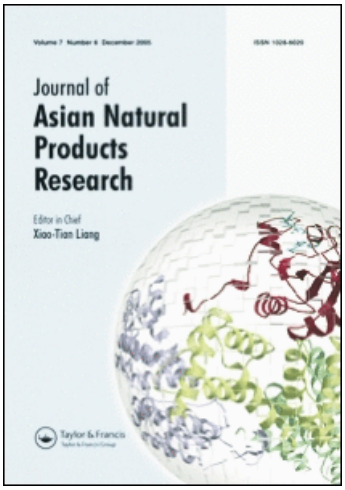

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Three new derivatives of anti-HIV-1 polyphenols isolated from *Salvia yunnanensis*

Zheng-Fu Zhang[a]; Zong-Gen Peng[a]; Lei Gao[a]; Biao Dong[a]; Jian-Rui Li[a]; Zhuo-Yong Li[a]; Hong-Shan Chen[a]
[a] Chinese Academy of Medical Sciences and Peking Union Medical College, Institute of Medicinal Biotechnology, Beijing, China

Online publication date: 27 July 2010

To cite this Article Zhang, Zheng-Fu , Peng, Zong-Gen , Gao, Lei , Dong, Biao , Li, Jian-Rui , Li, Zhuo-Yong and Chen, Hong-Shan(2008) 'Three new derivatives of anti-HIV-1 polyphenols isolated from *Salvia yunnanensis*', Journal of Asian Natural Products Research, 10: 5, 391 — 396
To link to this Article: DOI: 10.1080/10286020801966591
URL: http://dx.doi.org/10.1080/10286020801966591

Journal of Asian Natural Products Research
Vol. 10, No. 5, May 2008, 391–396

Three new derivatives of anti-HIV-1 polyphenols isolated from *Salvia yunnanensis*

Zheng-Fu Zhang, Zong-Gen Peng, Lei Gao, Biao Dong, Jian-Rui Li, Zhuo-Yong Li and Hong-Shan Chen*

Chinese Academy of Medical Sciences and Peking Union Medical College, Institute of Medicinal Biotechnology, Beijing 100050, China

(*Received 14 March 2007; final version received 29 June 2007*)

During the study of anti-HIV-1 active components of the aqueous extracts of the roots of *Salvia yunnanensis*, three new derivatives of polyphenols, namely: methyl salvianolate A (**2**), ethyl salvianolate A (**3**) and *cis*-lithospermic acid (**5**) were isolated along with two known polyphenols, salvianolic acid A (**1**) and lithospermic acid (**4**) their structures were elucidated on the basis of NMR and MS spectral analyses. The anti-HIV-1 activities of the 5 polyphenols were tested for the inhibition of P24 antigen in HIV-1 infected MT-4 cell cultures and HIV-1 replicative enzymes *in vitro*.

Keywords: *Salvia yunnanensis*; *Labiatae*; Polyphenols; Anti-HIV-1 activity

1. Introduction

Danshen is a well-known Chinese traditional herb, officially listed as *Salvia miltiorrhiza* in the Chinese Pharmacopoeia was widely used in the treatment of cerebro-cardiovascular diseases, hepatitis and hepatocirrhosis[1], its water-soluble extract possess a variety of biological activities including antioxidant,[2] antitumor,[3] and antiviral.[4,5]

The water-soluble extracts of *S. miltiorrhiza* and *Salvia yunnanensis* have been found to have potent effect against human immunodeficiency virus type 1 (HIV-1),[5] in our laboratory, and the polyphenols including lithospermic acid and lithospermic acid B were reported to be the active components of *S. miltiorrhiza* or the *Salvia* genus,[6] these prompted us to evaluate the active constituents of *S. yunnanensis*. By the method of activity tracing, three new polyphenols, methyl salvianolate A (**2**), ethyl salvianolate A (**3**) and *cis*-lithospermic acid (**5**), along with two known polyphenols, salvianolic acid A (**1**) and lithospermic acid (**4**), were isolated and identified from the aqueous extracts of the roots of *S. yunnanensis* (Figure 1). The anti-HIV-1 activities of the five polyphenols were tested for the inhibition of P24 antigen in HIV-1-infected MT-4 cell cultures and HIV-1 replicative enzymes *in vitro*.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. ^{13}C NMR spectrum of **1** showed signals for two pairs of olefinic carbons (δ 147.4 and 115.5, 137.9 and 120.7), two carbonyl carbons (δ 173.6 and 168.7), one oxygenated methine carbon (δ 74.7), one methylene carbon (δ 37.9) and 18 aromatic carbons (δ 113.9–148.3). ^{1}H NMR spectrum of **1** showed signals for two pairs of two

*Corresponding author. Email: chenhs_10@163.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020801966591
http://www.informaworld.com

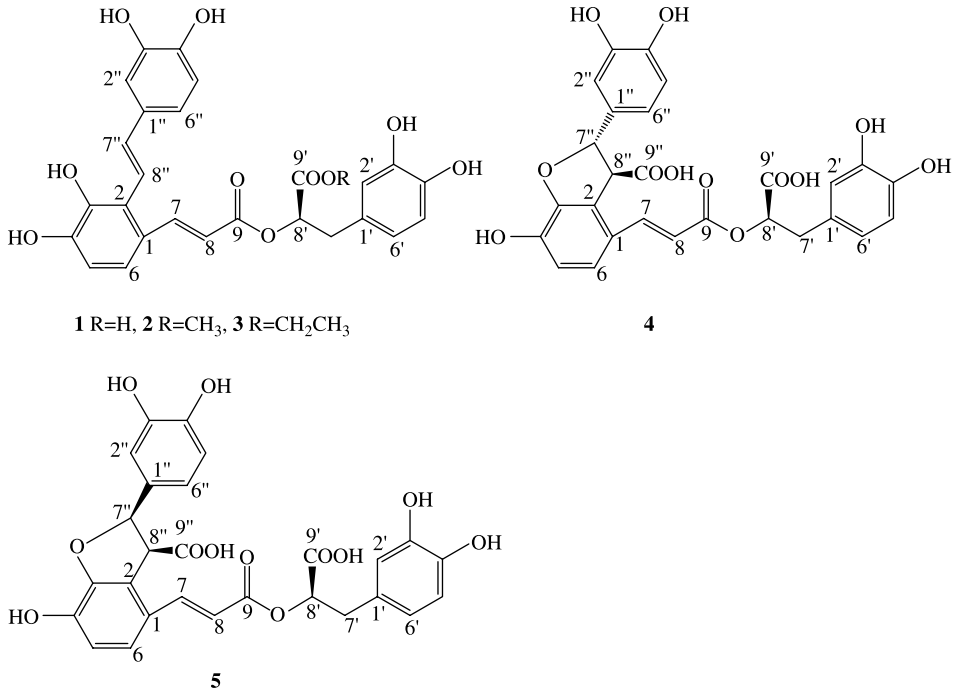

Figure 1. Structures of compounds **1**–**5**.

doublets due to *trans*-olefinic protons at δ 8.01, 6.23 ($J = 16$ Hz) and δ 7.08, 6.60 ($J = 16$ Hz), three double doublets in the aliphatic region (δ 5.11, $J = 8.5$, 3.5 Hz; δ 3.01, $J = 14$, 3.5 Hz; δ 2.90, $J = 14$, 8.5 Hz) for a —CH(O)—CH_2— unit, and the signals at δ 6.43–7.06 belonging to three aromatic ring protons. The full assignment of the ^{1}H and ^{13}C chemical shifts of **1** established by 2D NMR techniques are listed in Tables 1 and 2. By comparison of the NMR spectral data of **1** with those of salvianolic acid A,[7,8] compound **1** was identified to be salvianolic acid A.

Compound **2** was obtained as a yellow amorphous powder. The structure of **2** was readily apparent as a methyl ester of salvianolic acid A from its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). The only difference between the NMR spectra of **2** and those of salvianolic acid A was the appearance of a three-proton singlet at δ 3.63 and its correlated carbon signal at δ 52.7 in the HMQC spectrum, suggesting that a methyl ester group was present. Moreover, the carbonyl group of C-9′ at δ 173.6 in salvianolic acid A was upfield shifted to δ 172.2 in **2**, which indicated that the methoxy group was linked at the C-9′. This linkage was also affirmed by the fact that the C-9′ was correlated with the methyl group (δ 3.63) in the HMBC spectrum. The full assignment of the ^{1}H and ^{13}C NMR signals of **2** (Tables 1 and 2) was established using HMQC and HMBC experiments. Its HRESIMS showed a base peak at *m/z* 507.1325, consistent with the $[M - H]^-$ ion, expected for the molecular formula $C_{27}H_{24}O_{10}$. Compound **2** was identified as methyl salvianolate A.

Compound **3** was obtained as a yellow amorphous powder. Its NMR spectra were quite similar to those of methyl salvianolate A. The only difference between their NMR spectra was that there was a ethyl ester group in **3**, instead of a methyl ester group in methyl salvianolate A, and the carbonyl group of C-9′ at δ 172.2 in methyl salvianolate A was upfield shifted to δ 171.8 in **3**. Thus, compound **3** was identified as ethyl salvianolate A. The assignment of the chemical

Table 1. ^{13}C NMR spectral data for compounds **1**–**5** (125 MHz).

C	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
1	126.1	126.0	126.0	126.6	126.0
2	128.4	128.5	128.5	129.4	130.3
3	144.4	144.4	144.4	149.9	150.4
4	148.3	148.3	148.3	145.5	145.8
5	114.8	114.8	114.8	120.4	119.0
6	120.1	120.1	120.1	124.3	125.0
7	147.4	147.6	147.6	145.5	145.4
8	115.5	115.2	115.3	118.5	118.3
9	168.7	168.5	168.5	171.2	170.8
1′	129.3	128.7	128.7	132.6	131.7
2′	117.4	117.3	117.4	120.4	120.1
3′	146.1	146.2	146.2	147.0	147.2
4′	145.2	145.3	145.3	145.9	146.7
5′	116.3	116.3	116.3	119.4	120.2
6′	122.0	122.0	122.0	124.9	124.8
7′	37.9	37.9	37.9	39.6	39.1
8′	74.7	74.5	74.9	78.5	77.2
9′	173.6	172.2	171.8	178.8	177.2
1″	131.4	131.3	131.3	135.9	131.2
2″	114.0	114.0	113.9	116.2	117.0
3″	146.5	146.5	146.5	147.3	146.7
4″	146.7	146.8	146.8	147.2	145.8
5″	116.5	116.4	116.4	119.2	118.9
6″	120.5	120.4	120.4	120.7	122.1
7″	137.9	137.9	120.7	90.4	89.5
8″	120.7	120.7	114.8	59.6	56.6
9″				179.2	177.1
OCH_3		52.7	62.4		
OCH_2CH_3			14.4		

[a] Recorded in CD_3OD.
[b] Recorded in D_2O.

Table 3. The inhibition activities of compounds **1**–**5** and AZT on HIV-1 P24 in MT-4 cell cultures.

Samples	EC_{50} (μg/ml)	TC_{50} (μg/ml)	SI
1	2.07 ± 0.65	7.61 ± 0.17	3.7
2	1.62 ± 1.00	6.96 ± 0.69	4.3
3	1.44 ± 0.41	7.38 ± 0.35	5.1
4	3.99 ± 0.08	32.52 ± 1.14	7.9
5	6.11 ± 1.65	86.41 ± 0.60	14.1
AZT	0.000083 ± 0.000022	>0.64	>7727

structure of **3** (Tables 1 and 2) was also supported by HRESIMS, which showed a base peak at *m*/*z* 521.1427 for the expected $[M - H]^-$ ion.

Compound **4** was obtained as a colourless amorphous powder. ^1H NMR spectrum of **4** showed two doublets assigned to *trans*-olefinic protons at δ 7.22 and 5.88 ($J = 16$ Hz), two ABX-spin systems (Table 1) in the aromatic region, two doublets due to two *ortho*-aromatic protons at δ 6.57 and 6.52 ($J = 8.5$ Hz), and three double doublets attributable to a $-CH(OH)-CH_2-$ unit at δ 2.81, 2.87 and 4.85 ($J = 14$, 8 and 4 Hz). Furthermore, ^1H NMR spectrum showed a pair of mutually coupled aliphatic proton signals at δ 5.64 and 3.80 ($J = 4$ Hz), consistent with the presence of a dihydrobenzofuran moiety. The coupling constant of 4 Hz indicated that H-7″

Table 2. ^1H NMR spectral data for compounds **1**–**5** (500 MHz, *J* in Hz).

H	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
5	6.71 d (8.0)	6.70 d (8.0)	6.70 d (8.0)	6.52 d (8.5)	6.86 d (8.5)
6	7.05 d (8.0)	7.06 d (8.0)	7.07 d (8.0)	6.57 d (8.0)	6.95 d (8.0)
7	8.01 d (16.0)	8.01 d (16.0)	8.01 d (16.0)	7.22 d (16.0)	7.36 d (16.0)
8	6.23 d (16.0)	6.23 d (16.0)	6.23 d (16.0)	5.88 d (16.0)	6.13 d (16.0)
2′	6.66 d (1.5)	6.60 d (2.0)	6.61 d (2.0)	6.74 d (1.5)	6.84 d (1.5)
5′	6.59 d (8.0)	6.58 d (8.0)	6.59 d (8.0)	6.65 d (9.0)	6.84 d (8.5)
6′	6.48 dd (8.0/1.5)	6.44 dd (8.0/2.0)	6.46 dd (8.0/2.0)	6.52 dd (8.0/1.5)	6.67 dd (8.0/1.5)
7′α	3.01 dd (3.5/14.0)	2.94 dd (5.5/14.0)	2.94 dd (5.5/14.0)	2.87 dd (4.0/14.0)	3.01 br
7′β	2.90 dd (8.5/14.0)	2.91 dd (7.5/14.0)	2.91 dd (7.5/14.0)	2.81 dd (8.0/14.0)	2.97 br
8′	5.11 dd (3.5/8.5)	5.12 dd (5.5/7.5)	5.08 dd (5.5/7.5)	4.85 dd (4.0/8.0)	5.14 br
2″	7.00 d (1.0)	6.98 d (1.0)	6.99 d (2.0)	6.66 d (1.0)	7.00 d (1.0)
5″	6.69 d (8.0)	6.69 d (8.0)	6.68 d (8.0)	6.55 d (8.0)	6.91 d (8.0)
6″	6.82 dd (8.0/1.0)	6.81 dd (8.0/1.0)	6.81 dd (8.0/2.0)	6.46 dd (8.0/1.5)	6.82 dd (8.0/1.5)
7″	6.60 d (16.0)	6.57 d (16.0)	6.56 d (16.0)	5.64 d (4.0)	5.72 d (9.0)
8″	7.08 d (16.0)	7.08 d (16.0)	7.08 d (16.0)	3.80 d (4.5)	4.39 d (9.5)
OCH_3		3.63 s	4.08 q		
OCH_2CH_3			1.13 t		

[a] Recorded in CD_3OD.
[b] Recorded in D_2O.

Table 4. The inhibition activities of compounds **1**–**5** on HIV-1 replicative enzymes *in vitro*.

Samples	IC_{50} (μg/ml)		
	Reverse transcriptase	Protease	Integrase
NVP	0.11 ± 0.04		
IND		4.23 ± 2.43 nM	
S-y			0.88 ± 0.06
1	59.28 ± 1.17	12.23 ± 1.93	13.69 ± 8.21
2	50.58 ± 0.04	10.73 ± 2.18	7.58 ± 3.08
3	56.38	12.03 ± 3.01	14.54 ± 6.28
4	>100	25.39 ± 4.95	17.87 ± 9.24
5	43.64 ± 5.26	25.20 ± 4.27	5.25 ± 1.65

and H-8″ in the dihydrobenzofuran ring were *trans*-oriented.[9–11] ^{13}C NMR spectrum of **4** showed signals for three carbonyl carbons (δ 171.2, 178.8 and 179.2), 18 aromatic carbons (δ 116.2–150.0), two olefinic carbons (δ 145.5 and 118.5), two oxygenated methine carbons (δ 78.5 and 90.4), one methine carbon (δ 59.6) and one methylene carbon (δ 39.6). NMR spectral data of **4** were similar to those of lithospermic acid in the literature.[12,13] Compound **4** was therefore identified as lithospermic acid and the full assignments of ^{1}H and ^{13}C chemical shifts of **4** are shown in Tables 1 and 2, respectively.

Compound **5** was obtained as a brownish amorphous powder. The NMR spectral data (Tables 1 and 2) revealed that compound **5** possesses structural features similar to lithospermic acid (**4**). However, the magnitude of the coupling constant value of a pair of dihydrobenzofuran proton signals that appeared at δ 5.72 and 4.39 (each 1H, d, $J = 9$ Hz) was larger than that of lithospermic acid (δ 5.64 and 3.80, each 1H, d, $J = 4$ Hz). This fact clearly indicated that the relative configuration for H-7″ and H-8″ in the dihydrobenzofuran ring was *cis*-oriented; that is, the ring was either 20*S*, 21*R* or 20*R*, 21*S*.[14] Thus, compound **5** was deduced as a stereoisomer of **4**, identified as *cis*-lithospermic acid.

All the five polyphenols were evaluated for anti-HIV-1 IIIB activities in MT-4 cell cultures, and their results are shown in Table 3. Compound **1** and its methyl and ethyl derivatives showed some anti-HIV-1 activities but higher cytotoxicities in MT-4 cell cultures. For compound **1**, the EC_{50} value was 2.07 ± 0.65 μg/ml, the TC_{50} value was 7.61 ± 0.17 μg/ml, SI was 3.7; for compound **2**, the EC_{50} value was 1.62 ± 1.00 μg/ml, the TC_{50} value was 6.96 ± 0.69 μg/ml and SI was 4.3; for compound **3**, the EC_{50} value was 1.44 ± 0.41 μg/ml, the TC_{50} value was 7.38 ± 0.35 μg/ml and SI was 5.1. While compounds **4** and **5** were less toxic but also less active, their TC_{50} values were 32.53 ± 1.14 and 86.41 ± 0.60 μg/ml, their EC_{50} values were 3.99 ± 0.08 and 6.11 ± 1.65 μg/ml, respectively, and the SI of **5** was 14.10, higher than that of **4**. In comparison with the clinically used anti-HIV-1 drug AZT, the five polyphenols were weaker HIV-1 inhibitors.

The inhibitions of the five polyphenols were also detected *in vitro* on HIV-1 replicative enzymes, the results of which are shown in Table 4. On HIV-1 reverse transcriptase (HIV-1 RT), the IC_{50} values of the five polyphenols were 59.28 ± 1.17, 50.58 ± 0.04, 56.38, >100 and 43.64 ± 5.26 μg/ml, respectively; on HIV-1 protease, the IC_{50} values were 12.23 ± 1.93, 10.73 ± 2.18, 12.03 ± 3.01, 25.39 ± 4.95 and 25.20 ± 4.27 μg/ml, respectively. In comparison with the positive controls of the clinically effective HIV-1 RT inhibitors nevirapine (NVP) and indinavir (IND), they were poor inhibitors. As there was no clinically approved HIV-1 integrase inhibitor on the market, a polysaccharide sulfate S-y, which proved to be an HIV-1 integrase inhibitor many times in our laboratory, was

used as a positive control. In comparison with the EC_{50} value of S-y of 0.88 ± 0.06 μg/ml, the five polyphenols were weaker HIV-1 integrase inhibitors, and the IC_{50} values of **1**–**5** were 13.69 ± 8.21, 7.58 ± 3.08, 14.54 ± 6.28, 17.87 ± 9.24 and 5.25 ± 1.65 μg/ml, respectively.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Shimadzu UV-260 spectrophotometer. The optical values were determined on a Perkin–Elmer 341 polarimeter. NMR spectra were recorded on a Varian Unity INOVA-500 spectrometer with tetramethylsilane as an internal reference. ESIMS was carried out on a Waters Micromass ZQ 2000 mass spectrometer. HPLC was performed with a Shimadzu LC-10Avp instrument equipped with an SPD-10Avp (UV–vis) detector and a YMC-Pack Pro C18 (5 μm, Φ 4.6 × 150 mm) column; MeOH–H_2O–CH_3COOH (25:75:0.005) were used as a mobile phase. Water and EtOH were used as the eluent for Sephadex LH-20 CC, Diaion HP20 CC and ODS CC.

3.2 Plant material

The roots of *S. yunnanensis* were purchased in July 2004 from the Yunnan Chinese Medicinal Corporation, Kun Ming, China, and were identified by Professor Chen Hubiao, a botany specialist in the Peking University Health Science Center. A voucher specimen (No. 200408 dali) has been deposited at the Department of Virology, the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China.

3.3 Extraction and isolation

The roots (4 kg) of *S. yunnanensis*, after washing with water and drying in the shade for several days, were powdered and extracted three times with 10 l of water at 80°C for 30 min each time, and the concentrated aqueous extract was partitioned into a water fraction (300 g) and a 50% ethanol fraction (160 g) by chromatography on a Diaion HP20 column. The 50% ethanol fraction (140 g) was further fractionated on a Sephadex LH20 column into three subfractions, I (0–30% ethanol; 90.8 g), II (30–50% ethanol; 37 g) and β (50–80% ethanol; 12 g). Three subfractions were then chromatographed, respectively, on an ODS column with aqueous ethanol, and the fractions were collected in 20 ml volumes and were monitored by HPLC. Fractions containing the same pure compounds were combined and concentrated on a rotary evaporator, and the residue was finally freeze-dried. By the ODS column purification of subfraction I yielded lithospermic acid (**4**, 500 mg) and *cis*-lithospermic acid (**5**, 50 mg). Subfraction β yielded salvianolic acid A (**1**, 500 mg), methyl salvianolate A (**2**, 10 mg) and ethyl salvianolate A (**3**, 30 mg).

3.3.1 Methyl salvianolate A (2)

Yellow amorphous powder. HPLC Rt 3.1 min, $[\alpha]_D^{20}$ + 35 (EtOH; *c* 0.110). UVλ_{max}^{MeOH} (nm) (log ε): 204 (4.72), 289 (4.44), 336 (4.36). ^1H and ^{13}C NMR (CD_3OD) spectral data: Tables 1 and 2. HRESIMS *m/z*: 507.1325 $[M - H]^-$ (calcd for $C_{27}H_{23}O_{10}$, 507.1291).

3.3.2 Ethyl salvianolate A (3)

Yellow amorphous powder. HPLC Rt 2.9 min, $[\alpha]_D^{20}$ + 35 (EtOH; *c* 0.098). UVλ_{max}^{MeOH} (nm) (log ε): 204 (4.70), 289 (4.45), 335 (4.33). ^1H and ^{13}C NMR (CD_3OD) spectral data: Tables 1 and 2. HRESIMS *m/z*: 521.1427 $[M - H]^-$ (calcd for $C_{28}H_{25}O_{10}$, 521.1448).

3.3.3 Cis-lithospermic acid (5)

Brownish amorphous powder. HPLC Rt 6.6 min, $[\alpha]_D^{20}$ + 41 (MeOH; *c* 0.08). UVλ_{max}^{MeOH} (nm) (log ε): 253 (4.28), 288 (4.22), 309 (4.38). ^1H and ^{13}C NMR (D_2O) spectral data: Tables 1 and 2. HRESIMS *m/z*: 537.1047 $[M - H]^-$ (calcd for $C_{27}H_{21}O_{12}$, 537.1033).

3.4 HPLC analysis of compounds 2 and 3 in the water extract of S. yunnanensis

The dried roots (50 mg) were extracted with water three times, and the water extract was analyzed by HPLC. HPLC analysis was performed with a Shimadzu LC-10Avp instrument equipped with an SPD-10Avp (UV–vis) detector and a YMC-Pack Pro C18 (5 μm, Φ 4.6 × 150 mm) column. The mobile phase composed of MeOH–H_2O–CH_3COOH (25:75:0.005) was eluted at a flow rate of 1 ml/min. The elutes were monitored at 280 nm. HPLC analysis showed peaks with the same retention time as that of compounds **2** and **3**, which suggested that the two components were naturally occurring compounds and not artifacts formed during extraction and separation.

3.5 Anti-HIV activity

3.5.1 Inhibition of HIV-1 P24 antigen in cell cultures

Different concentrations of the five polyphenols isolated from *S. yunnanensis* and a positive control Zeduvidine (AZT) were added to HIV-1 IIIB-infected MT-4 cells, and, after 4 days, the supernatants were tested for HIV-1 P24 titers by ELISA methods. The cells were tested for cytotoxicity by MTT methods. Their EC_{50}, TC_{50} and SI values were calculated and are listed in Table 3.

3.5.2 Inhibition of HIV-1 replicative enzymes in vitro

The HIV-1 RT was detected by the ^3H incorporation method with a positive control of clinically used non-nucleotide NVP. The HIV-1 protease was detected by the fluorescent method with a positive control of clinically used IND. The HIV-1 integrase was detected by the ELISA method with a positive control of laboratory-proved active polysaccharide sulfate S-y. Different concentrations of the five polyphenols were tested with three HIV-1 replicative enzymes, each with a positive control. The EC_{50} values were calculated and are listed in Table 4.

Acknowledgements

The authors are grateful to Professor Li Lianniang at the Institute of Materia Medica in Beijing for her assistance in the structure identification. This work was supported by the National High-tech R&D Program (863 Program; 2004AA2Z3342).

References

[1] Pharmacopoeia Committee of the Health Ministry of the People's Republic of china, *Pharmacopoeia of the People's Republic of China* (Chemical Industry Press, Beijing, China, 1977), **1**, pp. 52–53.
[2] Y.L. Liu and G.T. Liu, *Acta Pharm. Sin.* **37**, 81 (2002).
[3] Y. Zhen, L. Li, and J. Su. CN 1,110,139 (Cl. A61K31/19) (1995)..
[4] H.S. Chen, X.G. Yan, X.Q. Zhang, L. Teng, Z. Li, Y.Z. Lu, X.X. Wu, and X.H. Chen, *Acta Acdemiae. Med. Sin.* **18**, 3 (1996).
[5] H.S. Chen, X.G. Yan, X.Q. Zhang. CN: 9510590215..
[6] I.S. Abd-Elazem, H.S. Chen, R.B. Bates, and R.C. Huang, *Antiviral Res.* **55**, 91 (2002).
[7] C.X. Zhou, H.W. Luo, and M. Niwa, *J. Chin. Pharm. Univ.* **30**, 411 (1999).
[8] L.N. Li, R. Tan, and W.M. Chen, *Planta Med.* **50**, 227 (1984).
[9] A.R. Martin, J.F. Caputo, and J.F. Caputo, *J. Org. Chem.* **39**, 1808 (1974).
[10] C.B. Ai and L.N. Li, *J. Nat. Prod.* **51**, 145 (1988).
[11] T. Tanaka, S. Morimoto, G.I. Nonaka, I. Nishioka, T. Yokozawa, H.Y. Chung, and H. Oura, *Chem. Pharm. Bull.* **37**, 340 (1989).
[12] Z.J. Wu, M.A. Ouyang, and C.R. Yang, *Acta Bot. Yunnan.* **21**, 357 (1999).
[13] C.X. Zhou, H.W. Luo, and M. Niwa, *J. Chin. Pharm. Univ.* **30**, 411 (1999).
[14] C. Koukoulitsa, A. Karioti, M.C. Bergonzi, G. Pescitelli, L. Di Bari, and H. Skaltsa, *J. Agric. Food Chem.* **54**, 5388 (2006).