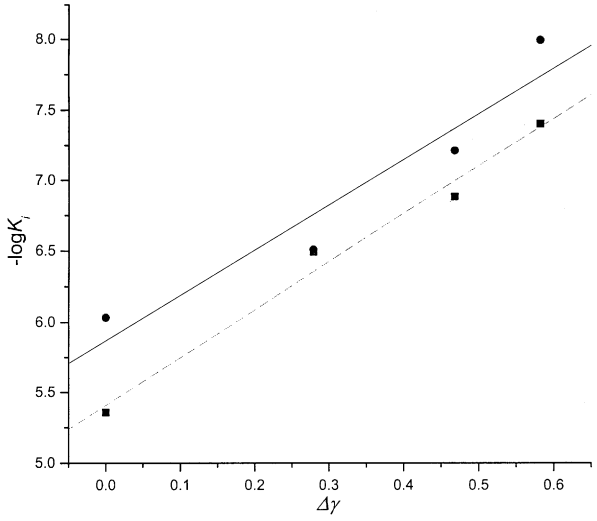

Figure 4. Correlations of the $-\log K_i$ values of AChE and BChE inhibitions by cage amines **1**–**4** against $\Delta\gamma$. Squares and circles are the $-\log K_i$ values of AChE and BChE, respectively. Straight lines are linear least-squares fit to eq 1 and give $s = 3.4 \pm 0.3$ and $s = 3.2 \pm 0.7$ for AChE (dashed line) and BChE (solid line) inhibitions, respectively.

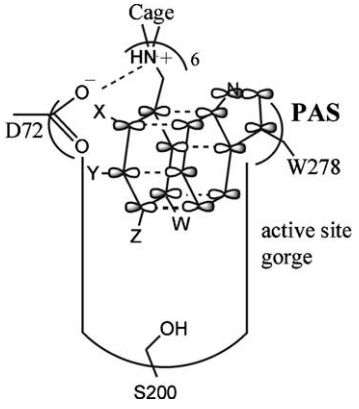

Figure 5. Stopper-bottle type interaction between cage amines **1**–**4** (stopper) and AChE (bottle). PAS is located at the entrance of active site gorge and mimics the bottle mouth. The π–π interaction between W278[6,7] and cage amine phenyl group and the ionic interaction between D72 anion[6,7] and the protonated quaternary amine cation of cage amine are proposed.

both enzymes in a similar way for substituent steric effect of cage amines. Positive *s* values indicate that bulky substituted cage amines fit well into the entrance of active site gorge, PAS, through the well known π–π interaction between cage amine phenyl group and W278 of AChE or Y332 of BChE (Fig. 5).[8,12] This interaction for AChE is slightly better than that for BChE probably because tryptophan provides more π electrons than tyrosine.

Since the $-\log K_i$ values do not correlate with Hammett substituent constant, σ, electronic effects of cage amine substituents are unimportant. Thus, interactions between cage amines and PAS are mostly steric effects. On the other hand, the ionic interaction between the aspartate anion of PAS (D72 of AChE or D70 of BChE)[13] and the protonated quaternary amine cation may occur (Fig. 5).

Overall, the relationship between cholinesterases and cage amines **1**–**4** mimics that between bottles and stoppers.

Acknowledgements

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