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Preparation of secolycorines against acetylcholinesterase

Shoei-Sheng Lee,[a,*] Uppala Venkatesham,[a] Chitneni Prasad Rao,[a] Sio-Hong Lam[a] and Jung-Hsin Lin[a,b,c]

[a]*School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Rd., Sec. 1, Taipei 100, Taiwan, ROC*
[b]*Division of Mechanics, Research Center for Applied Sciences, Academia Sinica, Taipei 115, Taiwan, ROC*
[c]*Institute for Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, ROC*

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Abstract—5,6-Secolycorines possessing a 5,6-dihydrophenanthridine skeleton were facilely prepared from lycorine through chemical transformations, mainly including N-alkylation, Hofmann degradation type reaction, reductive cleavage of trichloroethylcarbonyl moiety, and hydrogenation. Several secolycorine derivatives showed potent inhibitory activity against acetylcholinesterase with the IC_{50} value at micromolar range and are more potent than galanthamine.

1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative, and irreversible disorder that causes intellectual impairment and cognitive dysfunction.[1] In the last decade, treatment for AD has been based on the 'cholinergic hypothesis'.[2] This hypothesis suggested that patients with AD suffer from a deficit of cholinergic function in the brain such as decrease in hippocampal and cortical levels of the acetylcholine (ACh) and associated enzyme choline transferase. Inhibition of acetylcholinesterase (AChE), an enzyme responsible for the metabolic breakdown of ACh, can restore the level of ACh in the brain.[3] Nowadays, four AChE inhibitors, including tacrine, donepezil, rivastigmine, and galantamine, are used clinically for the treatment of mild to moderate disease stage, which is a short interval in the course of AD.[4] Hence the development of drugs for more serious AD is essential. Lycorine (**1**) is the first Amaryllidaceous alkaloid isolated in 1877 from *Narcissus pseudonarcissus*.[5] It has a tetracyclic pyrrole[*d*,*e*]phenanthridine (galanthan) skeleton and has been demonstrated to possess several biological activities, such as inhibition of protein and DNA synthesis,[6] cell growth and division,[7] and antiviral activity.[8,9] It also displays weak activity (IC_{50} 450 μM) against AChE.[10] Assoanine was found to be the most active AChE inhibitor (IC_{50} 3.87 μM) among more than 20 lycorine related alkaloids.[11,12] Its higher activity was explained by the presence of an aromatic ring C, providing a planar-like skeleton.[11,12] Whether the intact D-ring is essential for anti-AChE activity, however, had not been reported yet. Based upon these, we planned to prepare a series of *N*-alkyl-5,6-secolycorine derivatives, possessing a planar-like dihydrophenanthridine moiety devoid of D-ring, and evaluate their anti-AChE activity. Since lycorine (**1**) possesses a skeleton similar to the target designed and was found to be a major alkaloid in *Crinum asiaticum* var *sinicum* in our preliminary study, it was chosen as starting material in this study. The following describes the outcome of this effort.

2. Results and discussion

Lycorine (**1**) was facilely isolated from the MeOH extract of *C. asiaticum* var *sinicum*, simply by filtering the precipitate obtained during alkalinization of the acid soluble fraction as described in Section 5. Peracetylation of **1** by Ac_2O/py yielded diacetyllycorine (**2**),[13] δ_{Ac-Me} 2.00 and 2.20. N-Alkylation of **2** with various alkyl halides ($C_nH_{2n+1}X$, n = 1–6, X = I or Br) under refluxing CH_3CN gave the corresponding *N*-alkyl quaternary ammonium salts **3a**–**f** as diastereomers in excellent yields (Table 1). While reacting with iodomethane under refluxing $CHCl_3$, **3a** was obtained in a poor yield. Sever-

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* Corresponding author. Tel./fax: +886 2 23916127; e-mail: shoeilee@ha.mc.ntu.edu.tw

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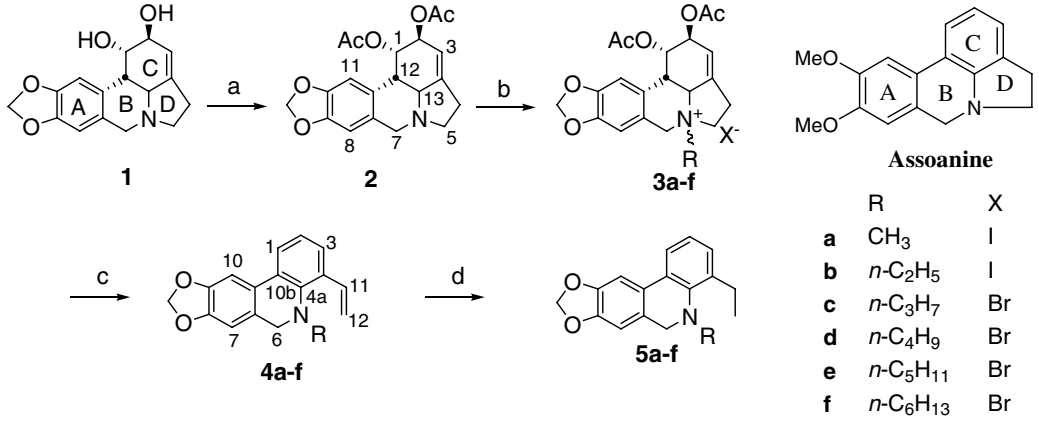
Table 1. Synthesis of diastereomeric mixtures of *N*-alkyl quaternary ammonium salts **3a–f**, and yields (%) of secolycorines **4a–f** and **5a–f**

Compound[a]	Reaction conditions for preparing **3a–f**		Yield		
	Temp. (°C)	RX[b]	Sub	4[c,d]	5 (h)[e]
1	40	CH_3I	**a**	50	66 (3.5)
2	70	C_2H_5I	**b**	76	54 (7)
3	70	*n*-C_3H_7Br	**c**	75	55 (4)
4	82	*n*-C_4H_9Br	**d**	72	61 (16)
5	82	*n*-$C_5H_{11}Br$	**e**	88	52 (17)
6	82	*n*-$C_6H_{13}Br$	**f**	84	60 (24)

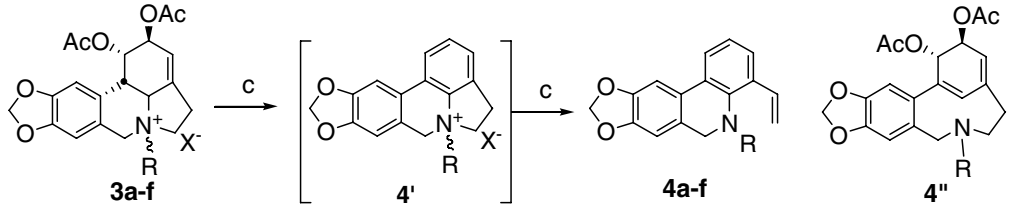
[a] The reactions for preparing **3a–b**, **d–f** were carried out in MeCN for 24 h while for **3c** 36 h.
[b] The low boiling reagents (methyl, ethyl, and propyl halides) were added in excess with frequent intervals.
[c] Two-step yields from **2** to **4a–f**.
[d] Reactions were carried out for 3.5–4 h both for the conversion of **2–3** and **3–4**.
[e] Compounds **4b–c** were reacted in cyclohexane and the rest were run in acetone.

al attempts to run the Hofmann degradation of **3a–f** were made using various conditions. It was found that the optimal reaction conditions were using potassium-*t*butoxide as base and under refluxing *t*butanol (Table 1). Thus **3a** afforded **4a** (50%). Relative to that of **2**, the ^{1}H NMR spectrum of **4a** exhibited additional two AMX systems, one in the aromatic region appearing at δ 7.55 (1H, dd, J = 7.7, 1.3 Hz), 7.44 (1H, dd, J = 7.7, 1.3 Hz), 7.13 (1H, t, J = 7.7 Hz), and the other in the olefinic region appearing at δ 5.29 (1H, dd, J = 11.0, 1.5 Hz), 5.72 (1H, dd, J = 17.8, 1.5 Hz), and 7.22 (1H, dd, J = 17.8, 11.0 Hz). The ^{1}H NMR data incorporating with the high resolution EI-MS, affording a molecular formula $C_{17}H_{15}NO_2$, established **4a** as 5-methyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine. Hence, under such reaction conditions, not only the Hofmann degradation but also elimination of two acetoxyl groups was undertaken. Under similar conditions, compounds **4b–f** were obtained from **3b–f**, respectively, in good yields (72–88%) (Scheme 1). The reason why another series of the Hofmann degradation products, that is, breakdown between C-13 and *N*,[6] were not obtained could be attributable to the reaction order. That is the elimination step took place first to form the dihydrophenanthridine (**4′**), followed by the Hofmann degradation to yield **4a–f** (Scheme 2). In addition, the C-13, *N*[6]-seco products **4″** could not be isolated because their skeleton was highly strained, making them decomposed easily. Respective catalytic hydrogenation of **4a–f** (H_2/Pd–C) yielded the corresponding 5-alkyl-4-ethyl-8,9-methylenedioxy-5,6-dihydrophenanthridines **5a–f** (Scheme 1). The ^{1}H NMR spectrum of **5a** showed signals for 4-ethyl group at δ 1.28 (3H, t) and 2.77 (2H, q), and *N*-Me at δ 2.45 (s), and the ^{13}C NMR showed signals of 4-ethyl group at δ 14.8 (q, C-12) and 23.2 (t, C-11), while those of C-4a and C-6 at δ 145.5 (s) and 55.3 (t).

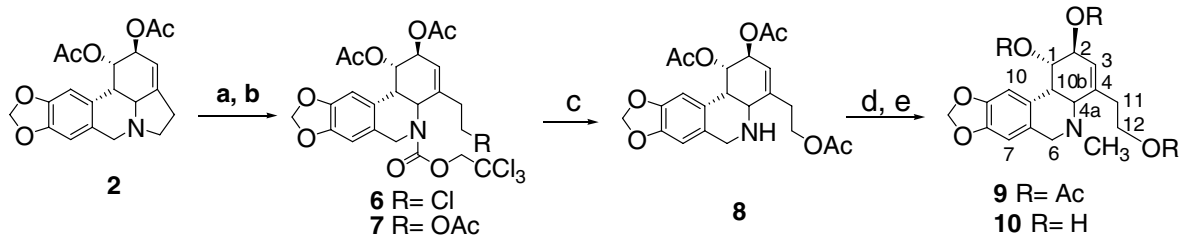
For comparison of anti-AChE activity with compounds **4a–f** and **5a–f**, the preparation of secolycorine derivatives with a partially saturated (**8-10**) and fully saturated ring C (**14–16**) was carried out (Schemes 3 and 4). Treatment of **2** with 2,2,2-trichloroethylchloroformate (TCECF) in the presence of potassium carbonate under



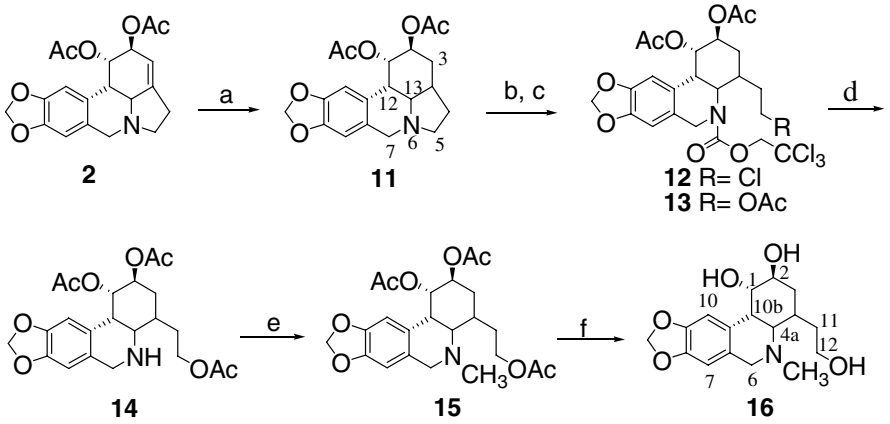
Scheme 1. Reagents and conditions: (a) Ac_2O, py, 50 °C, 13 h, 96%; (b) RX, CH_3CN, 24 h, reflux, 99%; (c) *t*BuOK, *t*BuOH, reflux, 4 h, 50–88%; (d) H_2, 10% Pd–C, acetone or cyclohexane, rt, 16 h, 52–66%.



Scheme 2. Reagents and conditions: (c) *t*BuOK, *t*BuOH, reflux, 4 h.



Scheme 3. Reagents and conditions: (a) $ClCO_2CH_2CCl_3$, K_2CO_3, dry toluene, reflux, 14 h; (b) NaOAc, DMSO, 105 °C, 58%; (c) Zn/90% AcOH, 50 °C, 6 h, 58%; (d) HCHO, $NaBH_3CN$, HOAc–MeOH, rt, 2 h, 90%; (e) K_2CO_3, MeOH–H_2O 9:1, 2 h, 72%.



Scheme 4. Reagents and conditions: (a) H_2 (300 psi)/10% Pd–C, AcOH, rt, 18 h, 60%; (b) $ClCO_2CH_2CCl_3$, K_2CO_3, dry toluene, reflux, 14 h, 57%; (c) NaOAc, DMSO, 105 °C, 6.5 h, 94%; (d) Zn/90% AcOH, 50 °C, 4 h, 43%; (e) HCHO/$NaBH_3CN$, AcOH–MeOH, rt, 2 h, 100%; (f) K_2CO_3, MeOH–H_2O 9:1, 2 h, 90%.

refluxing toluene yielded a major secolycorine carbamate **6**, obtained by cleaving C5–N^6 bond in the strained ring D. That a reductive cleavage of the N-protecting group in **6** with zinc dust in 90% HOAc regenerated **2** suggested the spacious vicinity between N^6 and the C-4 chloroethyl group as depicted in **6**. Nucleophilic displacement of the halide by an acetoxyl group in **6** with NaOAc in DMSO, affording **7**, followed by a reductive cleavage of the N-protecting group with freshly prepared zinc dust in 90% AcOH, yielded 5-acetoxy-diacetyl-5,6-secolycorine (**8**) (58%), whose ^{1}H NMR spectrum showed signals of three Ac–*Me* (δ 1.89, 1.98, and 2.04), H-4a (δ 3.43, d, J = 10.0 Hz), and H-6s (δ 3.98 and 4.07, each d, J_{gem} = 16.3 Hz). Reductive N-methylation of **8** (HCHO/$NaBH_3CN$) yielded **9** (90%), whose ^{1}H NMR spectrum ($CDCl_3$) showed signals of *N*-Me (δ 2.16), H-4a (δ 3.62, d, J = 10.7 Hz) and H-6s (δ 3.57 and 4.28, both doublet, J = 16.0 Hz). Subsequent O-deacetylation of **9** (K_2CO_3/$MeOH_{aq}$) gave the desired 5-hydroxy-*N*-methyl-5,6-secolycorine (**10**) (72%). The ^{1}H NMR spectrum of **10** (CD_3OD) showed the signals of H-4a (δ 3.69, d, J = 11.0 Hz), H-6s (δ 3.62/4.21, both d, J = 16.6 Hz), and H-12s (δ 3.66, 2H, m). Its high resolution EI-MS, affording a molecular formula $C_{17}H_{21}NO_5$, was also supportive for this structure assignment for **10**.

Starting from diacetyldihydrolycorine (**11**), obtained by catalytic hydrogenation of **2**, the 5,6-seco-dihydrolycorine **16** was prepared by a similar procedure as for the synthesis of **10** (Scheme 4). Treatment of **11** with TCECF, followed by NaOAc-DMSO and reductive cleavage of the N-protecting group (Zn–90% HOAc), yielded **14** (43%), whose ^{1}H NMR spectrum ($CDCl_3$) showed the signals of H-4a, H-6, and H-10b at δ 3.26 (dd), 3.95 (d) and 4.08 (d) (J_{gem} 15.6 Hz) and 3.19 (br d), respectively. Reductive N-methylation of **14** yielded **15** (~100%), whose ^{1}H NMR spectrum ($CDCl_3$) displayed the signals of N-*Me*, H-4a, H-6, and H-10b at δ 2.29 (s), 2.47 (dd), 3.35 and 3.73 (J_{gem} 14.4 Hz) and 3.25 (br d), respectively. O-Deacetylation under alkaline conditions gave **16** (90%), whose ^{1}H NMR spectrum (CD_3OD) exhibited the signals of N-*Me*, H-4a, H-6, and H-10b at δ 2.35 (s), 2.66 (dd), 3.41 (d) and 3.72 (d) (J_{gem} 14.7 Hz), and 3.27 (br d), respectively. The high resolution EI-MS of **16**, affording a molecular formula $C_{17}H_{23}NO_5$, was also supportive for this structure assignment.

3. Bioactivity

The anti-AChE activity of these prepared 5,6-secolycorine derivatives was evaluated by in vitro AChE inhibition assay, modified from Ellman's method.[14,15] The results were expressed as IC_{50} values and are summarized in Table 2. From these data, four conclusions are drawn. First, the cleavage of ring D does not decrease the anti-AChE activity. Instead, some advantages of such modification, including enhancing both potency

Table 2. Inhibitory effect of secolycorine derivatives against EelAChE (IC_{50}, μM) and the calculated binding free energies (ΔG_{calc}, kcal/mol) of compounds **4** and **5** series to EelAChE

Compound	IC_{50}[a]	Compound	IC_{50}[a]	ΔG_{calc}	Compound	IC_{50}[a]	ΔG_{calc}
1	nd[b]	**4a**	2.89	−9.36	**5a**	3.00	−9.28
3a	nd	**4b**	8.35	−8.91	**5b**	8.82	−9.28
9	nd	**4c**	10.59	−10.67	**5c**	9.98	−11.58
10	nd	**4d**	2.66	−10.30	**5d**	5.44	−11.54
15	nd	**4e**	<5	−11.80	**5e**	<5	−11.30
16	nd	**4f**	<5	−12.51	**5f**	<5	−10.10
Galanthamine	3.18						

[a] The IC_{50} values were calculated from the dose–response curve of six concentrations of each test compound in triplicate.
[b] No significant anti-AChE activity was observed at 10 μM.

and solubility relative to those of assoanine, are observed. For example, 4-ethyl-*N*-methyl-dihydrophenanthridine (**4a**) (IC_{50} 2.89 μM; for galanthamine IC_{50} 3.18 μM) is more potent than the reported assoanine (IC_{50} 3.87 μM; for galanthamine IC_{50} 1.07 μM).[11] Second, the length of N-substitution in dihydrophenanthridines of series **4** and **5** is critical to the activity. *N*-Methyl or *N*-butyl substitution seems to be optimal for anti-AChE activity, which decreases two to four times for *N*-ethyl (**4b**, **5b**) or *N*-propyl derivatives (**4c** and **5c**). With longer side chain (*N*-pentyl **4e/5e** and *N*-hexyl **4f/5f**), the bioassay, however, was hampered by the poor solubility in the test solution, even as the tartaric acid salts. From the saturated solution, the IC_{50} values of **4e** and **4f** were estimated to be less than 5 μM. Third, although the structures of 4-vinyl-5,6-dihydrophenanthridines (**4a**–**f**) are more planar than those of the corresponding 4-ethyl derivatives (**5a**–**f**), their anti-AChE activities seem to show no significant difference. Fourth, the increase of solubility with nonplanar ring C causes the dramatic loss of the anti-AChE activity as that observed for 5-hydroxy-5,6-secolycorine (**10**) and 5-hydroxy-5,6-seco-dihydrolycorine (**16**). Compounds **9** and **15**, the peracetyl derivatives of **10** and **16**, are much less polar but more bulky relative to the parent compounds. They did not show significant anti-AChE activity at 10 μM level similar to that observed for **10** and **16**. Accordingly, steric effect might be another factor to be clarified concerning anti-AChE activity.

4. Docking calculations

In order to elucidate the relationships between activities and the structures of these compounds, we performed docking calculations using AutoDock 3.05[16] and MEDock.[17] The IC_{50} values from the enzyme assay and the calculated binding free energies, obtained from docking of active compounds to an analogy to the eel AChE (EelAChE) (for details, see Section 5), are listed in Table 1. The IC_{50} values of some compounds cannot be determined due to poor solubility, which is a natural result of long hydrophobic moieties of these compounds. Due to the sequence mismatch between EelAChE and mAChE (mouse AChE), as well as the accuracy of the AutoDock scoring function, the predicted binding free energy does not match the experimental values on a number-by-number basis. However, an interesting correlation between experiments and simulation can be found for the effect of different lengths for the N-alkyl chain of the compounds **4a**–**f** and **5a**–**f**. Initially, the binding affinity decreases by increasing the *N*-alkyl chain length, but then increases again with even longer alkyl chain, although the predicted alkyl chain length for the minimum of binding affinity has been offset from that of experimental results. This trend is identical for both series of compounds, indicating that there is a second binding pocket just adjacent to that of the active site pocket. The second binding pocket should be mainly hydrophobic, while the first binding pocket should be more hydrophilic. On the other hand, based on the analysis using LIGPLOT,[18] it was also suggested that number of residues having hydrophobic contacts with the ligand can be considered as a good measure for prediction of binding affinities for compound **4** series. For example, the number of residues of AChE involved in the hydrophobic contacts with compound **4a** and compound **4d** are 12 and 11, respectively, while those for compound is **4b** and **4c** it is only 8 and 6 (Fig. 1), respectively, correlating very well with the reduction of their binding affinities. Although the IC_{50} values of compounds **4e**, **4f**, **5e**, and **5f** cannot be determined due to poor solubility, the incomplete inhibitory assays did indicate that their potencies should be at least as good as the best compound in each series. This suggests that hydrophobic interaction plays a key role for binding these compounds to AChE, and therefore the inhibitory effect of the these compounds. It should be noted that the *N*-alkyl chain needs be tailored to an optimal length in order to make a balance between the binding affinity and solubility.

5. Experimental

5.1. General

All reagents were used as purchased from the commercial suppliers without further purification. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. Chemical shifts are reported in δ ppm referenced to $CDCl_3$ for ^1H NMR (δ 7.26) and for ^{13}C NMR (δ 77.0). Infrared spectra were recorded on Jasco FT/IR-410 spectrometer and only the characteristic peaks were quoted. EI mass spectra were recorded on a Finnigan TSQ-700 mass spectrometer (70 eV) and HRMS spectra were recorded on a JEOL SX-102A mass spectrometer. Silica gel TLC was performed on aluminum sheet-backed TLC

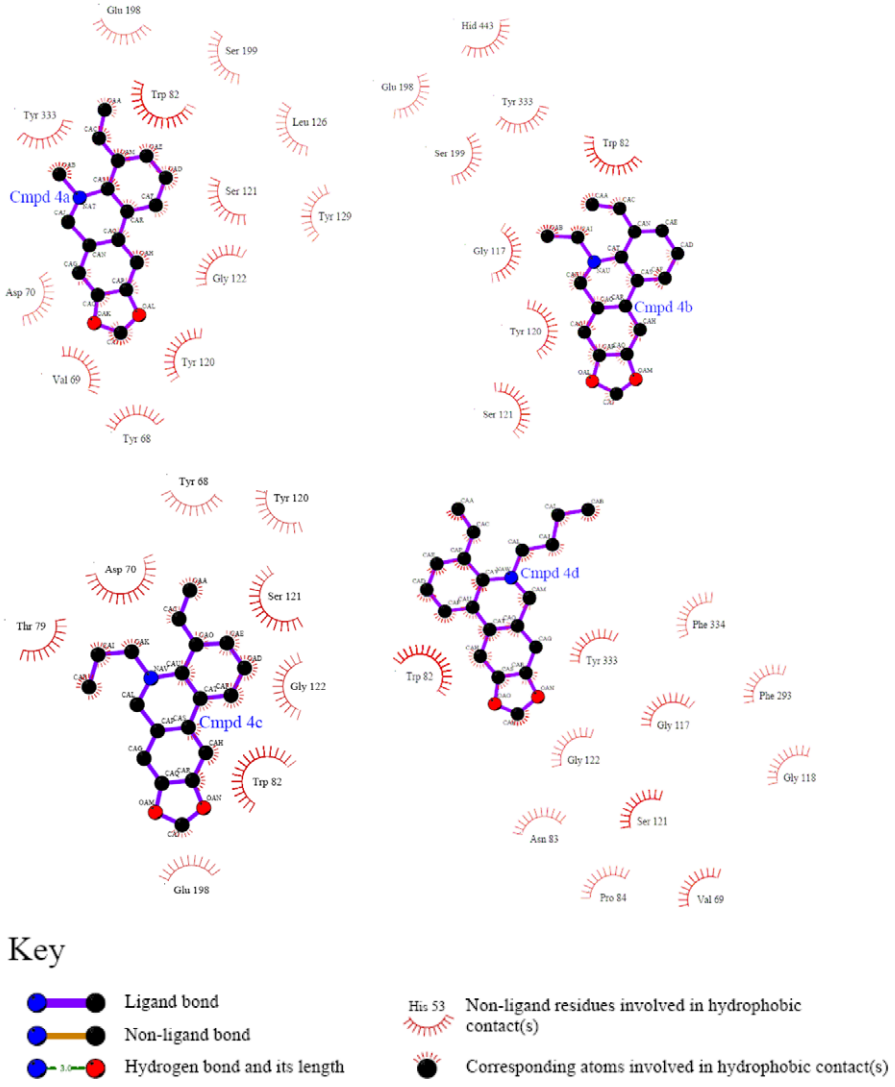


Figure 1. The diagrammatic representation of the interactions between **4** and EelAChE by LIGPLOT.[18]

plates (Merck Kieselgel 60F_{254}), visualized under UV 254 nm and Dragendorf's spray agent. Flash chromatography was performed on silica gel (230–400 mesh).

5.2. Isolation of lycorine (1)

The dry powdered leaves (2.84 kg) of *C. asiaticum* var. *sinicum*, collected in May 2004 in Pin-Lin area, Taipei County, Taiwan, were percolated with 95% EtOH (10 L × 5) at room temperature. Evaporation under reduced pressure gave an EtOH extract (1.02 kg) which was triturated with 2% $HOAc_{(aq)}$ (500 mL × 3). The combined acidic solutions were extracted with EtOAc (1.2 L × 3) to give an EtOAc soluble fraction (13.63 g). The aqueous layer was adjusted to pH 9 with 25% ammonia water, and the crystalline lycorine (**1**, 13.70 g), $R_f = 0.42$ [$CHCl_3$–MeOH–NH_4OH (85:15:0.1)], was collected by filtration.

5.3. Preparation of 5-alkyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4a–f)

5.3.1. Preparation of diacetyllycorine (2). The mixture of **1** (3.17 g, 11 mmol), pyridine (8.0 mL), and acetic anhydride (9.5 mL) in a sealed flask was stirred at 50 °C for 18 h, then abs. ethanol (25 mL) was added and was stirred for 3 h at room temperature. Condensation with the aid of toluene (4× 25 mL) afforded diacetyllycorine (**2**) (3.89 g, yield 96%),[13] $R_f = 0.72$ [$CHCl_3$–MeOH (94:6)].

5.3.2. General procedure for preparing diacetyllycorine *N*-alkyl salt (3a–f). To the solution of diacetyllycorine (**2**, 25 mg, 0.067 mmol) in acetonitrile (2 mL, LC grade) was added alkyl halide (0.5 mL, excess) via cannula. The reaction mixture was stirred under N_2 and heating conditions (see Table 1). After completion of the reaction, the reaction mixture was concentrated and the residue (**3a–f**) without further purification was subjected to Hofmann degradation reaction.

5.3.3. General procedure for preparing 5-alkyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4a–f). To one of compounds **3a–f** (1.5 mmol) dissolved in *tert*-BuOH (5 mL) was added potassium *tert*-butoxide (10.5 mmol, 7 equiv). The reaction mixture was refluxed under nitrogen. After the reaction was complete, water (50 mL) was added and the pH value was adjusted to 9

with saturated NH_4Cl solution. The resultant solution was partitioned with chloroform (3× 50 mL). The organic layer was dried over anhyd Na_2SO_4, concentrated to give a residue, which was subjected to silica gel flash column chromatography (FCC), eluted with hexane–$CHCl_3$ (25:75) to afford the corresponding secolycorine **4**.

5.3.4. General procedure for preparing 5-alkyl-4-ethyl-8,9-methylenedioxy-5,6-dihydro-phenanthridine (5a–f). To compound **4** (0.127 mmol) dissolved in a suitable solvent was added 10% Pd/C (20% w/w). The suspension was degassed under vacuum and then flushed with H_2 gas. This procedure was repeated three times and then the reaction was undertaken under H_2 (1 atm) at room temperature overnight. The reaction mixture was then filtered through a Celite pad and concentrated to give a residue, which was subjected to silica gel FCC, eluted with hexane–EtOAc (1:1), to give **5**.

5.3.4.1. 5-Methyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4a). Yield 50%; $R_f = 0.38$, hexane–$CHCl_3$ (4:6); ^1H NMR ($CDCl_3$) δ 2.49 (3H, s, N-*Me*), 4.01 (2H, s, H-6), 5.29 (1H, dd, $J = 11.0$, 1.5 Hz, H-12_E), 5.73 (1H, dd, $J = 17.8$, 1.5 Hz, H-12_Z), 5.96 (2H, s, –OCH_2O–), 6.69 (1H, s, H-7), 7.13 (1H, *t*-like, $J = 7.7$ Hz, H-2), 7.21 (1H, s, H-10), 7.22 (1H, dd, $J = 17.8$, 11.0 Hz, H-11), 7.44 (1H, dd, $J = 7.7$, 1.3 Hz, H-3), 7.55 (1H, dd, $J = 7.7$, 1.3 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 41.6 (q, N-*Me*), 54.9 (t, C-6), 101.0 (t, –O*C*H_2O–), 103.7 (d), 107.1 (d), 114.3 (t, C-12), 122.7 (d), 124.3 (d), 124.9 (d), 125.9 (s), 126.5 (s), 129.3 (s), 133.2 (s), 133.5 (d, C-11), 145.2 (s, C-4a), 147.3 (s) and 147.4 (s) (C-8 and C-9); EIMS *m/z* $[M]^+$ 265 (89), $[M-H]^+$ 264 (100), 250 (M^+-15) (14); HREIMS *m/z* $[M]^+$ 265.1083 (calcd for $C_{17}H_{15}NO_2$, 265.1097).

5.3.4.2. Common ^1H and ^{13}C NMR data for 5-alkyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4b–f). ^1H NMR ($CDCl_3$) δ 4.02–4.04 (2H, s, H-6), 5.26–5.27 (1H, dd, $J = 11.0$, 1.5 Hz, H-12_E), 5.69–5.71 (1H, dd, $J = 17.8$, 1.5 Hz, H-12_Z), 5.96 (2H, s, –OCH_2O–), 6.68–6.69 (1H, s, H-7), 7.11–7.13 (1H, *t*-like, $J = 7.7$ Hz, H-2), 7.20–7.21 (1H, s, H-10), 7.17–7.23 (1H, dd, $J = 17.8$, 11.0 Hz, H-11), 7.44–7.46 (1H, dd, $J = 7.7$, 1.3 Hz, H-3), 7.54–7.55 (1H, dd, $J = 7.7$, 1.3 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 49.8–50.2 (t, C-6), 100.9 (t, –O*C*H_2O–), 103.7 (d), 106.8–106.9 (d), 113.8–113.9 (t, C-12), 122.6 (d), 123.9 (d), 125.0–125.1 (d), 126.5 (s), 127.2–127.3 (s), 129.6 (s), 133.2 (s), 133.7–133.9 (d, C-11), 145.3–145.5 (s, C-4a), 147.2 (s) and 147.3 (s) (C-8 and C-9).

5.3.4.3. 5-Ethyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4b). Yield 76%; $R_f = 0.46$, hexane–$CHCl_3$ (4:6); other ^1H NMR ($CDCl_3$) δ 1.03 (3H, t, $J = 7.1$ Hz, H-2′), 2.73 (2H, q, $J = 7.1$ Hz, H-1′); other ^{13}C NMR ($CDCl_3$) δ 13.5 (q, C-2′), 47.4 (t, C-1′); EIMS *m/z* $[M]^+$ 279 (91), $[M-H]^+$ 278 (100), 250 $(M^+-29)^+$ (88); HREIMS *m/z* $[M]^+$ 279.1245 (calcd for $C_{18}H_{17}NO_2$, 279.1254).

5.3.4.4. 5-Propyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4c). Yield 75%; $R_f = 0.54$, hexane–$CHCl_3$ (4:6); other ^1H NMR ($CDCl_3$) δ 0.82 (3H, t, $J = 7.4$ Hz, H-3′), 1.49 (2H, m, H-2′), 2.65 (2H, m, H-1′); other ^{13}C NMR ($CDCl_3$) δ 11.4 (q, C-3′), 21.3 (t C-2′), 55.1 (t, C-1′); EIMS *m/z* $[M]^+$ 293 (90), $[M-H]^+$ 292 (73), 264 (M^+-29) (88), 250 (M^+-43) (100); HREIMS *m/z* $[M]^+$ 293.1409 (calcd for $C_{19}H_{19}NO_2$, 293.1410).

5.3.4.5. 5-Butyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4d). Yield 72%; $R_f = 0.54$, hexane–$CHCl_3$ (4:6); other ^1H NMR ($CDCl_3$) δ 0.85 (3H, t, $J = 7.3$ Hz, H-4′), 1.25 (2H, m, H-3′), 1.46 (2H, m, H-2′), 2.65 (2H, m, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-4′), 20.2 (t, C-3′), 30.4 (t, C-2′), 52.8 (t, C-1′); EIMS *m/z* $[M]^+$ 307 (72), $[M-H]^+$ 306 (53), 264 (M^+–C_3H_7) (91), 250 (M^+–C_4H_9) (100); HREIMS *m/z* $[M]^+$ 307.1570 (calcd for $C_{20}H_{21}NO_2$, 307.1567).

5.3.4.6. 8,9-Methylenedioxy-5-penty-4-vinyl-5,6-dihydrophenanthridine (4e). Yield 88%; $R_f = 0.53$, hexane–$CHCl_3$ (4:6); other ^1H NMR (400 MHz; $CDCl_3$) δ 0.84 (3H, t, $J = 7.2$ Hz, H-5′), 1.19 (4H, m, H-3′ and 4′), 1.49 (2H, m, H-2′), 2.63 (2H, m, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-5′), 22.5 (t, C-4′), 27.9 (t, C-3′), 29.1 (t, C-2′), 53.0 (t, C-1′); EIMS *m/z* $[M]^+$ 321 (52), $[M-H]^+$ 320 (41), 264 (M^+-57) (79), 250 (M^+-71) (100); HREIMS *m/z* $[M]^+$ 321.1714 (calcd for $C_{21}H_{23}NO_2$, 321.1723), $[M-H]^+$ 320.1648 (calcd for $C_{21}H_{22}NO_2$, 320.1645).

5.3.4.7. 5-Hexyl-8,9-methylenedioxy-4-vinyl-5,6-dihydrophenanthridine (4f). Yield 84%; $R_f = 0.53$, hexane–$CHCl_3$ (4:6); other ^1H NMR ($CDCl_3$) δ 0.84 (3H, t, $J = 6.8$ Hz, H-6′), 1.24 (6H, m, H-3′, 4′ and 5′), 1.47 (2H, m, H-2′), 2.63 (2H, t, $J = 7.5$ Hz, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-6′), 22.6 (t, C-5′), 26.6 (t, C-4′), 28.2 (t, C-3′), 31.7 (t, C-2′), 53.1 (t, C-1′); EIMS *m/z* $[M]^+$ 335 (60), $[M-H]^+$ 334 (48), 264 (M^+-71) (87), 250 (M^+-85) (100); HREIMS: *m/z* $[M]^+$ 335.1874 (calcd for $C_{22}H_{25}NO_2$, 335.1880).

5.3.4.8. 4-Ethyl-5-methyl-8,9-methylenedioxy-5,6-dihydrophenanthridine (5a). Yield 66%; $R_f = 0.28$, hexane–$CHCl_3$ (4:6); ^1H NMR ($CDCl_3$) δ 1.28 (3H, t, $J = 7.6$ Hz, H-12), 2.45 (3H, s, N-*Me*), 2.78 (2H, q, $J = 7.6$ Hz, H-11), 3.96 (2H, s, H-6), 5.96 (2H, s, –OCH_2O–), 6.69 (1H, s, H-7), 7.13 (1H, *t*-like, $J = 7.6$ Hz, H-2), 7.16 (1H, dd, $J = 7.6$, 2.1 Hz, H-3), 7.22 (1H, s, H-10), 7.47 (1H, dd, $J = 7.6$, 2.1 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 14.8 (q, C-12), 23.2 (t, C-11), 41.0 (q, N-*Me*), 55.3 (t, C-6), 100.9 (t, –O*C*H_2O–), 103.8 (d), 107.2 (d), 121.1 (d), 124.6 (d), 126.4 (s), 126.7 (d), 127.7 (s), 129.4 (s), 139.5 (s), 145.5 (s, C-4a), 147.2 (s) and 147.3 (s) (C-8 and C-9); EIMS: *m/z* 267 $[M]^+$ (50), 266 $[M-H]^+$ (100), 251 (13); HREIMS *m/z*$[M]^+$ 267.1243 (calcd for $C_{17}H_{17}NO_2$, 267.1254).

5.3.4.9. Common ^1H and ^{13}C NMR data for 5-alkyl-4-ethyl-8,9-methylenedioxy-5,6-dihydrophenanthridine (5b–f). ^1H NMR ($CDCl_3$) δ 1.26–1.29 (3H, t, $J = 7.6$ Hz, H-12), 2.75–2.77 (2H, q, $J = 7.6$ Hz, H-11), 3.97–3.99 (2H, s, H-6), 5.95–5.96 (2H, s, –OCH_2O–), 6.68–6.69 (1H, s, H-7), 7.11–7.13 (1H, *t*-like, $J = 7.6$ Hz, H-2), 7.16–7.17 (1H, dd, $J = 7.6$, 2.1 Hz, H-3), 7.21–7.22 (1H, s, H-10), 7.45–7.47 (1H, dd, $J = 7.6$, 2.1 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 14.6–14.7 (q, C-12), 23.3–23.4 (t, C-11), 49.4–50.2 (t, C-6), 100.8–100.9 (t, –O*C*H_2O–), 103.8 (d), 107.0–107.1 (d),

120.9–121.0 (d), 124.3–124.4 (d), 126.9–127.1 (s), 127.2–127.4 (s), 127.8–127.9 (d), 129.6–129.7 (s), 139.4 (s), 145.9–146.1 (s, C-4a), 147.0–147.1 (s) and 147.2–127.3 (s) (C-8 and C-9).

5.3.4.10. 4,5-Diethyl-8,9-methylenedioxy-5,6-dihydrophenanthridine (5b). Yield 54%; R_f = 0.40, hexane–$CHCl_3$ (4:6); other ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, J = 7.1 Hz, H-2′), 2.65 (2H, q, J = 7.1 Hz, H-1′); other ^{13}C NMR ($CDCl_3$) δ 13.6 (q, C-2′), 46.4 (t, C-1′); EIMS *m/z* 281 $[M]^+$ (76), 280 $[M-H]^+$ (100), 266 $[M\text{-}15]^+$ (18), 252 $[M\text{-}29]^+$ (18); HREIMS *m/z* $[M]^+$ 281.1403 (calcd for $C_{18}H_{19}NO_2$, 281.1410).

5.3.4.11. 4-Ethyl-8,9-methylenedioxy-5-propyl-5,6-dihydrophenanthridine (5c). Yield 55%; R_f = 0.48, hexane–$CHCl_3$ (4:6); other ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, J = 7.4 Hz, H-3′), 1.53 (2H, m, H-2′), 2.56 (2H, m, H-1′); other ^{13}C NMR ($CDCl_3$) δ 11.4 (q, C-3′), 21.3 (t, C-2′), 54.0 (t, C-1′); EIMS *m/z* $[M]^+$ 295 (77), $[M-H]^+$ 294 (44), 266 (M^+-29) (100), 252 (M^+-43) (21); HREIMS *m/z* $[M]^+$ 295.1567 (calcd for $C_{19}H_{21}NO_2$, 295.1567).

5.3.4.12. 5-Butyl-4-ethyl-8,9-methylenedioxy-5,6-dihydrophenanthridine (5d). Yield 61%; R_f = 0.50, hexane–$CHCl_3$ (4:6); other ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 7.3 Hz, H-4′), 1.31 (2H, m, H-3′), 1.53 (2H, m, H-2′), 2.61 (2H, m, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-4′), 20.2 (t, C-3′), 30.5 (t, C-2′), 51.9 (t, C-1′); EIMS *m/z* $[M]^+$ 309 (76), $[M-H]^+$ 308 (39), 266 (M^+-43) (100), 252 (M^+-57) (21); HREIMS *m/z* $[M]^+$ 309.1724 (calcd for $C_{20}H_{23}NO_2$, 309.1723).

5.3.4.13. 4-Ethyl-8,9-methylenedioxy-5-pentyl-5,6-dihydrophenanthridine (5e). Yield 52%; R_f = 0.48, hexane–$CHCl_3$ (7:13); other ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, t, J = 6.8 Hz, H-5′), 1.27 (4H, m, H-3′ and 4′), 1.52 (2H, m, H-2′), 2.57 (2H, t, J = 7.0 Hz, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-5′), 22.6 (t, C-4′), 27.9 (t, C-3′), 29.2 (t, C-2′), 52.1 (t, C-1′); ESIMS: $[M+H]^+$*m/z* 324.

5.3.4.14. 4-Ethyl-5-hexyl-8,9-methylenedioxy-5,6-dihydrophe-nanthridine (5f). Yield 60%; R_f = 0.49, hexane–$CHCl_3$ (7:13); other ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, J = 6.8 Hz, H-6′), 1.24 (6H, m, H-3′, 4′ and 5′), 1.51 (2H, m, H-2′), 2.56 (2H, t, J = 7.4 Hz, H-1′); other ^{13}C NMR ($CDCl_3$) δ 14.0 (q, C-6′), 22.6 (t, C-5′), 26.7 (t, C-4′), 28.2 (t, C-3′), 31.7 (t, C-2′), 52.2 (t, C-1′); ESIMS: $[M+H]^+$*m/z* 338.

5.4. Synthesis of 4aα,10bβ-1α,2β-dihydroxy-4-(2-hydroxyethyl)-5-methyl-8,9-methylenedioxy-1,2,4a,5,6,10b-hexahydrophenanthridine (10)

5.4.1. Preparation of the 5,6-secolycorine carbamate 6. To the suspension of **2** (4.83 g, 13.02 mmol), dry toluene (120 mL), and potassium carbonate (5.40 g, 39.1 mmol) was added 2,2,2-trichloroethylchloroformate (TCECF, 3.5 mL, 26.0 mmol) under nitrogen. The reaction mixture was heated for 20 h at 120 °C. After cooling to room temperature, the reaction mixture was washed with water (2× 100 mL) and brine (2× 50 mL) dried over anhyd Na_2SO_4, and concentrated under reduced pressure to give a liquid residue (12.1 g), which was subjected to silica gel FCC, eluted with hexane–$CHCl_3$ (1:3), to give a diacetyl-5,6-secolycorine carbamate (**6**) (4.31 g, 57%).

5.4.2. Preparation of the triacetyl-5,6-secolycorine 8. The solution of compound **6** (50 mg, 86 μmol) and anhydrous sodium acetate (50 mg) dissolved in dry DMSO (1.5 mL) was stirred at 105 °C for 6 h. After cooling, the reaction mixture was partitioned between water (35 mL) and toluene (3× 20 mL). The organic layer was dried over anhyd Na_2SO_4 and concentrated to give a residue, which was purified on a silica gel FCC, eluted with hexane–$CHCl_3$ (35:65), to afford **7** (30 mg, 58%). The suspension of secolycorine **7** (30 mg, 50 μmol) and activated Zn (105 mg, 1.60 mmol) in 90% acetic acid was stirred at room temperature for 4 h. Then the reaction mixture was diluted with chloroform (20 mL) and filtered through a Celite cake. The filtrate was washed with water (2× 5 mL), dried over anhyd Na_2SO_4, and concentrated to give a residue, which was flash chromatographed over a silica gel column, eluted with $CHCl_3$–MeOH (96:4), to afford **8** (8 mg, yield 37%).

5.4.3. Preparation of 5-hydroxy-*N*-methyl-5,6-secolycorine (10). To **8** (6 mg, 14 μmol) dissolved in MeOH (1 mL) were added formalin solution (55 rmuL, 37%), sodium cyanoborohydride (21 mg, 334 μmol), and acetic acid (0.2 mL) in sequence at room temperature. The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. The residue obtained was partitioned between chloroform (25 mL) and satd $NaHCO_3$ (2× 5 mL). The $CHCl_3$ layer was washed with brine solution (2× 5 mL), dried over anhyd Na_2SO_4 and concentrated to give a residue, which was purified by a flash silica gel column, eluted with chloroform, to give **9** (6.1 mg, 100%). The mixture of **9** (67 mg, 150 μmol), potassium carbonate (125 mg, 0.9 mmol), and MeOH–H_2O (9:1) (2.0 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the dry residue was subjected to alumina FCC (neutral), eluted with $CHCl_3$–MeOH (94:6), to give **10** (34.5 mg, yield 72%).

5.4.3.1. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-chloroethyl)-8,9-methylenedioxy-1,2,4a,5,6,10b-hexahydrophenanthridine-5-carboxylic acid 2,2,2-trichloroethyl ester (6). R_f = 0.63, hexane–$CHCl_3$ (2:8), developed three times; ^{1}H NMR ($CDCl_3$) δ 1.90 (3H, s), 2.07 (3H, s), 2.71 (2H, m), 3.58 (1H, m), 3.66 (2H, *t*-like, J = 6.2 Hz), 4.09 (1H, m), 4.37 (1H, m), 4.66 (1H, m), 4.76 (1H,d, J = 10.8 Hz), 5.13 (2H, br s), 5.70 (2H, m, H-1 and H-3), 5.92 (2H, s, –OCH_2O–), 6.56 (1H, s, H-7), 6.74 (1H, s, H-10); ESIMS *m/z* $[M+Na]^+$ 604: $[M+Na+2]^+$ 606: $[M+Na+4]^+$ 608: $[M+Na+6]^+$ 610: $[M+Na+8]^+$ 612 (80:100:50:8:1).

5.4.3.2. 4aα,10bβ-1α,2β-Diacetoxyl-4-(2-acetoxylethyl)-8,9-methylenedioxy-1,2,4a,5,6,11b-hexahydrophenanthridine (8). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.89 (3H, s), 1.98 (3H, s), 2.04 (3H, s) (3× Ac–*Me*), 2.47 (1H, m) and 2.70 (1H, m) (H-11), 2.89 (1H, br d, J = 10 Hz, H-10b), 3.43 (1H, br d, J = 10 Hz, H-4a), 3.98 (1H, d, J = 16.8 Hz) and 4.07 (1H, br d, J = 16.8 Hz) (H-6), 4.25 (2H, m, H-12), 5.06 (1H, s, H-2), 5.61 (2H, s, H-1 and 3), 5.85 (2H, s, –OCH_2O–), 6.45 (1H, s, H-7), 6.71 (1H, s, H-10).

5.4.3.3. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-acetoxylethyl)-5-methyl-8,9-methylenedioxy-1,2,4a,5,6,11b-hexahydrophenanthridine (9). R_f = 0.59, $CHCl_3$–MeOH (98:2); ^1H NMR ($CDCl_3$) δ 1.90 (3H, s), 2.00 (3H, s), 2.07 (3H, s) (3× Ac–*Me*), 2.16 (3H, s, N-*Me*), 2.38 (1H, m) and 2.68 (1H, m) (H-11), 3.15 (1H, d, J = 10.7 Hz, H-10b), 3.62 (1H, d, J = 10.7 Hz, H-4a), 4.17 (1H, m) and 4.29 (1H, m) (H-12), 3.57 (1H, d) and 4.28 (1H, d) (H-6, J_{gem} = 16.0 Hz), 5.06 (1H, br s, H-2), 5.68 (1H, br s, H-1), 5.72 (1H, br s, H-3), 5.88 (2H, s, –OCH_2O–), 6.49 (1H, s, H-7), 6.78 (1H, s, H-10); ^{13}C NMR ($CDCl_3$) δ 20.9 (q, Ac–*Me*), 21.0 (q, Ac–*Me*), 21.2 (q, Ac–*Me*), 30.2 (d, C-10b), 32.4 (t, C-11), 34.9 (q, N-*Me*), 56.9 (d, C-4a), 57.2 (t, C-6), 62.8 (t, C-12), 67.7 (d) and 69.7 (d) (C-1 and C-2), 100.9 (t, –O–CH_2O–), 104.9 (d), 107.2 (d), 121.6 (d, C-3), 126.2 (s), 128.1 (s), 143.4 (s, C-4), 146.5 (s), 146.6 (s), 169.8 (s), 170.1 (s), 170.9 (s) (3× Ac–*C*O); EIMS: *m*/*z* $[M]^+$ 445 (80), 430 (M^+-15) (11), 386 (M^+-59) (75), 385 (M^+-60) (63), 372 (M^+-73) (100).

5.4.3.4. 4aα,10bβ-1α,2β-Dihydroxy-4-(2-hydroxylethyl)-5-methyl-8,9-methylenedioxy-1,2,4a,5,6,11b-hexahydrophenanthridine (10). R_f = 0.44, $CHCl_3$–MeOH–NH_4OH (85:15:0.1); ^1H NMR (CD_3OD) δ 2.21 (3H, s, N-*Me*), 2.32 (1H, m) and 2.63 (1H, m) (H-11), 3.07 (1H, br d, J = 11.0 Hz, H-10b), 3.68 (2H, m, H-12), 3.69 (1H, d, J = 11.0 Hz, H-4a), 4.00 (1H, br s) and 4.50 (1H, br s) (H-1 and H-2), 3.62 (1H, d) and 4.21 (1H, d) (H-6, J_{gem} = 16.6 Hz), 5.68 (1H, br s, H-3), 5.88 (2H, s, –OCH_2O–), 6.56 (1H, s, H-7), 6.91 (1H, s, H-10); ^{13}C NMR (CD_3OD) δ 32.4 (d, C-10b), 35.1 (q, N-*Me*), 38.4 (t, C-11), 57.88 (t, C-6), 57.91 (d, C-4a), 62.2 (t, C-12), 70.4 (d) and 72.7 (d) (C-1 and C-2), 102.3 (t, –O*C*H_2O–), 106.1 (d) and 108.3 (d) (C-7 and C-10), 126.8 (d, C-3), 128.8 (s) and 129.6 (s) (C-6a and C-10a), 142.9 (s, C-4), 147.9 (s) and 148.4 (s) (C-8 and C-9); EIMS *m*/*z* $[M]^+$ 319 (79), 304 (M^+-15) (18), 288 (M^+-31) (100%). HREIMS *m*/*z* $[M]^+$ 319.1414 (calcd for $C_{17}H_{21}NO_5$, 319.1408).

5.5. Synthesis of 4α,4aα,10bβ-1α,2β-dihydroxy-4-(2-hydroxyethyl)-5-methyl-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine (16, 5-hydroxy-*N*-methyl-5,6-seco-dihydrolycorine)

5.5.1. Preparation of 5,6-seco-dihydrolycorine carbamate 12. To the suspension of diacetyldihdrolycorine (**11**, 225 mg, 603 μmol), dry toluene (50 mL), and potassium carbonate (3 equiv, 250 mg, 1.81 mmol) was added TCECF (0.16 mL, 1.21 mmol) under nitrogen. The reaction mixture was heated at 120 °C for 14 h. After similar workup for the preparation of **6**, the residue obtained was subjected to silica gel FCC, eluted with hexane–$CHCl_3$ (3:7), to give the 5,6-secodihydrolycorine carbamate **12** (200 mg, yield 57%).

5.5.2. Preparation of the triacetyl-5,6-seco-dihydrolycorine 14. The solution of compound **12** (50 mg, 86 μmol) and sodium acetate trihydrate (24 mg, ca. 2 equiv) in DMSO was stirred at 105 °C for 6.5 h. After similar workup for the preparation of **7**, the residue obtained was subjected to silica gel FCC, eluted with hexane–EtOAc (6:4), to yield **13** (49 mg, yield 94%). The suspension of **13** (153 mg, 252 μmol) and activated Zn (535 mg, 8.19 mmol) in 90% acetic acid was stirred at 50 °C for 4 h. After a similar workup as for the preparation of **8**, the residue obtained was subjected to silica gel FCC, eluted with $CHCl_3$–MeOH (95:5), to afford the product **14** (47 mg, yield 43%).

5.5.3. Preparation of 5-hydroxy-*N*-methyl-5,6-seco-dihydrolycorine (16). To **14** (23 mg, 0.053 mmol) dissolved in MeOH (1 mL) were added formalin solution (0.2 mL, 37%), $NaCNBH_3$ (81 mg, 1.27 mmol) and acetic acid (0.7 mL) in sequence at room temperature. The reaction mixture was stirred for 2 h. After similar workup as for the preparation of **9**, the residue obtained was subjected to silica gel FCC, eluted with $CHCl_3$–MeOH (97:3), to afford the product **15** (23.5 mg, yield 100%). The mixture of **15** (18 mg, 40.3 μmol), potassium carbonate (17 mg, 121 μmol), and MeOH–H_2O (9:1) (2.0 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the dry residue was flash chromatographed over an alumina column (neutral), eluted with $CHCl_3$–MeOH (95:5), to give **16** (11.6 mg, yield 90%).

5.5.3.1. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-chloroethyl)-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine-5-carboxylic acid 2,2,2-trichloroethyl ester (12). R_f = 0.54 hexane–EtOAc (1:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.07 (6H, s, 2× Ac–*Me*), 2.18 (2H, m), 2.83 (1H, m), 3.28 (1H, d, J = 12.5 Hz), 3.46 (1H, m), 3.52 (1H, m), 3.91 (1H, m), 4.08 (1H, m), 4.73 (1H, br s, H-2), 5.02 (2H, m), 5.65 (1H, br s, H-1), 5.90 (2H, br s, –OCH_2O–), 6.52 (1H, s, H-7), 6.68 (1H, s, H-10); EI-MS: *m*/*z* $[M]^+$ 583: $[M+2]^+$ 585: $[M+4]^+$ 587: $[M+6]^+$ 589: $[M+8]^+$ 591 [21:28:14:3:4:0.28, *theoretically* 21:28:14:3.1:0.26]. 463 (M^+–2HOAc): 465 (M^+–2HOAc + 2): 467 (M^+–2HOAc + 4): 469 (M^+–2HOAc + 6) (68.5: 91.1: 46.3: 11.5: 1.2, *theoretically* 68.5:91.3:10.1:0.85).

5.5.3.2. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-acetoxyethyl)-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine-5-carboxylic acid 2,2,2-trichloroethyl ester (13). R_f = 0.56 hexane–EtOAc (1:1); ^1H NMR ($CDCl_3$) δ 1.86 (2H, m), 2.03 (3H, s) and 2.07 (6H, s) (3× Ac–*Me*), 2.08 (1H, m), 2.72 (1H, m), 3.29 (1H, d, J = 12.3 Hz), 4.03 (1H, m), 4.11 (3H, m), 4.50 (1H, br s), 4.74 (1H, s), 4.96 (1H, m), 5.02 (1H, s), 5.66 (1H, br s), 5.91 (2H, s, –OCH_2O–), 6.53 (1H, s, H-8), 6.70 (1H, s, H-11); EIMS *m*/*z* $[M]^+$ 607: $[M+2]^+$ 609: $[M+4]^+$ 611: $[M+6]^+$ 613 (53.9:55.2:19.4:3.0), 487 (M^+–2HOAc): 489 (M^+–2HOAc + 2): 491 (M^+–2HOAc + 4): 493 (M^+–2HOAc + 6) (81.8:100:46.1:10.3).

5.5.3.3. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-acetoxyethyl)-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine (14). ^1H NMR ($CDCl_3$) δ 1.87 (3H, s), 2.02 (3H, s) and 2.09 (3H, s) (3× Ac–*Me*), 1.91 (2H, m), 2.20 (1H, m), 3.19 (1H, br d, J = 11.6 Hz, H-10b), 3.26 (1H, dd, J = 11.6, 4.3 Hz, H-4a), 3.95 (1H, d) and 4.08 (1H, d) (H-6s, J_{gem} = 15.6 Hz), 4.07 (1H, dt, J = 11.0, 4.3 Hz) and 4.19 (1H, dt, J = 11.0, 6.4 Hz) (H-5s), 4.34 (1H,

N*H*, D_2O exchangeable), 5.00 (1H, m, H-2), 5.54 (1H, br s, H-1), 5.88 (2H, s, –OC*H*$_2$O–), 6.46 (1H, s, H-7), 6.69 (1H, s, H-10); EI–MS *m*/*z* $[M]^+$ 433 (29), 432 (16), 373 (32), 372 (73), 314 (38), 313 (14), 254 (68), 204 (100); HREIMS *m*/*z* $[M]^+$ 433.1721 (calcd for $C_{22}H_{27}NO_8$, 433.1731).

5.5.3.4. 4aα,10bβ-1α,2β-Diacetoxy-4-(2-acetoxyethyl)-5-methyl-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine (15). R_f = 0.66, $CHCl_3$–MeOH (23:2); ^{1}H NMR ($CDCl_3$) δ 1.81 (1H, dt, *J* = 15.8, 4.4 Hz, H-3$_{ax}$), 1.86 (3H, s), 2.01 (3H, s) and 2.09 (3H, s) (3× Ac–*Me*), 2.00 (2H, m, H-4s), 2.13 (1H, br d, *J* = 15.8 Hz, H-3$_{eq}$), 2.21 (1H, m, H-4), 2.29 (3H, s, N-*Me*), 2.47 (1H, dd, *J* = 11.0, 4.0 Hz, H-4a), 3.25 (1H, br d, *J* = 11.0 Hz, H-10b), 3.35 (1H, d) and 3.73 (1H, d) (H-6s J_{gem} = 14.0 Hz), 4.13 (2H, m, H-12), 4.94 (1H, m, H-2), 5.51 (1H, br s, H-1), 5.85 (1H, br s) and 5.86 (1H, br s) (–OC*H*$_2$O–), 6.48 (1H, s, H-7), 6.68 (1H, s, H-10); ^{13}C NMR ($CDCl_3$) δ 20.9 (q), 21.0 (q), and 21.4 (q) (3× Ac–*Me*), 24.5 (t, C-11), 27.0 (t, C-3), 31.6 (d, C-4), 36.3 (d, C-10a), 41.6 (q, N-*Me*), 59.6 (t, C-6), 62.1 (d, C-4a), 63.7 (t, C-12), 69.3 (d) and 70.7 (d) (C-1 and C-2), 100.8 (t, –O*C*H$_2$O–), 105.4 (d) and 106.2 (d) (C-7 and C-10), 125.9 (s) and 128.5 (s) (C-6a and C-10a), 146.0 (s) and 146.7 (s) (C-8 and C-9), 169.5 (s), 169.6 (s) and 171.1 (s) (3× Ac–*C*O); EIMS *m*/*z* $[M]^+$ 447 (41), 388 (7), 328 (7), 260 (40), 218 (100); HRFABMS *m*/*z* $[M+H]^+$ 448.1952 (calcd for $C_{23}H_{29}NO_8$+H, 448.1966).

5.5.3.5. 4aα,10bβ-1α,2β-Dihydroxy-4-(2-hydroxyethyl)-5-methyl-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine (16). R_f = 0.34, $CHCl_3$–MeOH–NH_4OH_{aq} (85:15:0.1); ^{1}H NMR (CD_3OD) δ 1.76 (1H, m, H-11), 1.87 (1H, dt, *J* = 15.1, 4.0 Hz, H-3$_{ax}$), 1.99 (1H, br d, *J* = 15.0 Hz, H-3$_{eq}$), 2.15 (1H, ddt, *J* = 13.9, 11.7, 4.6 Hz, H-11), 2.29 (1H, m, H-4), 2.35 (3H, s, N-*Me*), 2.66 (1H, dd, *J* = 11.3, 4.1 Hz, H-4a), 3.27 (1H, br d, *J* = 11.4 Hz, H-10b), 3.41 (1H, d) and 3.72 (1H, d) (H-6s, J_{gem} = 14.7 Hz), 3.69 (1H, ddd, *J* = 10.6, 9.0, 4.8 Hz) and 3.76 (1H, ddd, *J* = 10.6, 5.7, 4.9 Hz) (H-12s), 3.88 (1H, *q*-like, *J* = 2.8 Hz, H-2), 4.31 (1H, br s, H-1), 5.87 (2H, s, –OC*H*$_2$O–), 6.53 (1H, s, H-7), 6.85 (1H, s, H-10); ^{13}C NMR (CD_3OD) δ 29.5 (t, C-11), 29.8 (t, C-3), 32.5 (d, C-4), 37.8 (d, C-10b), 41.7 (q, N-*Me*), 60.8 (t, C-6), 61.4 (t, C-12), 63.5 (d, C-4a), 71.8 (d) and 74.0 (d) (C-1 and C-2), 102.1 (t, –O*C*H$_2$O–), 106.7 (d), 107.1 (d) (C-7 and C-10), 129.6 (s) and 129.7 (s) (C-6a and C-10a), 147.2 (s) and 148.4 (s) (C-8 and C-9); EIMS *m*/*z* $[M]^+$ 321 (14), 218 (100), 188 (28); HREIMS *m*/*z* $[M]^+$ 321.1581 (calcd for $C_{17}H_{23}NO_5$, 321.1571).

5.6. Biological assay

AChE inhibitory activity was measured by modification of Ellman's method.[14,15] This colorimetric method is based on the amount of thiocholine produced when the substract ACh is hydrolyzed by the enzyme. In the 96-well plates, 13 μL acetylthiocholine iodide (ATCI, 15 mM in water), 64 μL of 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB, 3 mM in PBS containing 50 mM Tris–HCl, 0.1 M NaCl, and 0.02 M $MgCl_2{\cdot}6H_2O$, pH 8), 10 μL of sample (10 mM in MeOH diluted with water to 1–10 μM) or blank (1% MeOH in water) were added. The reaction was started after 13 μL acetylcholinesterase (0.22 U/ml in 50 mM Tris–HCl containing 0.1% bovine serum albumin) was added to the 96-well plate. Absorbance of the yellow anion product due to the spontaneous hydrolysis of substrate was measured at 405 nm for 12 min on a Microtiter plate reader (uQuant, Bio-TEK). Galanthamine used as a positive control. The inhibition percentage (%) was calculated by the following equation:

$$\text{Inhibition } (\%) = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$$

5.7. Docking calculations of active compounds to EelAChE

The docking calculations, using AutoDock 3.05[16] and MEDock,[17] of the active compounds to the eel AChE (EelAChE) were performed. The structure of EelAChE was taken from the Protein Data Bank[19] (accession code: 1C2B), which was obtained by fitting the structure of the mouse AChE (mAChE) structure (accession code: 1MAA) into a low resolution X-ray electron density map. The ligand files were prepared on the PRODRG 2 server (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/).[20] For the docking protocol, the number of generation in both evolutionary algorithms was set as large as 5000, so that the evolution of each run can reach a converged state. The iteration number for the local search algorithm, that is, the Solis-Wet searcher, is set to 600. The grid center is set at the amine nitrogen position of the Serine 203, which forms the catalytic triad with GLU 334 and HIS 447. The docked conformations were analyzed with LIGPLOT 4.4.2.[18]

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