Pergamon

Tetrahedron 58 (2002) 10315–10321

TETRAHEDRON

Reaction of tanshinones with biogenic amine metabolites in vitro

Lin-Kun An,[a,b] Xian-Zhang Bu,[a] Hai-Qiang Wu,[a] Xin-Dong Guo,[a] Lin Ma[a,b] and Lian-Quan Gu[a,b,*]

[a]*Department of Chemistry, School of Chemistry and Chemical Engineering, Zhongshan (Sun Yat-Sen) University, Guangzhou 510275, People's Republic of China*

[b]*School of Pharmaceutical Sciences, Zhongshan (Sun Yat-Sen) University, Guangzhou 510275, People's Republic of China*

Received 7 August 2002; revised 7 October 2002; accepted 31 October 2002

Abstract—The reaction of cryptotanshinone and tanshinone IIA with several biogenic amine metabolites involved in the pathogenic pathways of hepatic encephalopathy are investigated and eleven 1,2,3,4,-tetrahydrophenanthrene derivatives, **2**–**10**, **14** and **16**, are obtained. The probable mechanisms on reaction are discussed.

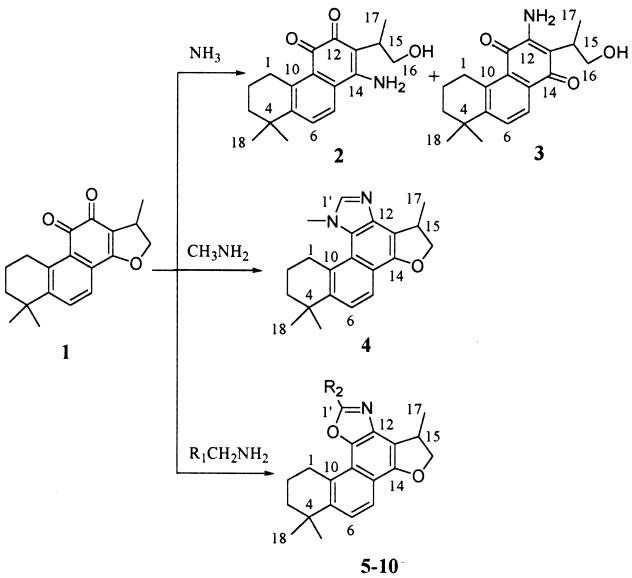
1. Introduction

Salvia miltiorrhiza Bunge, a well-known traditional Chinese medicinal herb, have attracted particular attention from medicinal chemists and clinicians, because of its reputed therapeutic effects in the treatment of coronary heart and cerebrovascular diseases as well as neurasthenic insomnia.[1,2] While many compounds with antibacterial, anti-fungal, anti-inflammatory, anti-neoplastic, and anti-platelet aggregation activities have been identified from this medicinal herb,[3–8] the active ingredient responsible for its tranquilizing effect has not been established.[1] The major components of the herb are tanshinones, including cryptotanshinone (**1**) and tanshinone IIA (**13**). In our previous work, it was found that cryptotanshinone tends to react with aqueous ammonia and alkyl amines.[9] This result led us to do some animal studies, which showed that tanshinones could decrease the ammonia concentration in plasma and the brain, that alleviates the symptoms of hepatic encephalopathy (HE).[10]

HE is a serious neuropsychiatric complication of both acute and chronic liver disease.[11] It was suggested that the abnormally high concentration of ammonia in plasma and cerebrospinal fluid as well as neurotransmission failure were responsible for HE.[12–17] In our attempt to explore the nature of the effect of tanshinones on HE, the interaction of typical tanshinones, **1** and **13**, with several biogenic amine metabolites involved in the pathogenic pathways of HE,[14–17] such as ammonia, 2-phenyl ethylamine, tyramine, 4-aminobutyric acid, 2-amino-1-phenyl ethanol and DL-noradrenaline, have been investigated systematically. In this paper, we report the chemical reaction of **1** and **13** with the biogenic amine metabolites mentioned above in vitro, and propose the probable mechanisms of these reactions.

2. Results

The reaction of **1** with aqueous ammonia in ethanol at room temperature, gave two major products **2** and **3** (Scheme 1).



Scheme 1. Reaction of compound **1** with amines.

Keywords: tanshinone; hepatic encephalopathy; biogenic amine metabolite.

* Corresponding author. Tel.: +86-20-84110272; fax: +86-20-84110272; e-mail: cedc42@zsulink.zsu.edu.cn

0040–4020/02/$ - see front matter
PII: S0040-4020(02)01414-X

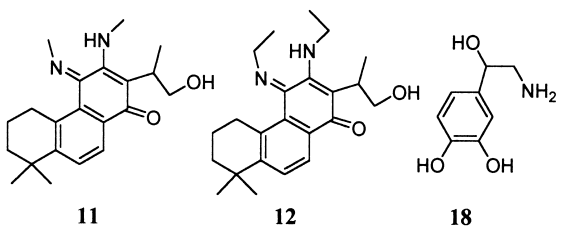
Mass spectra and elemental analysis showed that both have the same formula, $C_{19}H_{23}NO_3$. The infrared spectrum exhibited absorption bands at 3364, 3324, and 1671 cm^{-1} (α,β-unsaturated ketone) (compound **2**); 3500, 3333, and 1699 cm^{-1} (α,β-unsaturated ketone) (compound **3**). The UV spectrum showed characteristic maximum at 279, 349, 458 nm (compound **2**), and at 272, 294, 347 nm (compound **3**). These spectral properties suggested the presence of a 1,2-naphthoquinone moiety in compound **2** and a 1,4-naphthoquinone moiety in compound **3**. ^{13}C NMR and DEPT spectra showed the presence of a methylene group at δ_C 64.6 ppm (**2**) and δ_C 65.4 ppm (**3**). Signals for two protons with an ABX type splitting were found at δ_H 3.69 (dd, J=10, 5 Hz) and δ_H 3.77 (dd, J=10, 6 Hz) (**2**), δ_H 3.70 (dd, J=11, 5 Hz) and δ_H 3.79 (dd, J=11, 8 Hz) (**3**). Compared with compound **1**, there was a methylene group at δ_C 81.5 and δ_H 4.90 (t, J=9 Hz, 1H), 4.37 (dd, J=10, 6 Hz, 1H). This means that the dihydrofuran ring of **1** was opened during the reaction. Therefore, compounds **2** and **3** were assigned as 1-amino-2-(1-hydroxy-2-propyl)-8,8-dimethyl-5,6,7,8-tetrahydro phenanthrene-3,4-dione and 3-amino-2-(1-hydroxy-2-propyl)-8,8-dimethyl-5,6,7,8-tetrahydro phenanthrene-1,4-dione, respectively.

Reaction of **1** with aqueous CH_3NH_2 in ethanol at room temperature gave the major product **4** (Scheme 1), which had molecular formula $C_{21}H_{24}N_2O$, by mass spectra and elemental analysis. The IR absorption showed no active hydrogen. Comparison of the ^{13}C NMR and DEPT spectra with that of **1**, compound **4** had an additional methyl and an additional methine but no carbonyl. The ^1H NMR of **4** showed two additional single peaks at δ_H 4.05 (3H) and δ_H 7.86 (1H). These properties were clearly indicative of the presence of an aromatic hydrogen and a methyl attached to an atom with high electronegativity (N). It seems that a ring had been formed at the position that the carbonyls occupied in compound **1**. Furthermore, the NMR spectra of **4** showed the presence of a methylene with δ_C 79.3 and δ_H 4.91 (t, J=9 Hz, 1H) and δ_H 4.37 (dd, J=10, 6 Hz, 1H). These properties were similar to those of **1**, implying the presence of a dihydrofuran ring in **4**. Compared with **1**, the ^{13}C NMR spectra also showed a low-field shifting of $\delta_{C\text{-}1}$, $\delta_{C\text{-}2}$ and $\delta_{C\text{-}3}$, from δ_C 29.6, 19.3, and 37.8 to δ_C 34.3, 21.3, and 40.0, respectively. The single peak of six protons in the geminal dimethyl group ($\delta_{H\text{-}18}$) was split into two single peaks (δ_H 1.38, 1.41). All of these were apparently attributed to an anisotropic effect on the cyclohexane moiety, which proved that the methyl on the imidazole ring was attached to the N atom at the C-11 position in compound **4**. Hence, the structure of **4** was assigned as 1,4,9,9-tetramethyl-4,5,9,10,11,12-hexahydro-1*H*-6-oxa-1,3-diazadicyclopenta [*a*,*c*] phenanthrene.

Using ethanol as solvent, cryptotanshinone could react with ethylamine derivatives. The reactions of **1** with ethylamine, 2-phenyl ethylamine, tyramine, 4-aminobutyric acid and 2-amino-1-phenyl ethanol gave major products of **5**–**10**, respectively (Scheme 1). The ^{13}C NMR and IR data showed that products **5**–**9** had no *o*-quinone carbon present. Comparison of the NMR data with that of **1**, the basic framework of **1** had no apparent change except the quinone moiety during reacting. At the same time, **5**–**9** had an additional R_1 moiety, which was from the amine. However, ^{13}C NMR-DEPT showed that **5**–**9** had no additional methylene than **1**, which was present in the α-position of the amino group in the starting amines, but had an additional quarternary carbon (C-1′). FAB-MS and elemental analysis indicate that the molecular formula of **5**–**9** was $C_{21}H_{23}NO_2$, $C_{27}H_{27}NO_2$, $C_{27}H_{27}NO_3$, $C_{23}H_{25}NO_4$, and $C_{27}H_{27}NO_3$, respectively. The analytical data suggested that an oxazole ring had formed at the position occupied by the two carbonyls in **1**. The HMBC and HMQC correlation of H-15 with C-12 of **5**–**9** was found, and the $\delta_{C\text{-}12}$ of each product was assigned as 135.6, 135.2, 136.8, 136.7, and 136.3, respectively, which indicated that the N atom in the oxazole ring was attached to C-12. Therefore, **5**–**9** were assigned as 2,4,9,9-tetramethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene, 2-benzyl-4,9,9-trimethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene, 2-(4′-hydroxy-benzyl)-4,9,9-trimethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene, 2-(2-carboxy-ethyl)-4,9,9-trimethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene and 2-(1-hydroxy-benzyl)-4,9,9-trimethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene, respectively.

Compound **10**, one of the two major products obtained from reaction of **1** with 2-amino-1-phenyl ethanol, was much interesting. FAB-MS and elemental analysis indicated that its formula was $C_{20}H_{21}NO_2$. The NMR data of **10** was similar to **9**, except that it had an additional methine but no R_1 group (C_6H_5CHOH-) and an additional quarternary carbon signal compared with **1**. All data implying that it was a α-hydroxybenzyl-cleaved product, 4,9,9-trimethyl-4,5,9,10,11,12-hexahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene.

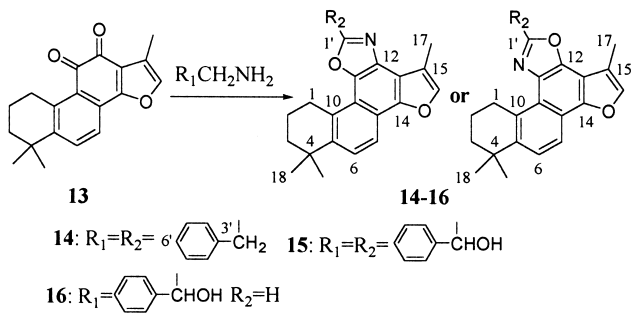
The reaction of **1** with CH_3NH_2 and $C_2H_5NH_2$ also gave the minor products **11** and **12**, respectively. FAB-MS showed that the molecular weight of **11** was 340, and that of **12** was 368. But no other analysis was performed owing to limited availability. The two minor products had been tentatively assigned the structures as shown in Fig. 1.



11 **12** **18**

Figure 1.

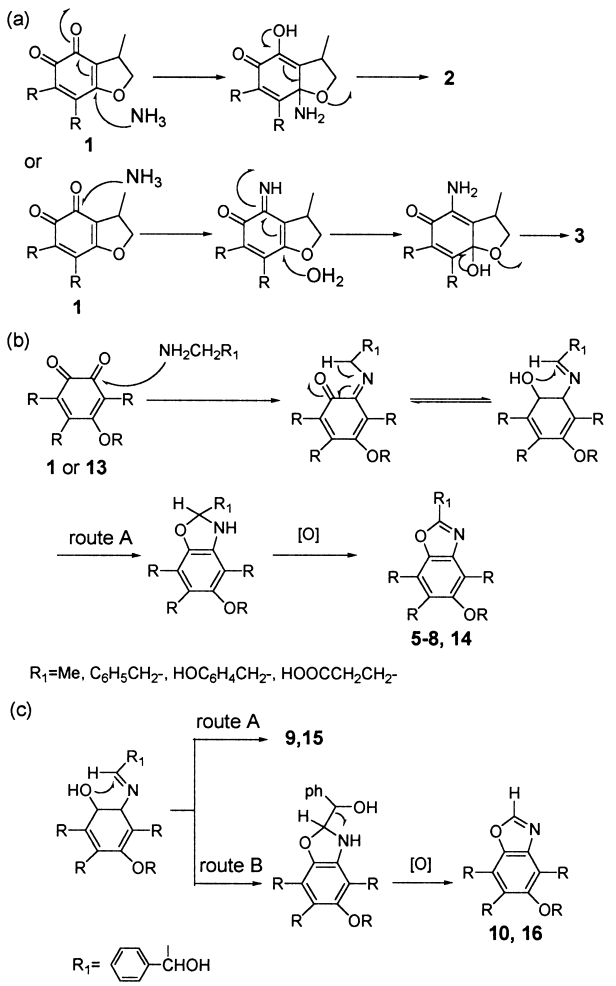
In the same way, tanshinone IIA was reacted with 2-phenyl ethylamine and 2-amino-1-phenyl ethanol, but the reaction did not take place unless refluxing in ethanol at 85°C, and major products **14** and **16** were obtained, respectively (Scheme 2). Similar to the **5**–**9** and **10**, **14** and **16** were assigned as R_1-uncleaved and R_1-cleaved oxazole ring derivatives, respectively, according to their NMR data. However, the exact carbon atom attached by N and O atoms in the oxazole ring could not be confirmed because the position of C-11 and C-12 was difficult to be assigned exactly based on HMBC and HMQC. Therefore, **14** and **16**



Scheme 2. Reaction of compound **13** with amines.

were assigned as 2-phenyl-4,9,9-trimethyl-9,10,11,12-tetrahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene or 2-phenyl-4,9,9-trimethyl-9,10,11,12-tetrahydro-3,6-dioxa-1-aza-dicyclopenta [*a*,*c*] phenanthrene and 4,9,9-trimethyl-9,10,11,12-tetrahydro-1,6-dioxa-3-aza-dicyclopenta [*a*,*c*] phenanthrene or 4,9,9-trimethyl-9,10,11,12-tetrahydro-3,6-dioxa-1-aza-dicyclopenta [*a*,*c*] phenanthrene.

The reaction of **13** with 2-amino-1-phenyl ethanol gave a minor product **15** at the same time. FAB-MS showed the molecular weight of **15** was 411. No other spectrum was recorded owing to limited availability. The probable structure was tentatively assigned as shown in Scheme 2.



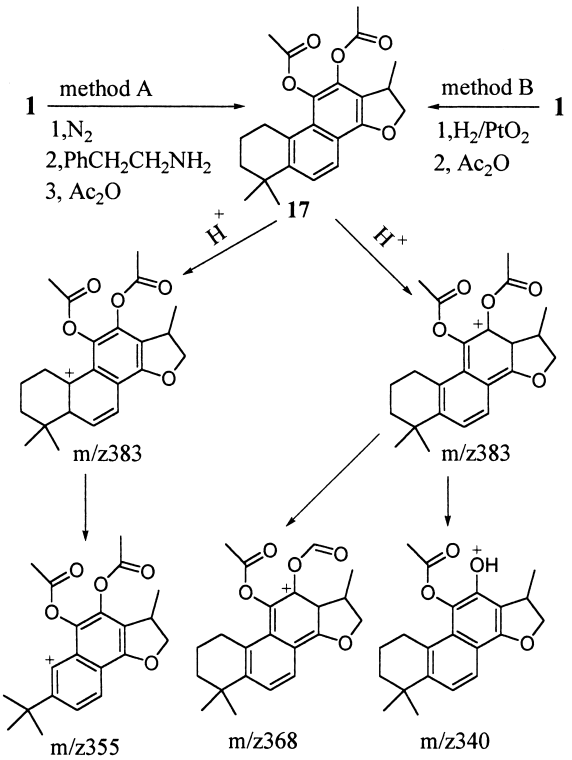
Scheme 3. The mechanisms on reaction of tanshinones with amines.

3. Discussion

The dihydrofuran ring was opened when compound **1** reacted with ammonia, and the amino group was attached to C-14 (**2**) or C-12 (**3**). This means that a 1,4-addition reaction or a 1,2-addition reaction was taken place. Therefore, the mechanism shown in Scheme 3(a) was proposed.

An oxazole ring was formed during the reaction of tanshinones with alkyl amines and at the same time, a molecule of H_2O and two H atoms were lost. According to the result, the mechanism shown in Scheme 3(b) was proposed. The amine group of the starting amine attacked the *o*-quinone moiety of tanshinone during the nucleophilic substituted reaction, and an imine intermediate (M) was formed. Afterwards, the imine intermediate may run a cyclization-oxidation reaction through route A. However, if the starting amine was 2-amino-1-phenyl ethanol, the imine intermediate will proceed through two routes (A and B) shown in Scheme 3(c), which gave two products, α-hydroxybenzyl-uncleaved and α-hydroxybenzyl-cleaved product.

An experiment was designed to determine whether the oxidant was atmosphere oxygen or tanshinone. Thus, **1** and 2-phenyl ethylamine were suspended in ethanol under nitrogen, and the mixture was stirred at 37°C for 10 h. The major product **6** was obtained from this reaction. Therefore, it means the oxidant was probably tanshinone itself. However, the reduced form of tanshinone (the catechol form) was not obtained because it was very sensitive to air. To capture the reduced form of tanshinone, an experiment was conducted. Under nitrogen, material **1** and 2-phenyl ethylamine were suspended in ethyl acetate, stirred for 10 h, and then acetic anhydride was added (Scheme 4, Method



Scheme 4. Compound **17** and its MS–MS fragmentation patterns.

A). The reacted residue was detected by electrospray MS-MS, compound **6** and another product (**X**) were found. The MS-MS data of product **X** indicated that it had several fragments: *m/z* 383 [M+1], 368, 355 and 340. No further analysis was conducted because the yield was very low. Comparison of the standard compound **17** obtained through Method B, the compound **X** had the same fragments as **17**, which means that compound **X** was **17**. This result identified that tanshinones could act as the oxidant. In fact, the reduced form of tanshinone is easily oxidized by oxygen, which could be proved during catalytic hydrogenation of **1**. Under hydrogen and catalytic Pd/C, an orange solution of **1** in ethanol became colorless after 2 min. The colorless solution immediately became orange after exposure to air. TLC did not detect any other component existing in the resolution but compound **1**. Therefore, the reactive oxidant could be the tanshinone itself when the reaction was under nitrogen, or else oxygen could act as the oxidant when the reaction was exposed to air.

Noticeably, reaction of **1** with 2-amino-1-phenyl ethanol gave a R_1-uncleaved and a R_1-cleaved oxazole ring derivatives, **9** and **10**, while using **13** as start material, the major product was **16**, only limited R_1-uncleaved product **15** was obtained. Using DL-noradrenaline (**18**, Fig. 1) as starting material, the reaction with **1** or **13** gave major products **10** or **16**, respectively, which indicated that α-hydroxybenzyl group was eliminated easily during the cyclization-oxidation reaction.

The fact that tanshinones can react with those biogenic amine metabolites involved in the pathogenesis HE imply, to some extent, that tanshinones may remove those compounds in vivo, which may contribute to our primary results, tanshinones can alleviate the symptoms of HE. Further biochemical studies are currently in progress.

4. Experimental

4.1. General

Cryptotanshinone and tanshinone IIA were isolated from the Chinese medicinal herb, *S. miltiorrhiza* Bunge. Aqueous ammonia solution (33%), methylamine solution in water (33%) and ethylamine solution in water (70%) were commercially available locally; 2-phenyl ethylamine, tyramine and 4-amino butyric acid were purchased from ACROS ORGANICS. 2-amino-1-phenyl ethanol and DL-noradrenaline were purchased from SIGMA Chemical Co. All chemicals were not refined before usage.

Melting points were uncorrected and were determined using a XT-4 apparatus. ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{13}C HMQC and HMBC spectra were measured on a Varian UNITY INOVA 500 MHz spectrometer using TMS as an internal standard. For the electrospray (ESI) MS analysis a Finnigan LCQ Deca XP ion trap mass spectrometer equipped with a Microsoft Windows NT data system and an ESI interface was used. FAB-MS was measured on a VG ZAB-HS analytical spectrometer. Elementary analysis was recorded on an Elementar Vario EL elementary analysis device. IR absorption was recorded on a Bruker EQUINOX-55 spectrophotometer. UV absorption was recorded on a SHIMADZU UV-2501 PC spectrophotometer.

4.1.1. Separation of cryptotanshinone (1) and tanshinone IIA (13). Supercritical extract of Chinese medicinal herb, *S. miltiorrhiza* Bunge, was purified by silica gel column chromatography by stepwise elution using ethyl acetate/petroleum ether (5:95–10:90) mixture as eluent. Obtained orange needle crystals **1** and red needle crystals **13**.

Compound **1**. FAB-MS *m/z* (rel. int.): 297 $[M+1]^+$ (100).^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.31 (s, 6H), 1.36 (d, J=6 Hz, 6H), 1.64 (m, 2H), 1.78 (m, 2H), 3.22 (t, J=7 Hz, 2H), 3.62 (m, 1H), 4.37 (dd, ABX, J=9, 6 Hz, 1H), 4.90 (t, J=9 Hz, 1H), 7.51 (d, AB, J=8 Hz, 1H), 7.64 (d, AB, J=8 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 18.7q, 19.3t, 29.6t, 31.7q, 34.6d, 34.7s, 37.8t, 81.5t, 118.2s, 122.5d, 126.1s, 128.8s, 132.5d, 143.6s, 152.3s, 170.8s, 175.5s, 184.0s.

Compound **13**. FAB-MS *m/z* (rel. int.): 295 $[M+1]^+$ (79), 136 (100). ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.28 (s, 6H), 1.62 (m, 2H), 1.72 (m, 2H), 2.23 (d, J=1.3 Hz, 3H), 3.15 (t, J=6 Hz, 2H), 7.19 (q, J=1.3 Hz, 1H), 7.52 (d, AB, J=8 Hz, 1H), 7.61 (d, AB, J=8 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 8.9q, 19.3t, 30.1t, 32.0q, 34.9s, 38.2t, 120.4d, 120.8s, 121.5d, 127.1s, 128.0s, 134.1d, 141.9s, 145.1s, 150.3s, 162.4s, 176.5s, 184.4s.

4.2. Preparation of compounds 2–12

4.2.1. Compounds 2 and 3. To a solution of **1** (50 mg, 169 mmol) in ethanol (8 ml), aqueous ammonia (33%, 15 ml) was added. The mixture was stirred at room temperature for 10 h. Solvent was evaporated under vacuum, residue was purified by silica gel column chromatography and stepwise elution using ethyl acetate/-petroleum ether mixture (30:70–95:5) as eluent. Dark red solid **2** and yellow solid **3** were obtained.

Compound **2**. 26 mg, yield 50%. $C_{19}H_{23}NO_3$, calcd: C, 72.82%; H, 7.40%; N, 4.47%; O, 15.32%; found: C, 72.78%; H, 7.43%; N, 4.15%. Mp 168–188°C. FAB-MS *m/z* (rel. int.): 314 $[M+1]^+$ (100), 296 $[M+1-18]^+$ (10). UV λ_{max} (EtOH): 458, 349, 279, 242, 217 nm. IR ν_{max} (KBr): 3364, 3324, 1671, 1588, 1506, 1459, 1417, 1027, 680 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.14 (d, J=7.5 Hz, 3H), 1.31 (s, 6H), 1.61 (m, 2H), 1.75 (m, 2H), 3.07 (m, 2H), 3.12 (m, 1H), 3.69 (dd, J=10, 5 Hz, 1H), 3.77 (dd, J=10, 6 Hz, 1H), 5.02 (s, 1H), 7.73 (d, AB, J=9 Hz, 1H), 7.84 (s, 2H), 7.91 (d, AB, J=8.5 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 14.0q, 18.9t, 29.5t, 31.2q, 32.5d, 34.0s, 37.6t, 64.6t, 112.8s, 121.9d, 129.3s, 131.2s, 131.8d, 139.7s, 149.3s, 154.8s, 176.9s, 186.3s. The structure was confirmed by DEPT, HMQC and HMBC.

Compound **3**. 15 mg, yield 28%. $C_{19}H_{23}NO_3$, calcd: C, 72.82%; H, 7.40%; N, 4.47%; O, 15.32%; found: C, 72.76%; H, 7.45%; N, 4.49%. Mp 62–64°C. FAB-MS *m/z* (rel. int.): 314 $[M+1]^+$ (57), 296 $[M+1-18]$ (30), 57 (100). UV λ_{max} (EtOH): 347, 294, 272, 240, 217 nm. IR ν_{max} (KBr): 3500, 3333, 1699, 1642, 1564, 1459, 1415, 1380, 1330, 1327, 1201, 1026 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$,

TMS) δ 1.10 (d, J=6 Hz, 3H), 1.31 (s, 6H), 1.67 (m, 2H), 1.79 (m, 2H), 3.09 (m, 2H), 3.12 (m, 1H), 3.70 (dd, J=11, 5 Hz, 1H), 3.79 (dd, J=11, 8 Hz, 1H), 4.81 (s, 1H), 6.74 (s, 2H), 7.80 (d, AB, J=8 Hz, 1H), 7.88 (d, AB, J=8 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 14.4q, 19.7t, 29.7t, 31.8q, 33.6d, 34.9s, 38.3t, 65.4t, 116.4s, 124.4d, 128.0s, 133.1d, 139.4s, 149.0s, 151.3s, 182.0s, 184.2s. The structure was confirmed by DEPT, HMQC and HMBC.

4.2.2. Compounds 4 and 11. **4** and **11** were prepared according to the procedure of **2** and **3**, using **1** (50 mg, 169 mmol) and methylamine in water (33%, 10 ml) as starting materials, and stepwise elution using ethyl acetate/petroleum ether (10:90–30:70) mixture as eluent. Colorless needle crystals **4** and solid **11** were obtained.

Compound **4**. 20 mg, yield 37%. $C_{21}H_{24}N_2O$, calcd: C, 78.71%; H, 7.55%; N, 8.74%; O, 4.99%; found: C, 78.65%; H, 7.63%; N, 8.68%. FAB-MS m/z (rel. int.): 321 $[M+1]^+$ (90), 55 (100). IR ν_{max} (KBr): 2961, 2930, 2863, 1684, 1384 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.38 (s, 3H), 1.41 (s, 3H), 1.56 (d, J=6.5 Hz, 3H), 1.69 (m, 2H), 1.76 (m, 2H), 3.27 (m, 2H), 4.05 (s, 3H), 4.10 (m, 1H), 4.37 (dd, J=10, 6 Hz, 1H), 4.91 (t, J=9 Hz, 1H), 7.51 (d, AB, J=8.5 Hz, 1H), 7.86 (s, 1H), 7.93 (d, AB, J=8.5 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 19.7q, 21.3t, 32.7q, 34.3t, 35.1s, 37.1d, 38.6q, 40.0t, 79.3t, 115.2s, 118.3s, 120.6d, 123.0s, 124.2d, 126.3s, 129.4s, 140.0s, 143.2s, 145.7d, 152.3s. The structure was confirmed by DEPT and HMQC.

Compound **11**. FAB-MS m/z (rel. int.): 341 $[M+1]^+$ (25), 55 (100). No further analysis was conducted because the yield was very low.

4.2.3. Compound 5. **5** was prepared according to the procedure of **2** and **3**, using **1** (50 mg, 169 mmol) and ethylamine in water (70%, 10 ml) as starting materials, and ethyl acetate/petroleum ether (5:95) mixture as eluent. Colorless needle crystals **5** were obtained, 25 mg, yield 46%. $C_{21}H_{23}NO_2$, calcd: C, 78.47%; H, 7.21%; N, 4.36%; O, 9.96%; found: C, 78.55%; H, 7.14%; N, 4.41%. FAB-MS m/z (rel. int.): 322 $[M+1]^+$ (90), 55 (100). IR ν_{max} (KBr): 2954, 2918, 2852, 1568, 1458, 1397, 1121, 956 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.37 (s, 3H), 1.38 (s, 3H), 1.52 (d, J=6.5 Hz, 3H), 1.76 (m, 2H), 1.98 (m, 2H), 2.70 (s, 3H), 3.44 (t, J=6 Hz, 2H), 4.04 (m, 1H), 4.38 (dd, ABX, J=9, 6 Hz, 1H), 4.91 (t, J=9 Hz, 1H), 7.49 (d, AB, J=9 Hz, 1H), 7.84 (d, AB, J=9 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 14.9q, 19.5t, 19.8q, 29.3t, 31.7q, 34.4s, 36.8d, 38.6t, 79.4t, 115.2s, 116.8s, 117.8s, 119.7d, 124.1d, 129.4s, 135.6s, 143.0s, 143.7s, 153.0s, 162.9s. The structure was confirmed by DEPT, HMQC and HMBC.

4.2.4. Compound 6. **1** (50 mg, 169 mmol) and 2-phenyl ethylamine (22 mg, 182 mmol) were suspended in ethanol (8 ml). The mixture was stirred under 37°C for 10 h. Solvent was evaporated under vacuum, residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (5:95) mixture as eluent. Colorless crystals **6** were obtained, 35 mg, yield 52%. $C_{27}H_{27}NO_2$, calcd: C, 81.58%; H, 6.85%; N, 3.52%; O, 8.05%; found: C, 81.56%; H, 6.89%; N, 3.49%. Mp 94.5–96°C. FAB-MS m/z (rel. int.): 398 $[M+1]^+$ (70), 397 (100). UV λ_{max} (MeOH) 349.0, 333.5, 318.5, 255.0, 231.0 nm. IR ν_{max} (KBr): 3029, 1603, 1553, 1454, 1241 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 1.35 (s, 6H), 1.53 (d, J=7 Hz, 3H), 1.74 (m, 2H), 1.95 (m, 2H), 3.37 (t, J=6 Hz, 2H), 4.05 (m, 1H), 4.37 (dd, ABX, J=6, 9 Hz, 1H), 4.38 (s, 2H), 4.90 (t, J=9 Hz, 1H), 7.25–7.28 (m, 1H), 7.32–7.35 (m, 2H), 7.40–7.43 (m, 2H), 7.47 (d, AB, J=9 Hz, 1H), 7.83 (d, AB, J=9 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, TMS) δ 19.5t, 19.9q, 29.2t, 31.5q, 31.6q, 34.3s, 35.3t, 36.8d, 38.5t, 79.4t, 115.2s, 117.0s, 119.6s, 119.8d, 124.3d, 127.1d, 128.7d, 128.8d, 129.4s, 135.2s, 135.3s, 143.1s, 143.8s, 153.2s, 164.1s. The structure was confirmed by DEPT, HMQC and HMBC.

4.2.5. Compound 7. **1** (50 mg, 169 mmol) and tyramine (26 mg, 190 mmol) were suspended in ethanol (8 ml). The mixture was stirred under reflux for 8 h. Solvent was evaporated under vacuum, residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (20:82) mixture as eluent. A light yellow solid **7** was obtained, 12 mg, yield 17%. $C_{27}H_{27}NO_3$, calcd: C, 78.42%; H, 6.58%; N, 3.39%; O, 11.61%; found: C, 78.51%; H, 6.64%; N, 3.31%. FAB-MS m/z (rel. int.): 414 $[M+1]^+$ (4), 55 (100). UV λ_{max} (MeOH): 349.5, 333.5, 319.0, 255.5, 230.5 nm. IR ν_{max} (KBr): 3067, 3017, 1612, 1556, 1514, 1456 cm^{-1}. ^{1}H NMR (500 MHz, acetone, TMS) δ 1.35 (s, 3H), 1.354 (s, 3H), 1.51 (d, J=7 Hz, 3H), 1.75 (m, 2H), 1.94 (m, 2H), 3.39 (t, J=7 Hz, 2H), 4.00 (m, 1H), 4.28 (s, 2H), 4.34 (dd, ABX, J=7, 9 Hz, 1H), 4.92 (t, J=9 Hz, 1H), 6.83 (d, AB, J=9 Hz, 2H), 7.29 (d, AB, J=9 Hz, 2H), 7.53 (d, AB, J=9 Hz, 1H), 7.80 (d, AB, J=9 Hz, 1H), 8.30 (s, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 19.8q, 20.2t, 29.9t, 31.8q, 31.9q, 34.8t, 34.9s, 37.6d, 39.3t, 79.9t, 116.3d, 116.4s, 117.7s, 120.4s, 120.6d, 124.9d, 127.4s, 130.1, 130.8d, 136.8s, 143.6s, 144.5s, 153.9s, 157.4s, 165.9s. The structure was confirmed by DEPT, HMQC and HMBC.

4.2.6. Compound 8. To a mixture of **1** (50 mg, 169 mmol) and 4-aminobutyric acid (19 mg, 185 mmol) in ethanol (8 ml), aqueous NaOH resolution (2 ml, 2%) was added. The mixture was stirred under reflux for 5 h. Solvent was evaporated under vacuum, residue solution was acidified with diluted hydrochloric acid to pH 2, filtrated, light brown solid obtained. The solid was purified by silica gel column chromatography using ethyl acetate/petroleum ether/acetic acid (20:80:0.5) mixture as eluent. Light brown solid **8** was obtained, 9 mg, yield 14%. $C_{23}H_{25}NO_4$, calcd: C, 72.80%; H, 6.64%; N, 3.69%; O, 16.87%; found: C, 72.83%; H, 6.69%; N, 3.60%. Mp 192–193°C. FAB-MS m/z (rel. int.): 380 $[M+1]^+$ (3), 55 (100). UV λ_{max} (MeOH) 348.0, 332.0, 318.0, 253.0, 230.0 nm. IR ν_{max} (KBr): 1718, 1560, 1455, 1406 cm^{-1}. ^{1}H NMR (500 MHz, acetone, TMS) δ 1.36 (s, 3H), 1.37 (s, 3H), 1.50 (d, J=7 Hz, 3H), 1.76 (m, 2H), 1.97 (m, 2H), 3.01 (t, J=7 Hz, 2H), 3.33 (t, J=7 Hz, 2H), 3.44 (t, J=6.5 Hz, 2H), 4.00 (m, 1H), 4.34 (dd, ABX, J=7, 9 Hz, 1H), 4.92 (t, J=9 Hz, 1H), 7.53 (d, AB, J=9 Hz, 1H), 7.80 (d, AB, J=9 Hz, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 19.7q, 20.2t, 24.7t, 29.9t, 30.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 79.9t, 116.3s, 117.7s, 120.4s, 120.6d, 124.9d, 130.1s, 136.7s, 143.5s, 144.4s, 153.9s, 166.1s, 173.3s. The structure was confirmed by DEPT, HMQC and HMBC.

4.2.7. Compounds 9 and 10. **9** and **10** were prepared according to the procedure of **6**, using **1** (50 mg, 169 mmol)

and 2-amino-1-phenyl ethanol (35 mg, 256 mmol) as starting materials, and stepwise elution using ethyl acetate/petroleum ether (5:95–15:85) mixture as eluent. Light yellow solid **9** (18 mg) and **10** (15 mg) were obtained, yield 35% and 21%, respectively.

According to the procedure, the reaction of **1** (50 mg, 169 mmol) with **18** (34 mg, 201 mmol) gave major product **10** (9 mg, yield 17%).

Compound **9**. $C_{27}H_{27}NO_3$, calcd: C, 78.42%; H, 6.58%; N, 3.39%; O, 11.61%; found: C, 78.44%; H, 6.65%; N, 3.34%. FAB-MS *m/z* (rel. int.): 414 $[M+1]^+$ (50), 55 (100). UV λ_{max} (MeOH): 315.0, 335.5, 319.0, 256.0, 232.5, 208.5 nm. IR ν_{max} (KBr): 3064, 3031, 1603, 1557, 1454, 1403 cm^{-1}. ^1H NMR (500 MHz, acetone, TMS) δ 1.35 (s, 6H), 1.51 (d, *J*=7 Hz, 3H), 1.76 (m, 2H), 1.95 (m, 2H), 2.87 (br, 1H), 3.41 (t, *J*=7 Hz, 2H), 4.02 (m, 1H), 4.35 (dd, ABX, *J*=6.5, 9 Hz, 1H), 4.92 (t, *J*=9 Hz, 1H), 6.18 (s, 1H), 7.30–7.34 (m, 1H), 7.38–7.42 (m, 2H), 7.55 (d, AB, *J*=9 Hz, 1H), 7.66–7.68 (m, 2H), 7.80 (d, AB, *J*=9 Hz, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 19.8q, 20.1t, 29.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 70.9d, 80.0t, 116.5s, 118.1s, 120.5s, 120.6d, 125.3d, 127.6d, 128.8d, 129.2d, 130.2s, 136.3s, 141.4s, 143.6s, 144.6s, 154.1s, 167.0s. The structure was confirmed by DEPT, HMQC and HMBC.

Compound **10**. $C_{20}H_{21}NO_2$, calcd: C, 78.15%; H, 6.89%; N, 4.56%; O, 10.41%; found: C, 78.19%; H, 6.94%; N, 4.51%. FAB-MS *m/z* (rel. int.): 308 $[M+1]^+$ (9), 55 (100). UV λ_{max} (MeOH) 348.5, 334.0, 252.0, 230.0 nm. IR ν_{max} (KBr): 3069, 1602, 1505, 1457, 1399 cm^{-1}. ^1H NMR (500 MHz, acetone, TMS) δ 1.36 (s, 3H), 1.37 (s, 3H), 1.53 (d, *J*=7 Hz, 3H), 1.76 (m, 2H), 1.98 (m, 2H), 3.45 (t, *J*=7 Hz, 2H), 4.04 (m, 1H), 4.36 (dd, ABX, *J*=7, 9 Hz, 1H), 4.95 (t, *J*=9 Hz, 1H), 7.58 (d, AB, *J*=9 Hz, 1H), 7.83 (d, AB, *J*=9 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 19.7q, 20.1t, 29.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 80.0t, 116.5s, 118.4s, 120.4s, 120.6d, 125.4d, 130.3s, 135.7s, 143.0s, 144.7s, 153.5d, 154.2s. The structure was confirmed by DEPT, HMQC and HMBC.

4.3. Preparation of compounds 14–16

Mixture of **13** (50 mg, 170 mmol) and the amine in ethanol (8 ml) was stirred under reflux for 25 h. Solvent was evaporated under vacuum, the residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether mixture as eluent.

4.3.1. Compound 14. Using 2-phenyl ethylamine (23 mg, 190 mmol) as the starting material and ethyl acetate/petroleum ether (3:97) mixture as eluent. Colorless crystals **9** were obtained, 39 mg, yield 58%. $C_{27}H_{25}NO_2$, calcd: C, 82.00%; H, 6.37%; N, 3.54%; O, 8.09%; found: C, 81.8%; H, 6.40%; N, 3.51%. Mp 132–133°C. FAB-MS *m/z* (rel. int.): 396 $[M+1]^+$ (100%). UV λ_{max} (MeOH): 343.0, 327.0, 312.5, 280.0, 264.5, 257.0 nm. IR ν_{max} (KBr): 3031, 1603, 1551, 1454, 1382, 1238 cm^{-1}. ^1H NMR (500 MHz, acetone, TMS) δ 1.38 (s, 6H), 1.76 (m, 2H), 1.97 (m, 2H), 2.54 (d, *J*=1.5 Hz, 3H), 3.44 (t, brd, *J*=6.5 Hz, 2H), 4.46 (s, 2H), 7.26–7.30 (m, 1H), 7.35–7.39 (m, 2H), 7.48–7.51 (m, 2H), 7.67 (d, AB, *J*=9 Hz, 1H), 7.77 (q, *J*=1.5 Hz, 1H), 8.12 (dt, *J*=9 Hz, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 9.3q, 20.2t, 30.0t, 32.0q, 35.0s, 35.6t, 39.2t, 117.1s, 117.2s, 118.4s, 118.6s, 119.0d, 126.1d, 127.8d, 129.5d, 129.8d, 130.8s, 133.9s, 136.8s, 142.6d, 144.1s, 145.2s, 150.0s, 165.2s. The structure was confirmed by DEPT, HMQC and HMBC.

4.3.2. Compounds 15 and 16. Using 2-amino-1-phenyl ethanol (35 mg, 256 mmol) as starting materials, and stepwise elution using ethyl acetate/petroleum ether (5:95–10:90) mixture as eluent. Light yellow solid **15** and solid **16** (21 mg, yield 40%) were obtained.

Using **18** (34 mg, 204 mmol) as the starting material instead of 2-amino-1-phenyl ethanol, the reaction gave major product **16** (7 mg, yield 14%).

Compound **15**. FAB-MS *m/z* (rel. int.): 412 $[M+1]^+$ (12), 154 (100). No further analysis was conducted because the yield was very low.

Compound **16**. $C_{20}H_{19}NO_2$, calcd: C, 78.66%; H, 6.27%; N, 4.59%; O, 10.48%; found: C, 78.65%; H, 6.29%; N, 4.56%. Mp 134–138°C. FAB-MS *m/z* (rel. int.): 306 $[M+1]^+$ (60), 55 (100). UV λ_{max} (MeOH): 342.0, 326.5, 261.5, 255.0 nm. IR ν_{max} (KBr): 3066, 1629, 1505, 1452, 1382 cm^{-1}. ^1H NMR (500 MHz, acetone, TMS) δ 1.41 (s, 6H), 1.81 (m, 2H), 2.02 (m, 2H), 2.56 (d, *J*=1.5 Hz, 3H), 3.54 (t, brd, *J*=6.5 Hz, 2H), 7.74 (d, *J*=9 Hz, 1H), 7.81 (q, *J*=1.5 Hz, 1H), 8.16 (dt, *J*=1, 9 Hz, 1H), 8.64 (s, 1H). ^{13}C NMR (500 MHz, acetone, TMS) δ 9.3q, 20.1t, 30.1t, 32.0q, 35.1s, 39.2t, 117.2s, 117.3s, 118.5s, 119.0d, 119.1s, 126.5d, 131.2s, 132.8s, 142.8d, 144.3s, 145.0s, 150.2s, 153.5d. The structure was confirmed by DEPT, HMQC and HMBC.

4.3.3. Preparation of compound 17. *Method A*. Under nitrogen, solution of **1** (50 mg, 169 mmol) and 2-phenyl ethylamine (22 mg, 182 mmol) in ethyl acetate (12 ml) was stirred under 37°C for 10 h, added acetic anhydride (2 ml), stirred at room temperature for 24 h, added water (10 ml). The mixture was extracted with ethyl acetate three times, and organic layer was washed with water, saturated aqueous $NaHCO_3$ and water respectively, and dried over Na_2SO_4. Solvent was evaporated under vacuum, and the residue was analyzed by LC-MS/MS. Compound **17**, MS/MS *m/z*: 383 $[M+1]^+$ (65), 368 (100), 355 (90), 340 (25).

Method B. To a solution of **1** (50 mg, 169 mmol) in ethyl acetate (20 ml), palladium charcoal (10%, 10 mg) was added. The mixture was stirred at room temperature for 10 min in a hydrogen atmosphere, and dripped acetic anhydride (2 ml), stirred at room temperature for 20 h, added water (10 ml). The mixture was extracted with ethyl acetate three times, and organic layer was washed with water, saturated aqueous $NaHCO_3$ and water respectively, and dried over Na_2SO_4. Solvent was evaporated under vacuum, the residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (10:90) mixture as eluent. Colorless solid **17** was obtained, 53 mg, yield 82%. $C_{23}H_{26}O_5$, calcd: C, 72.23%; H, 6.85%; O, 20.92%; found: C, 72.27%; H, 6.93%. FAB-MS *m/z* (rel. int.): 383 $[M+1]^+$ (35), 149 (100). MS/MS *m/z*: 383 $[M+1]^+$ (45), 368 (100), 355 (80), 340 (25). ^1H NMR

(acetone) δ: 7.73 (d, AB, J=9 Hz, 1H), 7.50 (d, AB, J=9 Hz, 1H), 4.88 (t, J=9 Hz, 1H), 4.31 (dd, J=9, 6 Hz, 1H), 3.73 (m, 1H), 3.17 (s, br, 2H), 2.36 (s, 3H), 2.34 (s, 3H), 1.82 (m, 2H), 1.67 (m, 2H), 1.33 (s, 6H), 1.29 (d, J=7 Hz). ^{13}C NMR (acetone) δ 169.7s, 168.3s, 155.1s, 145.4s, 139.4s, 133.5s, 131.5s, 128.1s, 126.0d, 120.3d, 119.6s, 118.6s, 80.1t, 39.1t, 37.7d, 35.4s, 32.0q, 31.9q, 30.3t, 20.9q, 20.6t, 20.4q, 19.1q. UV λ_{max} (MeOH): 336.0, 322.0, 307.0, 229 nm. IR ν_{max} (KBr): 3067, 1767, 1605, 1514, 1466, 1409, 1374 cm^{-1}.

Acknowledgements

The program is sponsored by the Guangzhou City Science Foundation (2000-Z-021-01) and Guangdong Provincial Science Foundation (2KM04103S).

References

1. Chang, H. M.; Chui, K. Y.; Tan, F. W. L.; Yang, Y.; Zhong, Z. P.; Lee, C. M.; Sham, H. L.; Wang, H. N. C. *J. Med. Chem.* **1991**, *34*, 1675–1692.
2. Yagi, A.; Fujimoto, K.; Tanonaka, K.; Hirai, K.; Takeo, S. *Planta Med.* **1989**, *55*, 51–54.
3. Lee, D. S.; Lee, S. H.; Noh, J. G.; Hong, S. D. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 2236–2239.
4. Moujir, L.; Gutierrez-Navarro, A. M.; San Andres, L.; Luis, J. G. *Phytochemistry* **1993**, *34*, 1493–1495.
5. Lee, D. S.; Hong, S. D. *J. Microbiol. Biotechnol.* **1998**, *8*, 89–91.
6. Kang, H. S.; Chung, H. Y.; Jung, J. H.; Kang, S. S. *Arch. Pharmacal. Res.* **1997**, *20*, 496–500.
7. Weng, X. C.; Gordon, M. H. J. *Agric. Food Chem.* **1992**, *40*, 1331–1336.
8. Chang, H. M.; Cheng, K. P.; Choang, T. F.; Chow, H. F.; Chui, K. Y.; Hon, P. M.; Tan, F. W. L.; Yang, Y.; Zhong, Z. P.; Lee, C. M.; Sham, H. L.; Chan, C. F.; Cui, Y. X.; Wang, H. N. C. *J. Org. Chem.* **1990**, *55*, 3537–3543.
9. Bu, X. Z.; Huang, Z. S.; Zhang, M.; Ma, L.; Xiao, G. W.; Gu, L. Q. *Tetrahedron Lett.* **2001**, *42*, 5737–5740.
10. Gu, L. Q.; Bu, X. Z.; Ma, L. PCT CN 0100861, 24 May, 2001.
11. Mousseau, D. D.; Butterworth, R. F. *Proc. Soc. Exp. Biol. Med.* **1994**, *206*, 329–344.
12. Raabe, W. *Neurochem. Pathol.* **1987**, *6*, 145–166.
13. Fan, P.; Lavoie, J.; Le, N. L. O.; Szerb, J. C.; Butterworth, R. F. *Neuroscience* **1990**, *37*, 324–327.
14. Bahjat, A.; Faraj, V. M.; Camp, J. D.; Ansley, J. S.; Farouk, M. A.; Eugene, J. M. *J. Clin. Invest.* **1981**, *67*, 395–402.
15. Fischer, J. E.; Baldessarini, R. J. *Lancet* **1971**, *2*, 75–80.
16. Sourkes, T. L. *J. Neurol. Transm.* **1978**, *14*, 79–86.
17. Schafer, D. F.; Jones, E. A. *Lancet* **1982**, *1*, 18–20.