Nitrogen-Containing Compounds from *Salvia miltiorrhiza*

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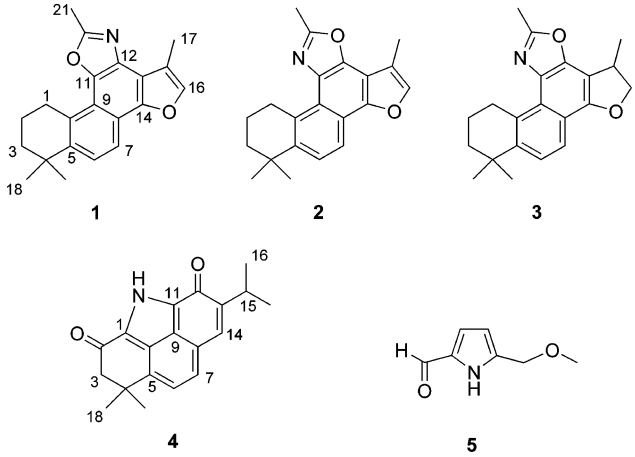
Received March 18, 2005

Five new N-containing compounds, neosalvianen (**1**), salvianen (**2**), salvianan (**3**), salviadione (**4**), and 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde (**5**), were isolated from *Salvia miltiorrhiza*. Their structures were mainly established by spectroscopic methods. Neosalvianen (**1**) and its analogues (**6a**, **6b**) were synthesized for spectroscopic data comparison. Compounds **1**, **2**, **4**, and **6a** were evaluated for their cytotoxic activities against selected cancer cell lines. Among these components, salvianen (**2**) exhibited the most potent cytotoxicity with a CD_{50} range of 30.4−39.5 μM against HeLa (cervical epitheloid carcinoma), HepG2 (hepatocellular carcinoma), and OVCAR-3 (ovarian adenocarcinoma) cell lines in a dose-dependent manner. The cytotoxicities of the tested compounds were not specific and showed similar activities to the selected cancer cell lines.

The traditional Chinese medicine "Danshen", the dried root of *Salvia miltiorrhiza* Bunge (Labiatae), has been used for the treatment of menstrual disorders, menostasis, menorrhalgia, insomnia, arthritis, and coronary heart diseases, particularly angina pectoris and myocardial infarction.[1−3] Numerous diterpenoid tanshinones have been isolated from *S. miltiorrhiza*, and several were shown to possess various biological and pharmacological activities including antitumor,[3−7] antimicrobial,[8,9] antioxidant,[10−13] antiinflammatory,[14,15] and antiplatelet aggregation[16−18] activities.

This paper describes the isolation and identification of five new N-containing compounds, neosalvianen (**1**), salvianen (**2**), salvianan (**3**), salviadione (**4**), and 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde (**5**), from *S. miltiorrhiza*. In addition, two known components, tanshinone IIA and cryptotanshinone, previously reported from this plant, were also isolated. Compound **1** and its analogues (**6a**, **6b**) were synthesized for spectroscopic data comparison. Furthermore, some selected compounds were assayed for cytotoxic and antimicrobial properties.



Results and Discussion

The concentrated EtOH extract of *S. miltiorrhiza* was chromatographed on silica gel. Repeated chromatography of the fractions resulted in the isolation of five new compounds (**1**−**5**). The structures of these new compounds were elucidated from ^{1}H and ^{13}C NMR spectra with the aid of COSY, HMQC, and HMBC experiments.

Compound **1** was obtained as colorless needles with a molecular formula of $C_{21}H_{21}NO_2$ determined by HREIMS ([M]$^+$, *m/z* 319.1570). The IR spectrum of **1** indicated the existence of an oxazole ring (1571 and 822 cm^{-1}). The ^{1}H NMR spectrum exhibited three aromatic protons at δ 8.17 and 7.61 (1H each, d, J = 9.0 Hz) and 7.57 (1H, q, J = 1.0 Hz), three aliphatic methylene protons at δ 3.51 (2H, t, J = 6.0 Hz), 2.02, and 1.79, and three tertiary methyl signals at δ 2.81 (3H, s), 1.41 (6H, s), and 2.60 (3H, d, J = 1.0 Hz) (Table 1). The ^{13}C and DEPT NMR spectra of **1** showed 20 carbon signals (Table 2), which were assigned to three methyls, three saturated methylenes, three aromatic methines, and 11 quaternary (one saturated at δ 34.4 and 10 aromatic) carbons. The connectivities of ^{1}H and ^{13}C signals were determined by HMQC and revealed that the signals at δ_H 1.41 and δ_C 31.8 were attributed to two equivalent methyl groups. The COSY spectrum showed the correlations of protons at δ 2.02 (H-2) with those at δ 3.51 (H-1) and 1.79 (H-3), indicating three consecutive methylene groups. On the basis of the HMBC spectrum, the selected correlations of protons to carbons are shown in Figure 1. However, assignment of the oxazole ring orientation of **1** was still ambiguous because no ^{1}H−^{13}C correlation signal was observed at C-11 and C-12 in the HMBC spectrum. To study the orientation of N and O atoms in the oxazole ring, compound **1** was therefore synthesized.

The synthetic compound **1** was obtained by oxidation of **6a** in the presence of DDQ using benzene as the solvent (Scheme 1).[19] Compound **6a** was prepared by reaction of cryptotanshinone in ethanol with aqueous ethylamine solution.[20] Ethylamine preferentially attacked the carbonyl carbon at C-12 of cryptotanshinone and then cyclized with C-11 to afford the oxazole ring.[20,21] However, the three-bond coupling of H-15 to nitrogen attached at C-12 (δ_C 135.5) was not observed in HMBC experiments with coupling

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10.1021/np0500934 CCC: $30.25
Published on Web 06/29/2005

Table 1. ^{1}H NMR (500 MHz) Data of **1**−**4** and **6a** in $CDCl_3$

position	**1** δ (*J*)[a]	**2** δ (*J*)	**3** δ (*J*)	**4** δ (*J*)	**6a** δ (*J*)
1	3.51 t (6.0)	3.75 m	3.68 m		3.42 t (6.0)
2	2.02 m	2.00 m	1.96 m		1.97 m
3	1.79 m	1.76 m	1.76 m	2.95 s	1.76 m
6	7.61 d (9.0)	7.61 d (8.5)	7.49 d (9.0)	7.30 d (7.5)	7.52 d (8.0)
7	8.17 d (9.0)	8.16 d (8.5)	7.83 d (9.0)	7.66 d (7.5)	7.85 d (8.0)
14				7.56 s	
15			3.99 m	3.54 m	4.08 m
16	7.57 q (1.0)	7.53 q (1.0)	a 4.36 dd (2.0, 9.0)	1.31 d (7.0)	a 4.40 dd (6.0, 8.5)
			b 4.92 t (9.0)		b 4.92 t (8.5)
17	2.60 d (1.0)	2.50 d (1.0)	1.49 d (7.0)	1.31 d (7.0)	1.56 d (6.5)
18	1.41 s	1.39 s	1.36 s[b]	1.49 s	1.36 s[b]
19	1.41 s	1.39 s	1.37 s[b]	1.49 s	1.37 s[b]
21	2.81 s	2.81 s	2.74 s		2.72 s

[a] *J* = coupling constant in hertz. [b] Interchangeable.

Table 2. ^{13}C NMR (125 MHz) Data of **1**−**4** and **6a** in $CDCl_3$

position	**1** δ	**2** δ	**3** δ	**4** δ	**6a** δ
1	29.4	30.7	30.5	128.2	29.3
2	19.5	19.7	19.6	188.0	19.5
3	38.6	38.8	38.8	57.4	38.6
4	34.4	34.6	34.6	41.3	34.4
5	143.1	143.6	144.2	145.3	143.7
6	125.0	124.8	124.0	121.0	124.2
7	118.4	118.3	119.7	129.7	119.7
8	117.8	118.1	117.0	123.9	116.8
9	117.5	122.3	124.9	124.3	119.6
10	130.1	132.7	132.0	127.9	129.5
11	144.4	134.0	130.5	126.9	143.0
12	132.6	148.5	145.9	175.9	135.5
13	116.3	111.3	109.3	151.4	115.2
14	149.5	150.6	154.8	129.1	153.1
15	116.0	114.7	36.4	27.4	36.8
16	141.1	141.1	79.5	22.8	79.4
17	9.4	9.3	19.2	22.8	19.8
18	31.8	32.0	31.8[a]	30.1	31.6[a]
19	31.8	32.0	31.9[a]	30.1	31.7[a]
20	162.6	159.8	159.0		163.0
21	14.9	14.5	14.5		14.9

[a] Interchangeable.

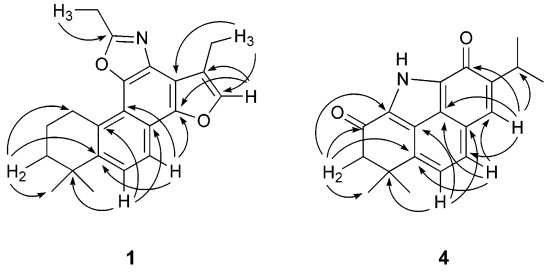
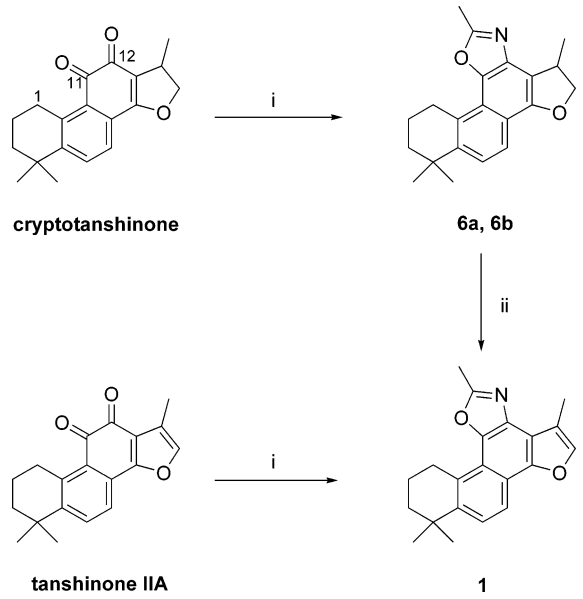


Figure 1. Selected HMBC correlations for compounds **1** and **4**.

constants of 2.5, 4, 6, 8, and 10 Hz. To confirm the orientation of N and O atoms in the oxazole ring of **6a**, compound **6b** was prepared from ^{15}N-enriched ethylamine. The ^{13}C NMR spectrum showed a two-bond coupling of C-13 (δ_C 115.2, d, $^2J_{C-N}$ = 5.6 Hz) with ^{15}N, indicating that the nitrogen atom was located at C-12.[22] Thus, the structure of **6a** was established, and it was named neosalvianan. Furthermore, compound **1** was also prepared by treatment of tanshinone IIA in EtOH with aqueous ethylamine solution. The ^{1}H and ^{13}C NMR spectra of synthetic **1** were identical to those of naturally occurring material (**1**). The structure of compound **1** was therefore confirmed, and **1** was given the trivial name neosalvianen.

Compound **2** was obtained as brown needles with a molecular formula of $C_{21}H_{21}NO_2$ determined by HREIMS ([M]$^+$, *m/z* 319.1581). The ^{13}C and DEPT NMR spectra of

Scheme 1. Syntheses of **1**, **6a**, and **6b**[a]



[a] (i) 70% aqueous $EtNH_2$, EtOH for **6a**; $Et^{15}NH_2$, EtOH for **6b**; (ii) DDQ, benzene.

2 showed the presence of 20 carbon signals (Table 2) and were similar to those of **1**. The ^{1}H NMR spectrum exhibited three aromatic protons at δ 8.16 and 7.61 (1H each, d, *J* = 8.5 Hz) and 7.53 (1H, q, *J* = 1.0 Hz), three methylene protons at δ 3.75, 2.00, and 1.76, and three tertiary methyl signals at δ 2.81 (3H, s), 1.39 (6H, s), and 2.50 (3H, d, *J* = 1.0 Hz) (Table 1). A set of equivalent methyl groups at δ_H 1.39 and δ_C 32.0 was observed in the HMQC spectrum. The COSY spectrum of **2** indicated that the protons at δ 2.00 (H-2) correlated with those at δ 3.75 (H-1) and 1.76 (H-3). Analysis of the COSY, HMQC, and HMBC data (Experimental Section) revealed that **2** was similar to **1**. The IR spectrum exhibited absorption bands at 1591 and 821 cm^{-1} for the oxazole ring. The EIMS of **2** gave a molecular ion peak at *m/z* 319 and major fragment peaks at *m/z* 304, 263, and 235, suggesting [M − CH_3]$^+$, [M − CH_3 − C_2H_3N]$^+$, and [M − CH_3 − C_2H_3N − CO]$^+$, respectively. By comparison with the spectra of **1**, the above data suggested that the N atom in the oxazole ring of **2** was alternatively attached to C-11, resonating at δ 134.0. The structure of **2** was therefore assigned, and it was named salvianen.

Compound **3** was obtained as pale green plates with a molecular formula of $C_{21}H_{23}NO_2$ determined by HREIMS ([M]$^+$, *m/z* 321.1728). The ^{1}H NMR spectrum exhibited two aromatic protons at δ 7.83 and 7.49 (1H each, d, *J* = 9.0 Hz), one methine signal at δ 3.99, three methylene

protons at δ 3.68, 1.96, and 1.76, an oxygenated methylene at δ 4.92 (1H, t, J = 9.0 Hz) and 4.36 (1H, dd, J = 2.0 and 9.0 Hz), and four methyl groups at δ 2.74, 1.37, and 1.36 (3H each, s), and 1.49 (3H, d, J = 7.0 Hz) (Table 1). The ^{13}C and DEPT NMR spectra displayed 21 carbons (Table 2), which were assigned to four methyls (three tertiary and one secondary), four aliphatic methylenes (one oxygenated at δ 79.5), three methines (one aliphatic at δ 36.4 and two aromatic), and 10 quaternary (one aliphatic at δ 34.6 and nine aromatic) carbons. In the COSY spectrum, it showed correlations of the methylene protons at δ 1.96 (H-2) with those at δ 3.68 (H-1) and 1.76 (H-3). In addition, the correlations of the methine signal at δ 3.99 (H-15) with the oxygenated methylene protons at δ 4.36 (H-16a) and 4.92 (H-16b) and the methyl group at δ 1.49 (H-17) were also observed. Analysis of the COSY, HMQC, and HMBC data (Experimental Section) indicated that **3** was partially similar to **2**. The long-range correlations between H-16 and C-13, C-14, and C-17 in the HMBC spectrum revealed the presence of a 3-methyldihydrofuran ring. Further comparisons of the COSY, HMQC, and HMBC data with those of **6a** showed that **3** was similar to synthetic **6a**. In contrast to **6a**, the N atom in the oxazole ring of **3** was attached to C-11, resonating at δ 130.5. The structure of **3** was therefore determined, and it was given the trivial name salvianan.

Compound **4** was obtained as an orange solid with a molecular formula of $C_{19}H_{19}NO_2$ determined by HREIMS ($[M]^+$, *m/z* 293.1419). The ^{1}H NMR spectrum exhibited three aromatic protons at δ 7.66 and 7.30 (1H each, d, J = 7.5 Hz) and 7.56 (1H, s), one aliphatic methine at δ 3.54, one aliphatic methylene at δ 2.95 (2H, s), and two methyl signals at δ 1.49 (6H, s) and 1.31 (6H, d, J = 7.0 Hz) (Table 1). The ^{13}C NMR spectrum of **4** showed 17 carbon signals (Table 2). The DEPT NMR spectrum displayed two methyls, one aliphatic methylene, four methines (one aliphatic and three aromatic), and 10 quaternary (one aliphatic, seven aromatic, and two carbonyl) carbons. The connectivities of ^{1}H−^{13}C signals were determined by the HMQC spectrum, which revealed two sets of two equivalent methyl groups (δ_H 1.49, δ_C 30.1; δ_H 1.31, δ_C 22.8). The structure elucidation was further facilitated through analyses of the COSY and HMBC spectra. The COSY spectrum showed correlation between the methine proton at δ 3.54 (H-15) and the two equivalent methyl protons at δ 1.31 (H-16 and H-17), which indicated the presence of an isopropyl group. Some selected HMBC correlations are shown in Figure 1. However, it was difficult to assign the C-11 carbon on the basis of the HMBC spectrum by standard pulse sequence (J_{H-C} = 8 Hz). When the ^{1}H−^{13}C coupling constant of 2.5 Hz was applied, the four-bond correlations from H-7 and H-14 to C-11 were observed in the HMBC spectrum, hence confirming the assignment of C-11. The ^{1}H NMR spectrum of **4** in DMSO-d_6 exhibited a broad signal at δ 14.23, which indicated the existence of the intramolecular hydrogen bonding between the pyrrole N−H and the carbonyl groups. The EIMS of **4** gave a molecular ion at *m/z* 293 and major fragment ions at *m/z* 278, 265, 250, and 237, suggesting $[M - CH_3]^+$, $[M - CO]^+$, $[M - CH_3 - CO]^+$, and $[M - 2CO]^+$, respectively. The IR spectrum of **4** showed the presence of a pyrrole NH stretch (3429 cm^{-1}) and conjugated ketones (1679 and 1582 cm^{-1}). The structure of **4** was therefore assigned; **4** was given the trivial name salviadione.

Compound **5** was obtained as a pale brown oil with a molecular formula of $C_7H_9NO_2$ determined by HREIMS ($[M]^+$, *m/z* 139.0637). The IR spectrum indicated the presence of a pyrrole NH stretch band (3263 cm^{-1}) and a conjugated aldehyde (1652 cm^{-1}). The EIMS of **5** gave a molecular ion at *m/z* 139 and major fragment ions at *m/z* 110 and 108, indicating $[M - CHO]^+$ and $[M - OCH_3]^+$, respectively. The ^{1}H NMR spectrum exhibited one aldehyde proton at δ 9.45, two aromatic protons at δ 6.89 and 6.20, one oxygenated methylene at δ 4.17, one methoxy group at δ 3.37, and one N−H proton at δ 9.62. The ^{13}C NMR and DEPT spectra showed one methoxy (δ 58.4), one oxygenated methylene (δ 67.0), one aldehyde methine (δ 178.8), and four aromatic carbons. Of the four aromatic carbons, two quaternary carbons were deshielded (δ 137.6 and 132.7) and two protonated carbons were upfield (δ 121.5 and 109.8), which suggested that the pyrrole ring was 2,5-disubstituted. The HMBC spectrum showed that the aldehyde proton and the oxygenated methylene protons correlated with C-3 and C-4, respectively. The ^{1}H and ^{13}C NMR data were comparable with those of 5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde.[23] Thus, the structure of this new compound was deduced as 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde.

Table 3. Cytotoxicities of Selected Compounds against Human Cancer Cell Lines

compound	CD_{50} (μM)		
	HeLa	HepG2	OVCAR-3
1	63.9	59.2	74.6
2	32.3	30.4	39.5
4	178	137	169
6a	108	121	126
crytotanshinone	17.2	29.7	9.12
tanshinone IIA	8.50	26.5	9.52
cisplatin	18.3	27.7	31.7

The cytotoxicities of N-containing compounds **1**, **2**, **4**, and **6a** against the HeLa (cervical epitheloid carcinoma), HepG2 (hepatocellular carcinoma), and OVCAR-3 (ovarian adenocarcinoma) cell lines have not been previously reported. The cytotoxic evaluation of these compounds showed that **2** was the most potent, with a CD_{50} range of 30.4−39.5 μM against the selected cell lines (Table 3), which is comparable with the positive control, cisplatin. Compounds **1**, **2**, **4**, and **6a** were further subjected to evaluation of antibacterial activity against Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* and Gram-negative *Escherichia coli*. No significant activity was observed for the dosage up to 100 μg/disk of the tested compounds.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-S9 micro-melting point apparatus and are uncorrected. UV spectra were measured with a Hitachi U-3310 spectrophotometer. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer in $CDCl_3$ with TMS as an internal standard. ^{1}H, ^{13}C, COSY, HMQC, HMBC, and DEPT spectra were obtained using standard Varian pulse sequences. EIMS spectra were measured with a direct insertion probe on a Finnigan GCQ spectrometer at 30 eV. HREIMS data were taken on a Finnigan MAT 95S mass spectrometer. Silica gel (Kieselgel 60, 70−230 mesh, Macherey-Nagel) was used for column chromatography. TLC was carried out on aluminum sheets precoated with silica gel 60 F_{254} (layer thickness 0.2 mm, Merck). The chromatograms were visualized under UV light (254 or 365 nm) or by spraying with 5% phosphomolybdic acid in 5% H_2SO_4 containing a trace of ceric sulfate, followed by heating on a hot plate (120 °C). Ethylamine-^{15}N hydrochloride was purchased from Aldrich Chemical Co.

Plant Material. The dried root of *S. miltiorrhiza* was purchased from the Cherng-Chi Chinese herbal shop in Taipei in January 2002. A voucher specimen (NRICM 02006) was deposited in the herbarium of the National Research Institute of Chinese Medicine, Taipei.

Extraction and Isolation. The dried root of *S. miltiorrhiza* (20 kg) was crushed and extracted with EtOH (3 × 100 L) at 60 °C for 24 h. The EtOH extracts were combined and concentrated in vacuo to 3 L. The concentrated extract was suspended in H_2O (15 L) and partitioned successively with EtOAc. After concentration of the EtOAc extract, the concentrate was mixed with 1.5 kg of silica gel (230−400 mesh). The air-dried mixture was divided into three equal parts, and each part was subjected to column chromatography (10 cm i.d. × 100 cm). Each column was then eluted with a stepwise gradient eluent of hexane/EtOAc (95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 25:75, 0:100) (15 L each). Fractions (1 L each) were collected, and similar fractions were combined to give 16 fractions (A−P). Fraction E was separated by column chromatography (2 cm i.d. × 100 cm) and eluted with hexane/EtOAc (95:5) to afford **2** (5 mg). Fraction F was rechromatograped on a column (1 cm i.d. × 100 cm) with $CHCl_3$/hexane (20:80) as the eluent to yield **3** (3 mg). Fraction G was separated by column chromatography (2 cm i.d. × 100 cm). Elution with hexane/EtOAc (100:0, 95:5, 90:10) (3 L each) gave **1** (15 mg). Fraction I was similarly rechromatograped on a column (2 cm i.d. × 100 cm) and eluted with hexane/EtOAc (95:5, 90:10, 85:15, 80:20, 75:25) (2 L each), affording **4** (10 mg) and **5** (35 mg).

Neosalvianen (1): colorless needles (EtOAc/hexane); 15 mg; mp 213−215 °C; TLC R_f 0.44 (15% EtOAc/hexane); UV (CH_3OH) λ_{max} (log ϵ) 201 (4.90), 255 (5.34), 263 (5.43) nm; IR (KBr) ν_{max} 2956, 2924, 2851, 1729, 1571, 1466, 1418, 1375, 1237, 1133, 989, 941, 822 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; COSY correlations H-2/H-1, H-3; H-6/H-7; H-16/H-17; HREIMS *m/z* 319.1570 (calcd for $C_{21}H_{21}NO_2$ 319.1567); EIMS *m/z* 319 $[M]^+$ (93), 304 (100), 276 (5), 263 (34), 235 (5).

Salvianen (2): brown needles (EtOAc/hexane); 5 mg; mp 90−92 °C; TLC R_f 0.60 (10% EtOAc/hexane); IR (KBr) ν_{max} 2957, 2923, 2851, 1813, 1591, 1456, 1376, 1125, 1073, 941, 821 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; COSY correlations H-2/H-1, H-3; H-6/H-7; H-16/H-17; HMBC correlations H-1/C-2, C-3, C-5, C-9, C-10; H-2/C-1, C-3, C-4, C-10; H-3/C-1, C-2, C-4, C-5, C-18; H-6/C-4, C-8, C-10; H-7/C-5, C-9, C-14; H-16/C-13, C-14, C-15; H-17/C-13, C-15, C-16; H-18/C-3, C-4, C-5; H-21/C-20; HREIMS *m/z* 319.1581 (calcd for $C_{21}H_{21}NO_2$ 319.1567); EIMS *m/z* 319 $[M]^+$ (93), 304 (100), 290 (25), 263 (39), 235 (34).

Salvianan (3): pale green plates (EtOAc/hexane); 3 mg; TLC R_f 0.55 (15% EtOAc/hexane); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; COSY correlations H-2/H-1, H-3; H-6/H-7; H-15/H-16a, H-16b, H-17; HMBC correlations H-1/C-2, C-3, C-5, C-9, C-10; H-2/C-1, C-3, C-4, C-10; H-3/C-1, C-2, C-4, C-5, C-18, C-19; H-6/C-4, C-8, C-10; H-7/C-5, C-9, C-14; H-15/C-13, C-14, C-17; H-16a/C-13, C-14, C-15, C-17; H-16b/C-13, C-14, C-15, C-17; H-17/C-13, C-15, C-16; H-18/C-3, C-4, C-5, C-19; H-19/ C-3, C-4, C-5, C-18; H-21/C-20; HREIMS *m/z* 321.1728 (calcd for $C_{21}H_{23}NO_2$ 321.1723); EIMS *m/z* 321 $[M]^+$ (100), 306 (96), 292 (40), 265 (15), 263 (14), 237 (71).

Salviadione (4): orange solid (EtOAc/hexane); 10 mg; mp 212−214 °C; TLC R_f 0.51 (40% EtOAc/hexane); IR (KBr) ν_{max} 3429, 2954, 2925, 2853, 1679, 1650, 1582, 1266 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; COSY correlations H-6/H-7; H-15/H-16; HREIMS *m/z* 293.1419 (calcd for $C_{19}H_{19}NO_2$ 293.1410); EIMS *m/z* 293 $[M]^+$ (100), 278 (50), 265 (41), 250 (32), 237 (25), 209 (15).

5-(Methoxymethyl)-1*H*-pyrrole-2-carbaldehyde (5): pale brown oil; 35 mg; TLC R_f 0.53 (40% EtOAc/hexane); IR (neat) ν_{max} 3263, 3017, 2927, 2854, 1652, 1496, 1422, 1373, 1216, 1186, 1093, 1041, 759, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.37 (3H, s, OCH_3), 4.17 (2H, s, OCH_2), 6.20 (1H, m, H-4), 6.89 (1H, m, H-3), 9.45 (1H, s, C*H*O), 9.62 (1H, br, N*H*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.8 (d, *C*HO), 137.6 (s, C-5), 132.7 (s, C-2), 121.5 (d, C-3), 109.8 (d, C-4), 67.0 (t, OCH_2), 58.4 (q, OCH_3); HREIMS *m/z* 139.0637 (calcd for $C_7H_9NO_2$ 139.0633); EIMS *m/z* 139 $[M]^+$ (92), 110 (100), 108 (83), 80 (52).

Synthesis of Neosalvianan (6a).[20] To a solution of cryptotanshinone (100 mg, 0.34 mmol) in EtOH (20 mL) was added $EtNH_2$ in H_2O (70%, 15 mL). After stirring at room temperature for 24 h, the mixture was concentrated and subjected to column chromatography with hexane/EtOAc (95:5) as the solvent to give **6a** (25 mg, 23%) as a colorless solid: mp 230−231 °C; UV (CH_3OH) λ_{max} (log ϵ) 230 (4.52), 252 (4.46) nm; IR (KBr) ν_{max} 2953, 2910, 1568, 1459, 1398, 1240, 1121, 988, 957, 932, 820 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; HMBC correlations H-1/C-2, C-3, C-5, C-9, C-10; H-2/C-1, C-3, C-4, C-10; H-3/C-1, C-2, C-4, C-5, C-18; H-6/C-4, C-8, C-10; H-7/C-5, C-9, C-14; H-16a/C-13, C-14, C-15, C-17; H-16b/C-13, C-14, C-17; H-17/C-13, C-15, C-16; H-18/C-3, C-4, C-5; H-21/C-20; EIMS *m/z* 321 $[M]^+$ (100), 306 (74), 277 (3), 265 (40), 236 (13), 221 (12).

Synthesis of ^{15}N-Enriched Neosalvianan (6b). To a solution of cryptotanshinone (100 mg, 0.34 mmol) in EtOH (15 mL) was added $Et^{15}NH_2$·HCl (260 mg, 3.3 mmol) in 2 N NaOH solution (1.6 mL). After stirring at room temperature for 22 h, the mixture was concentrated and subjected to column chromatography eluting with hexane/EtOAc (95:5) to give **6b** (15 mg, 15%) as a colorless solid. The ^1H and ^{13}C NMR data were the same as those of **6a** except H-21 (d, J_{H-N} = 2.0 Hz), C-13 (d, J_{C-N} = 5.6 Hz), C-20 (d, J_{C-N} = 2.2 Hz), and C-21 (d, J_{C-N} = 5.6 Hz), which coupled with ^{15}N.

Synthesis of Neosalvianen (1) from Neosalvianan (6a). To a solution of **6a** (18 mg, 0.056 mmol) in dry benzene (5 mL) was added DDQ (32 mg, 0.14 mmol).[19] After stirring at room temperature for 48 h, the mixture was filtered through Celite and concentrated. The crude residue was subjected to column chromatography eluting with hexane/EtOAc (95:5) to give **1** (10 mg, 56%) as a colorless solid. The ^1H, ^{13}C NMR, mp, and EIMS data were identical with those of natural product **1**.

Synthesis of Neosalvianen (1) from Tanshinone IIA. A solution of $EtNH_2$ in H_2O (70%, 10 mL) was added to a solution of tanshinone IIA (50 mg, 0.17 mmol) in EtOH (10 mL). The reaction mixture was stirred at room temperature for 48 h and then concentrated. The residue was subjected to column chromatography and eluted with hexane/EtOAc (95:5) to afford **1** (8 mg, 15%).

Cytotoxicity Assay. The cell line culture conditions[24] and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay for CD_{50} were carried out according to the procedures previously described.[25]

Antibacterial Activity. Bacteria, culture conditions, and the antibacterial activity evaluation for compounds **1**, **2**, **4**, and **6a** and the positive control were the same as previously outlined.[25]

Acknowledgment. We gratefully acknowledge the financial support through the National Science Council (NSC 93-2113-M-077-004 to C.M.S.) and NRICM for this work.

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NP0500934