STUDIES ON INHIBITORY ACTIVITY AGAINST ACETYLCHOLINESTERASE OF NEW BISBENZYLISOQUINOLINE ALKALOID AND ITS RELATED COMPOUNDS

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Abstract — A new phenolic bisbenzylisoquinoline (BBI) alkaloid named 2'-*N*-norfangchinoline was isolated from the root of *Stephania tetrandra* S. MOORE along withfangchinoline (**2**) and atherospermoline (**3**). The chemical structure of 2'-*N*-norfangchinoline was proved to be **4** by spectral analyses and chemical methods. Moreover three phenolic BBI alkaloidal compounds, 2,2'-*N,N*-dinorfangchinoline (**8**), 2'-*N*-noratherospermoline (**9**), 2-*N*-norfangchinoline (**10**) were derived from tetrandrine (**1**). And 12-*O*-acetyl atherospermoline (**11**) was obtained by partial acetylation of atherospermoline (**3**). Seven phenolic BBI compounds (**2**, **3**, **4**, **8**, **9**, **10**, and **11**) also have the inhibitory effect on acetylcholinesterase.

In a previous paper, we reported the isolation of thirteen known alkaloids, and the structural determination of four new BBI alkaloids from the root of *S. tetrandra*.[1] Recently in the screening test of the extracts of crude drugs on the inhibitory effect against acetylcholinesterase (AChE), we found the activity of anti-AChE on the methanol extract of the root of *S. tetrandra*, particularly on the phenolic alkaloidal fraction. In the chemical research on constituents of this plant, we have successively isolated a new phenolic BBI alkaloid named fenfangjine E (**4**) along with two known phenolic BBI alkaloids, fangchinoline (**2**), atherospermoline (**3**) and a known non-phenolic BBI alkaloid, tetrandrine (**1**). The active concentrations of these phenolic alkaloids as AChE inhibitors were all in the 10^{-6} M range. Non-phenolic alkaloid tetrandrine (**1**) and the non-phenolic alkaloidal fraction had no inhibitory activity against AChE. This paper presents the isolation and the structural determination of a new phenolic BBI alkaloid and the derivation of four active phenolic BBI alkaloidal compounds from **1** or **3**.

The powdered root was extracted with MeOH. The MeOH extract was partitioned between hexane and 90% MeOH. The 90% MeOH layer was partitioned between $CHCl_3$ and 2% NH_4OH. The $CHCl_3$ layer showed the inhibitory activity against AChE. The $CHCl_3$ layer was separated on

alumina column by elution with $CHCl_3$, followed with $CHCl_3$-MeOH (10:1) to give two fractions A-I and A-II. The fraction A-I gave two known alkaloids (**1**) and (**2**) by chromatographic separation. The fraction A-II was partitioned between $CHCl_3$ and 5% acetic acid. The 5% acetic acid layer was basified with 25% NH_4OH and extracted with $CHCl_3$ to give the phenolic alkaloidal fraction. Then repeated chromatographic separation of the $CHCl_3$ extract gave two phenolic BBI alkaloids (**3**) and new alkaloid fenfangjine E (**4**).

Fenfangjine E (**4**), $C_{36}H_{38}N_2O_6$, mp 162-164°C, [α] D +252.5°, was obtained as colorless needles from EtOH. The EIMS of **4** exhibited following fragments; *m/z* 594 $[M]^+$, 367 $[M-227]^+$ and 184. The IR spectrum (KBr) of **4** showed absorption of hydroxyl group at 3320 cm^{-1}. In the ^{1}H-NMR spectrum ($CDCl_3$) of **4**, one *N*-methyl signal (δ 2.31) and three methoxy signals(δ 3.35, 3.77 and 3.92) were observed. They were similar to those of **2** except for *N*-methyl signal of 2'-position. The *N*-methyl signal of 2'-position of **2** showed at δ 2.61, while that of compound (**4**) disappeared. This indicated **4** was the *N*-demethylated compound in 2'-position of **2**. On *O*-demethylation with $AlCl_3$ in dry CH_2Cl_2, 2'-*N*-nortetrandrine (cycleanorine) (**6**) afforded **4**. From the above data, compound (**4**) was elucidated as 2'-*N*-norfangchinoline.

Non-phenolic BBI alkaloid tetrandrine (**1**) which was main component of *S. tetrandra*, had no inhibitory effect against AChE at final concentration of 10μM. Three phenolic alkaloids (**2**, **3**, and **4**) exhibited the anti-AChE activity of 60.2%, 54.6%, 66.4% in the same assay, respectively. These alkaloids were common to have the phenolic hydroxyl group at 7-position. Therefore we derived three phenolic compounds from **1**. On treatment with methyl chloroformate[2] in THF, **1** afforded three carbamates which were separated by silica gel column chromatography. In succession on alkaline hydrolysis with 10% KOH in ethylene glycol, these carbamates yielded three *N*-demethylated compounds, 2,2'-*N*,*N*-dinortetrandrine (**5**), cycleanorine (**6**), and 2-*N*-nortetrandrine (**7**), respectively. On *O*-demethylation with $AlCl_3$ in dry CH_2Cl_2, **5** afforded compound (**8**). Treatment of **6** with $AlCl_3$ in dry CH_2Cl_2, gave two phenolic compounds (**4**) and (**9**) which were separated by silica gel column chromatography. And 2-*N*-nortetrandrine (**7**) was treated by the same methods to give compound (**10**).

Compound (**8**), $C_{35}H_{36}N_2O_6$, mp 222-224°C, [α] D +356.8°, was obtained as colorless needles. The EIMS of compound(**8**) showed following fragments; *m/z* 580 $[M]^+$, 353 $[M-227]^+$ and 177. The molecular ion peak at *m/z* 580 of **8** corresponds to loss of 28 mass units in comparison with that of **2** at *m/z* 608. The ^{1}H-NMR spectrum ($CDCl_3$) of **8** showed three methoxy groups at δ 3.35 (6'-OCH_3), 3.75 (6-OCH_3) and 3.94 (12-OCH_3) to disappear two *N*-methyl groups as compared with **2**. From the above data, compound (**8**) was elucidated as 2,2'-*N*,*N*-dinorfangchinoline.

Compound (**9**), $C_{35}H_{36}N_2O_6$, mp 220-222°C, [α] D +271.4°, was obtained as colorless needles. The EIMS of compound (**9**) showed following fragments; *m/z* 580 $[M]^+$, 367 $[M-213]^+$ and 184. It is known that BBI alkaloids show a characteristic fragmentation pattern in which cleavage occurs at two positions between the isoquinoline and benzyl groups.[3] The fragment at *m/z* 367 of **9** corresponds to plus of 14 mass units in comparison with the fragment at *m/z* 353 of **8**. Accordingly **9** was supposed to have hydroxyl group at 12-position. The ^{1}H-NMR spectrum ($CDCl_3$) of **9** showed

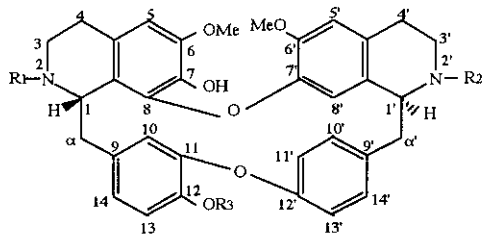
single *N*-methyl signal at δ 2.31 and two methoxy signals at 3.18 and 3.77. The signal at δ 3.18 of **9** suggested the existence of a methoxy group at 7-position in comparison with that of **2**. Acetylation of **9** with acetic anhydride in pyridine gave its triacetate (**9a**). In the ^{1}H-NMR spectrum ($CDCl_3$) of compound (**9a**), the acetoxy group at 7-position and the methoxy group at 6'-position were observed at δ 1.51 and 3.44, respectively. Therefore the signal at δ 3.18 of **9** was concluded the methoxy group at 6'-position. From the above data, **9** was elucidated as 2'-*N*-noratherospermoline.

Compound (**10**), $C_{36}H_{38}N_2O_6$, mp 165-168°C, [α] D +327.8°, was obtained as colorless needles. The EIMS of compound (**10**), *m/z* 594 $[M]^+$, 367 $[M-227]^+$ and 184, was all the same to these of **4**. In the ^{1}H-NMR spectrum ($CDCl_3$) of **10**, the signals of three methoxy groups (δ 3.35, 3.76 and 3.94) and single *N*-methyl group (δ 2.61) were observed, and these signals were closely similar to those of **4** except for *N*-methyl signal. Though two *N*-methyl signals of **2** showed at δ 2.32 (2-NCH_3) and 2.61 (2'-NCH_3), that of **10** was observed only at δ 2.61. From the above data, **10** was determined as 2-*N*-norfangchinoline.

It is known that acetylation of **3** with acetic anhydride in pyridine gave 7,12-*O*,*O*-diacetylatherospermoline.[4] We tried to derive 12-*O*-acetylatherospermoline from **3**. Treatment of **3** with acetic anhydride in dry CH_2Cl_2 gave its monoacetate (**11**). Compound (**11**), $C_{38}H_{40}N_2O_7$, [α]D +174.8°, was obtained as a white amorphous powder. The EIMS of compound (**11**) exhibited the molecular ion peak at *m/z* 636 and the fragments at *m/z* 381 and 191 were agreement with these of **3**. The ^{1}H-NMR spectrum of **11** showed the signals corresponding to two *N*-methyl groups at δ 2.34, 2.61 and two methoxy groups at δ 3.34, 3.76 and single acetoxy group at δ 2.31. In the ^{1}H-NMR spectrum of 7,12-*O*,*O*-diacetylatherospermoline, two acetoxy groups at 7 and 12-positions are known to exhibit at δ 1.62 and 2.33, respectively.[4] Accordingly **11** was supposed to have the hydroxyl group at 7-position and the acetoxyl group at 12-position. From the above data, **11** was elucidated as 12-*O*-acetylatherospermoline.

Molar concentration of seven BBI compounds required to give 50% inhibition against rat striatum AChE as

Table 1. The Inhibitory Effects of Phenolic BBI Compounds on Acetylcholinesterase



compounds	R1	R2	R3	Inhibition (IC50) AchE
fangchinoline (**2**)	Me	Me	Me	3.2×10^{-6} M
atherospermoline (**3**)	Me	Me	H	4.0×10^{-6} M
2'-*N*-norfangchinoline(**4**)	Me	H	Me	3.9×10^{-6} M
2,2'-*N*,*N*-dinorfangchinoline (**8**)	H	H	Me	5.8×10^{-6} M
2'-*N*-noratherospermoline (**9**)	Me	H	H	2.5×10^{-6} M
2-*N*-norfangchinoline (**10**)	H	Me	Me	6.2×10^{-6} M
12-*O*-acetylatherospermoline (**11**)	Me	Me	Ac	1.0×10^{-5} M

The source of acetylcholinesterase is rat striatum.

shown in Table 1. The IC_{50} value of phisostigmine was 1.7×10^{-6} M as positive control. The seven compounds (**2**, **3**, **4**, **8**, **9**, **10**, and **11**) of fangchinoline type (1-*S*, 1'-*S*) which have phenolic hydroxyl group at 7-position, were exhibited the inhibitory activity against AChE. Though we assayed two phenolic BBI alkaloids, homoaromoline (1-*S*, 1'-*R*)[5] and isofangchinoline (1-*R*,1'-*S*)[6] which are different in the configuration at 1 or 1'-position from **2**, these two alkaloids were observed no activity of anti-AChE.

The active seven BBI compounds have phenolic hydroxyl group at 7-position and also *S*,*S* configuration at 1 or 1'-position.

EXPERIMENTAL

Melting points were determined on a Yanaco MP-3 type and are uncorrected. Specific optical rotations were measured on a JASCO DIP-360 automatic polarimeter. IR spectra were taken with a Hitachi 270-30 type spectrophotometer. ^1H and ^{13}C-NMR spectra were measured on JEOL JNM-FX 200 and Bruker AM 500 spectrometers using TMS as an internal standard; chemical shifts are recorded in δ values. MS and HRMS were obtained with a JEOL DX-300 spectrometer. Plant material was purchased from Row Medical Trading Co., Ltd..

Extraction and Isolation

The dried root of *S. tetrandra* (15 kg) was milled and extracted twice with hot MeOH (50 L) for 2 h. The MeOH extract was concentrated to dryness and the residue (790 g) was partitioned with hexane and 90% MeOH. 2% NH_4OH was added to the 90% MeOH extract and the NH_4OH solution was extracted three times with $CHCl_3$ (2 L). The $CHCl_3$ extract was concentrated *in vacuo* to yield 331 g of fraction A. Fr. A was separated on alumina column (2 kg) by elution with $CHCl_3$, followed with $CHCl_3$-MeOH (10:1) to give two fractions, Fr. A-I (255 g) and Fr. A -II (34 g), respectively. Chromatography of Fr. A-I on silica gel by elution with $CHCl_3$-MeOH (40:1) yielded two main alkaloids, tetrandrine (**1**) and fangchinoline (**2**), and these compounds were recrystallized from MeOH and acetone to give 118 g and 56 g, respectively. Fr. A-II was partitioned with $CHCl_3$ and 5% acetic acid. The 5% acetic acid layer was basified with 25% NH_4OH and extracted with $CHCl_3$. Repeated chromatography of the $CHCl_3$ extract on silica gel by elution with $CHCl_3$-MeOH (30:1) and AcOEt-MeOH-H_2O (50:10:1) yielded atherospermoline (**3**) and compound (**4**). These compounds were recrystallized from acetone and EtOH to give 115 mg and 466 mg, respectively.

Tetrandrine(1): Colorless needles (from MeOH), mp 217-218°C, $[\alpha]_D^{25}$ +248.1° (*c*=1.16, $CHCl_3$). EIMS *m/z* : 622 $[M]^+$, 395 $[M-227]^+$, 198. IR (KBr) cm^{-1}: 2932, 1606, 1585, 1508. Tetrandrine (**1**) was identical with an authentic sample.

Fangchinoline (2) : Colorless needles (from acetone), mp 237-239°C, $[\alpha]_D^{25}$ +232.9° (*c*=1.29, $CHCl_3$). EIMS *m/z* : 608 $[M]^+$, 381 $[M-227]^+$, 191. IR (KBr) cm^{-1}: 3420 (OH), 2928, 1616, 1584, 1504. Fangchinoline (**2**) was identical with an authentic sample.

Atherospermoline (3) : Colorless needles (from acetone), mp 182-184°C, $[\alpha]_D^{26}$ +240.8°(*c*=0.24,

MeOH). EIMS *m/z* : 594 $[M]^+$,381 [M-213]$^+$, 191. IR (KBr) cm^{-1}: 3424(OH), 2936, 1616, 1596, 1504. Atherospermoline (**3**) was identical with an authentic sample.

Fenfangjine E (4): Colorless needles (from EtOH), mp 162-164°C, $[\alpha]_D^{26}$ +252.5° (*c*=0.45, $CHCl_3$). EIMS *m/z* : 594 $[M]^+$, 367 [M-227]$^+$, 184. HRMS : Calcd for $C_{36}H_{38}N_2O_6$ $[M]^+$ 594.2729. Found 594.2725. IR (KBr) cm^{-1} : 3320 (OH), 2936, 1616, 1586, 1504. ^{1}H-NMR : Table 2. ^{13}C-NMR : Table 3.

***N*-Demethylation of 1** : A solution of **1** (15 g, 24.1 mmol) in dry THF (500 mL) was stirred with an excess of methyl chloroformate (15 mL, 158.7 mmol) at rt for 5 h. The reaction mixture was basified with NH_4OH and evaporated to dryness. H_2O was added to the residue and the solution was extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated *in vacuo* and then separated on silica gel by elution with a gradient of $CHCl_3$-MeOH (150:1→50:1) to give three crude carbamates of **1**, tetrandrine 2,2'-*N*,*N*-dicarbamate (2.55 g), tetrandrine 2'-*N*-carbamate (6.14 g), tetrandrine 2-*N*-carbamate (1.21 g). The crude tetrandrine 2,2'-*N*,*N*-dicarbamate (2.55 g) was dissolved in a

Table 2. The ^{1}H-NMR Spectral Data for Phenolic BBI Compounds [a)]

	4	**5**[b)]	**6**[b)]	**7**[b)]	**8**	**9**	**10**	**11**
H-1	3.74d	4.01d		4.01d	3.99d	3.93d	4.00d	3.77d
	(1.6)	(9.0)		(9.0)	(9.0)	(11.0)	(9.0)	(10.0)
H-5	6.51s	6.50s	6.50s	6.52s	6.52s	6.48s	6.53s	6.50s
H-10	6.54d	6.43d	6.52	6.45d	6.43d	6.42d	6.46d	6.64d
	(9.5)	(2.0)	(s-like)	(2.0)	(1.9)	(1.7)	(1.9)	(1.6)
H-13	6.84d	6.88d	6.86m	6.88d	6.87d	6.73d	6.87d	6.96d
	(8.2)	(8.0)		(8.0)	(8.1)	(8.0)	(8.0)	(8.1)
H-14	6.86dd	6.76dd	6.86m	6.75dd	6.73dd	6.75dd	6.73dd	6.94dd
	(8.2,1.6)	(8.0,2.0)		(8.0,2.0)	(8.1,1.9)	(8.0,1.7)	(8.0,1.9)	(8.1,1.6)
H-1'	4.19dd	4.19dd	4.22dd		4.16dd	4.23dd	3.84dd	3.86dd
	(10.8,5.9)	(11.0,6.0)	(11.0,6.0)		(11.1,5.5)	(10.6,6.1)	(11.2,5.2)	(10.8,5.6)
H-5'	6.29s	6.31s	6.31s	6.31s	6.30s	6.29s	6.29s	6.28s
H-8'	6.05s	5.99s	6.00s	5.99s	6.03s	6.09s	6.03s	6.02s
H-10'	6.39dd	6.40dd	6.36dd	6.36dd	6.42dd	6.17dd	6.37dd	6.29dd
	(8.3,2.2)	(8.3,2.2)	(8.3,2.0)	(8.3,2.2)	(8.2,2.2)	(8.2,2.1)	(8.2,2.2)	(8.2,2.2)
H-11'	6.81dd	6.84dd	6.82dd	6.84dd	6.83dd	6.60dd	6.82dd	6.77dd
	(8.3,2.6)	(8.3,2.4)	(8.3,2.4)	(8.3,2.4)	(8.2,2.5)	(8.2,2.5)	(8.2,2.5)	(8.2,2.5)
H-13'	7.13dd	7.14dd	7.14dd	7.14dd	7.13dd	7.11dd	7.13dd	7.11dd
	(8.3,2.6)	(8.3,2.4)	(8.3,2.4)	(8.3,2.4)	(8.2,2.5)	(8.2,2.5)	(8.2,2.5)	(8.2,2.5)
H-14'	7.35dd	7.40dd	7.37dd	7.37dd	7.39dd	7.31dd	7.36dd	7.36dd
	(8.3,2.2)	(8.3,2.2)	(8.3,2.0)	(8.3,2.2)	(8.2,2.2)	(8.2,2.1)	(8.2,2.2)	(8.2,2.2)
2-NCH_3	2.31s	-	2.32s	-	-	2.31s	-	2.34s
2'-NCH_3	-	-	-	2.62s	-	-	2.61s	2.61s
6-OCH_3	3.77s	3.76s	3.76s	3.76s	3.77s	3.77s	3.76s	3.76s
7-OCH_3	-	3.25s	3,23s	3.22s	-	-	-	-
12-OCH_3	3.92s	3.95s	3.95s	3.95s	3.94s	-	3.92s	-
6'-OCH_3	3.35s	3.38s	3.39s	3.39s	3.35s	3.18s	3.35s	3.34s
12-$OCOCH_3$	-	-	-	-	-	-	-	2.31s

a) The spectra were obtained at 500 MHz in $CDCl_3$ solution. The data for each proton are shown in ppm from TMS and *J* values (Hz) are in parentheses.

b) The spectra were measured at 200 MHz.

Table 3. The ^{13}C-NMR Spectral Data for Phenolic BBI Compounds [a]

carbones	4	5[b]	6[b]	7[b]	8	9	10	11
1	61.4	61.4	53.8	53.9	53.9	61.1	53.9	61.2
3	44.3	44.1	39.8	39.9	39.7	43.7	39.8	44.2
4	22.0	22.1	28.9	28.8	28.6	21.6	28.6	21.8
4a	123.5	129.2	129.4	128.7	124.1	123.1	124.1	123.6
5	105.0	105.9	106.2	106.0	105.2	105.1	105.2	105.0
6	145.7	151.3	151.3	151.3	145.7	145.7	145.7	145.9
7	134.6	138.1	137.9	137.8	134.1	134.6	134.2	134.7
8	141.8	148.4	147.1	147.1	140.5	141.7	140.5	142.0
8a	123.6	123.1	123.7	123.5	124.2	123.5	124.1	123.6
9	135.0	134.9	134.0	134.1	134.5	133.2	134.5	138.1
10	116.3	116.2	115.8	115.8	115.9	116.3	115.9	121.7
11	147.1	147.0	146.8	146.9	147.2	147.8	147.2	151.3
12	149.5	149.4	150.1	150.1	150.2	143.8	150.2	141.4
13	111.6	111.6	111.6	111.6	111.7	115.4	111.7	117.7
14	122.8	122.8	122.3	122.3	121.7	123.3	122.3	123.3
α	41.9	41.9	37.1	37.1	37.1	41.8	37.1	42.3
1'	56.3	56.4	56.7	64.2	56.5	56.1	64.1	63.9
3'	42.2	42.5	42.8	45.3	42.4	42.0	45.5	45.4
4'	27.9	28.1	28.1	25.2	27.8	28.0	25.5	25.7
4'a[c]	130.1	128.8	128.7	128.0	130.3	130.0	128.0	128.8
5'	113.7	113.1	113.1	112.7	113.9	113.5	113.4	113.1
6'	148.7	148.5	148.3	148.6	148.5	148.8	148.6	149.0
7'	143.7	143.8	143.2	143.2	143.3	143.7	143.2	143.7
8'	119.8	119.4	118.7	119.4	119.1	119.8	119.1	120.8
8'a[c]	128.9	128.1	128.7	127.7	128.7	128.7	129.1	128.4
9'	135.1	134.9	135.5	135.5	135.6	135.1	135.7	135.5
10'[d]	130.3	130.2	130.3	130.3	130.5	130.4	130.3	130.1
11'[e]	122.0	121.9	121.6	121.6	122.3	121.9	121.6	121.7
12'	153.9	153.9	153.2	153.2	153.3	153.4	153.2	154.0
13'[e]	122.0	121.9	122.2	122.2	122.3	122.4	122.2	122.3
14'[d]	132.4	132.4	132.4	132.7	132.5	132.6	132.7	132.8
α'	38.4	38.6	38.5	38.2	38.4	38.4	38.1	37.7
2-NCH3	42.4	42.3	-	-	-	42.0	-	42.3
2'-NCH3	-	-	-	42.7	-	-	42.8	42.7
7-OCH3	-	60.3	60.4	60.4	-	-	-	-
6,12,6'-OCH3	56.2 (2C)	55.9 (2C)	55.9 (2C)	55.9 (2C)	56.3 (2C)	56.0 56.3	56.2 56.2 (2C)	56.2 (2C)
	56.3	56.2	56.2	56.2	56.6	-	(2C)	-
12-OCOCH3	-	-	-	-	-	-	-	20.8
12-OCOCH3	-	-	-	-		-	-	169.4

a) The spectra were obtained at 125 MHz in $CDCl_3$ solution. The data for each carbon are shown in ppm from TMS.

b) The spectra were measured at 40 MHz.

c,d,e) Signals within the vertical columns may be reversed.

solution of KOH in ethylene glycol (10%, 50 mL) and heated at 190°C for 1h. The cooled solution was acidified with 35% HCl and was allowed to stand for 5 min. The reaction mixture was basified with 25% NH_4OH and was extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated and separated on silica gel chromatography by elution with $CHCl_3$-MeOH (20:1) to furnish the crude 2,2'-*N*,*N*-dinortetrandrine (**5**), which was recrystallized from EtOH-H_2O as colorless needles

(232 mg, 1.62%). The other carbamates, tetrandrine 2'-*N*-carbamate and tetrandrine 2-*N*-carbamate, were treated by the same methods for derivation of 2,2'-*N*,*N*-dinortetrandrine (**5**) to give cycleanorine (**6**) (834 mg, 5.69%) and 2-*N*-nortetrandrine (**7**) (421 mg, 2.87%), respectively.

2,2'-*N*,*N*-Dinortetrandrine (5) : Colorless needles (from EtOH-H_2O), mp218-220°C, [α]$_D^{22}$ +334.8° (*c*=0.58, $CHCl_3$). EIMS *m/z* : 594 $[M]^+$, 367 $[M-227]^+$, 184. HRMS: Calcd for $C_{36}H_{38}N_2O_6$ $[M]^+$ 594.2729. Found 594.2700. IR (KBr) cm^{-1}: 2932, 1608, 1584, 1512. ^{1}H-NMR: Table 2. ^{13}C-NMR: Table 3.

Cycleanorine (6) : Colorless needles (from EtOH-H_2O), mp 166-167°C, [α]$_D^{22}$ +287.1° (c=0.77, $CHCl_3$). EIMS *m/z* : 608 $[M]^+$, 381 $[M-227]^+$, 191. HRMS: Calcd for $C_{37}H_{40}N_2O_6$ $[M]^+$ 608.2885. Found 608.2855. IR (KBr) cm^{-1}: 2936, 1608, 1584, 1510. ^{1}H-NMR: Table 2. ^{13}C-NMR: Table 3.

2-*N*-Nortetrandrine (7) : Colorless needles (from EtOH), mp 211-213°C, [α]$_D^{22}$ +313.2° (*c*=0.36, $CHCl_3$). EIMS *m/z* : 608 $[M]^+$,381 $[M-227]^+$, 191. HRMS: Calcd for $C_{37}H_{40}N_2O_6$ $[M]^+$ 608.2886. Found 608.2932. IR (KBr) cm^{-1}: 2932, 1608, 1584, 1510. ^{1}H-NMR: Table 2. ^{13}C-NMR: Table 3.

2,2'-*N*,*N*-Dinorfangchinoline (8): To a solution of **5** (232 mg, 0.39 mmol) in dry CH_2Cl_2 (30 mL) was added an excess of $AlCl_3$ (1 g, 7.5 mmol) and stirred at rt for 2h. The cooled solution, after addition with small amount of H_2O and MeOH, was basified with 25% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated and separated on silica gel chromatography by elution with $CHCl_3$-MeOH-NH_4OH (10:1:0.05) to furnish the crude 2,2'-*N*,*N*-dinorfangchinoline (**8**), which was recrystallized from EtOH as colorless needles (68 mg, 30.0%). mp 222-224°C, [α]$_D^{26}$ +356.8° (*c*=0.36, $CHCl_3$). EIMS *m/z* : 580 $[M]^+$, 353 $[M-227]^+$, 177. HRMS: Calcd for $C_{35}H_{36}N_2O_6$ $[M]^+$ 580.2573. Found 580.2579. IR (KBr) cm^{-1}: 3424 (OH), 2939, 1616, 1584, 1506. ^{1}H-NMR : Table 2. ^{13}C-NMR : Table 3.

2'-*N*-Noratherospermoline (9) : A solution of **6** (834 mg, 1.37 mmol) in dry CH_2Cl_2 (60 mL) was stirred with an excess of $AlCl_3$ (2 g, 15.0 mmol) at rt for 5 h. The cooled solution, after addition with small amout of H_2O and MeOH, was basified with 25% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated and separated on silica gel by using $CHCl_3$-MeOH-NH_4OH (50:10:1) to give the crude 2'-*N*-noratherospermoline (**9**), which was recrystallized from EtOH as colorless needles (71 mg, 8.9%). mp 220-222°C, [α]$_D^{26}$ +271.4° (*c*=0.26, $CHCl_3$). EIMS *m/z* : 580 $[M]^+$, 367 $[M-213]^+$, 184. HRMS : Calcd for $C_{35}H_{36}N_2O_6$ $[M]^+$ 580.2572. Found 580.2561. IR (KBr) cm^{-1}: 3312 (OH), 2940, 1616, 1592, 1504. ^{1}H-NMR : Table 2. ^{13}C-NMR : Table 3.

2-*N*-Norfangchinoline (10) : A solution of **7** (235 mg, 0.39 mmol) in dry CH_2Cl_2 (30 mL) was stirred with an excess of $AlCl_3$ (1 g, 7.5 mmol) at rt for 40 min. The cooled solution, after addition with small amount of H_2O and MeOH, was basified with 25% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract, after concentration, was separated on silica gel by using $CHCl_3$-MeOH (20:1) to furnish the crude 2-*N*-norfangchinoline (**10**), which was recrystallized from benzene as colorless needles (91 mg, 39.6%). mp 165-168°C, [α]$_D^{26}$ +327.8° (*c*=0.37, $CHCl_3$). EIMS *m/z* : 594 $[M]^+$, 367 $[M-227]^+$, 184. HRMS : Calcd for $C_{36}H_{38}N_2O_6$ $[M]^+$ 594.2729. Found 594.2718. IR (KBr) cm^{-1}: 3436 (OH), 2932, 1616, 1586, 1504. ^{1}H-NMR : Table 2. ^{13}C-NMR : Table 3.

12-*O*-Acetylatherospermoline (11) : To a solution of **3** (250 mg, 0.42 mmol) in dry CH_2Cl_2 (25

mL) was added acetic anhydride (1 mL, 9.8 mmol) and stirred at rt for 3 h. H_2O (20 mL) was added and the reaction mixture, after basification with 25% NH_4OH, was extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and separated on silica gel by using $CHCl_3$-MeOH (20:1) to afford 12-*O*-acetylatherospermoline(**11**) as a white amorpous pawder (210 mg, 78.4%). $[\alpha]_D^{26}$ +174.8° (*c*=0.54, $CHCl_3$). EIMS *m/z* : 636 $[M]^+$, 381 $[M-225]^+$, 191. HRMS: Calcd for $C_{38}H_{40}N_2O_7$ $[M]^+$ 636.2836. Found 636.2847. IR (KBr) cm^{-1} : 3524 (OH), 2936, 1766 (C=O), 1618, 1594, 1504. ^{1}H-NMR : Table 2. ^{13}C-NMR : Table 3.

Determination of AChE Activity

The inhibitory effects of the various compounds on AChE activity were compared with phisostigmine *in vitro* in homogenates of rat striatum. AChE activity was measured by HPLC with electrochemical detection method of Kaneda *et al.*[7]

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