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EXCERPTS OF DISSERTATION

Studies on the chemical constituents and bioactivities of five Schisandra medicinal species and Elsholtzia bodinieri*

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Abstract The chemical constituents and bioactivities of six medicinal plants have been studied. In the study one hundred and one compounds, including twenty-six new compounds were isolated. Among these compounds, sixteen unique highly oxidized nortriterpenoids, belonging to an unprecedented new nortriterpenoid skeleton with a biosynthetically modified eight-membered ring, and unusual nortriterpenoids or bisnortriterpenoid skeletons, have been isolated from three *Schisandra* species. Interestingly, to date, no naturally occurring triterpenoids have been found to have such a highly modified oxidized norcycloartane skeleton.

The bioactivities of compounds isolated from the genus *Schisandra* have been tested, including anti-HIV-1, anti-inflammatory, and cytotoxities against C8166, MT-4 and K562 cells activities. SM-10 and SM-26 were found to show promising anti-HIV-1 activity and the selectivity index were 174.08 and > 25.94, respectively.

Key words *Schisandra* species, *Elsholtzia bodinieri*, chemical constituents, anti-HIV activity, biogenetic origin

CLC Q946-33

Triterpenoids are the most ubiquitous, nonsteroidal secondary metabolites in terrestrial and marine flora and fauna. Their presence, even in nonphotosynthetic bacteria, has created interest from both an evolutionary and functional point of view. Although medicinal uses of the compounds from this class are rather limited, considerable recent work in this regard strongly indicates their great potential as drugs[1]. Moreover, despite the great diversity that already exists among the skeletons of triterpenoids, new variants continue to emerge. Some of the new triterpenoid structures possess novel skeletons and represent unique biosynthetic end products. The majority of

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triterpenoids possess the conventional skeleton arising from the cyclization of squalene-2,3-epoxide to yield fused polycyclic products. More unusual are the incompletely cyclized compounds, or those exhibiting cyclization within the chain, or two consecutive cyclizations rather than the cyclization beginning at one end. While triterpenoids with rearranged carbocyclic skeletons have been isolated quite frequently, there are some skeletons formed through extensive oxidation accompanied by various bond cleavages[2,3].

With the aim of identifying new natural compounds with interesting biological activities and of investigating the occurrence of natural terpenoids that could be used as natural sources of intermediates for the synthesis of high-added value compounds, we examined the plants of the genus *Schisandra* grown in Yunnan Province, which belongs to the economically and medicinally important family Schisandra ceae.

Sixteen highly oxygenated nortriterpenoids (Fig. 1), with three unprecedented carbon skeletons, of which we proposed the names "schisanartane", "schiartane", and "18-norschiartane (Fig. 3), have been isolated and characterized, some of which have potent anti-HIV-1 activity with low toxicity. The schisanartane skeleton triterpenoids are the main constituents of *Schisandra* species and are also a unique family of cycloartane derived triterpenoids because of their octacyclic backbone, which includes a 7/8/5 consecutive carbocycle and more than 12 chiral centers. These unusual ring assembly and highly oxygenated structure features are distinctive from any other naturally occurring triterpenoids. As a consequence, these structurally complex molecules have brought great interest and challenges to the chemists for total synthesis and biogenetic studies[4,5].

1 Investigation on chemical constituents of five Schisandra species and Elsholtiza bodinieri

The chemical constituents and bioactivities of five medicinal *Schisandra* plants, *Schisandra micrantha* A. C. Smith, *S. lancifolia* (Rehd. et Wils.) A. C. Smith, *S. henryi* var. *yunnanensis* var. *yunnanensis* A. C. Smitt, S. *Plena* A. C. Smith, and *S. sphenanthera* Rehd. et Wils, have been studied. Eighty seven compounds, including twenty-one new compounds were isolated. Their structures have been elucidated on the basis of extensive spectroscopic studies in conjunction with single-crystal X-ray diffraction analysis.

1.1 Study on chemical constituents from S. micrantha

Our investigation on the chemical constituents of the leaves and stems of *S. micrantha* resulted in the isolation of ten novel compounds, together with twenty-five known ones. Five novel nortriterpenoids, micrandilactones A, D-G(**1**～**5**) are the first examples of structurally unique highly oxidized norcycloartane skeleton with a biosynthetically modified eight-membered ring D isolated from the family *Schisandra*ceae. Two novel nortriterpenoids, micrandilactones B-C (**6**, **7**), represent another unprecedented highly oxidized norcycloartane skeleton (Fig. 1). Micranoic acids A and B (**8**,**9**) belong to a new group of octanortriterpenoids in which the entire C-17 side chains have been lost (Fig. 2). Micrantherin A is a new dibenzocyclooctadiene lignan[6～10].

1.2 Investigation on chemical constituents of S. lancifolia

In our present study, fifteen compounds, including five novel ones, were isolated from the leaves and stems of *S. lancifolia*. The skeletal type displayed by lancifodilactone A (**10**) is noticeable for it represents a new group of highly oxygenated bisnortriterpenoid. Lancifodilactones B-E (**11**～**14**) is a novel compound possessing the same schisanartane skeleton with that of micrandilactone A, of which was firstly isolated from *S. micrantha* (Fig. 1)[11,12].

1.3 Phytochemical study on S. henryi var. yunnanensis

The investigation of the leaves and stems of *S. henryi* var. *yunnanensis* led to the isolation of sixteen compounds. Among them, four novel nortriterpenoids, henridilactones A-D(**15**～**18**), are members of a unique unusual highly oxidized schisanartane skeletal class structurally related to micrandilactone A (Fig. 1)[13].

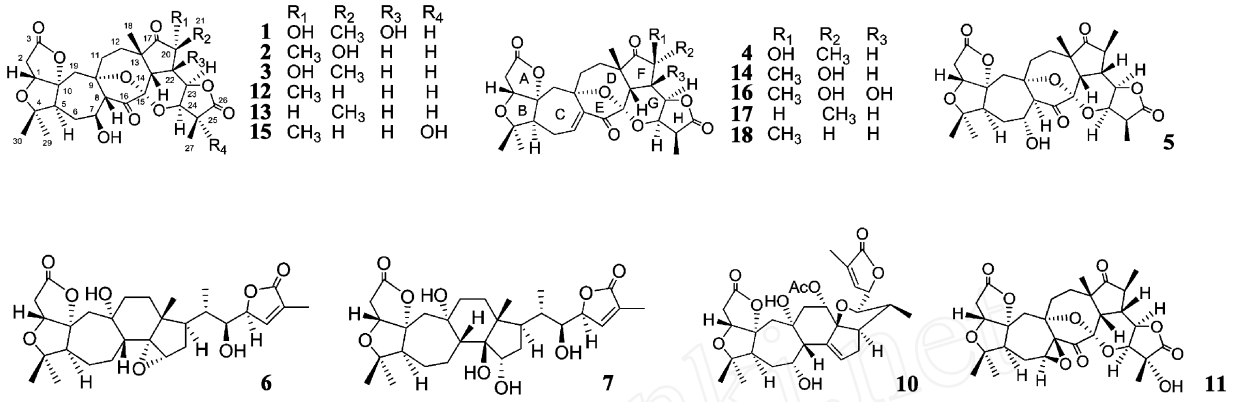

Fig. 1 Novel nortriterpenoids with new skeletons

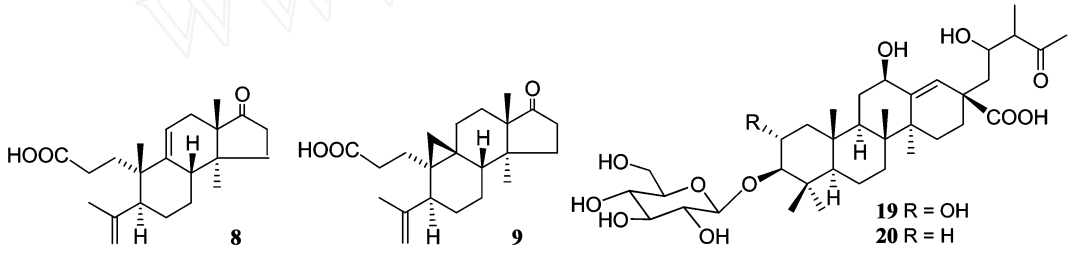

Fig. 2 Unusual Octanortriterpenoids and 18,19-seco-ursane glycosides

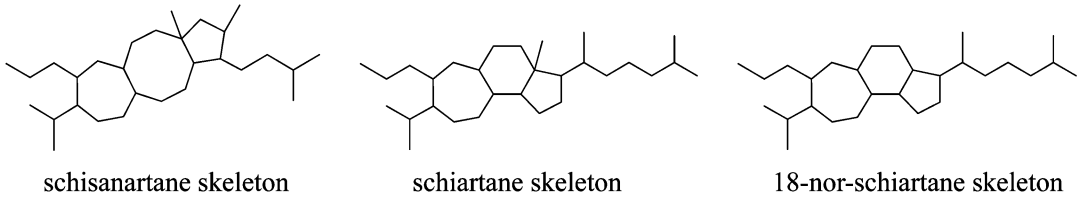

Fig. 3 Three new skeletons

1.4 Research on Chemical Constituents from S. plena and S. sphenanthera

The chemical constituents of the leaves and stems of *S. plena* are described for the first time. This investigation has resulted in the isolation of a new sesquiterpenoid, plenoxide. In addition, thirteen known compounds, including sesquiterpenoids, coumarins, flavanones, triterpenoids and steroids were also isolated. The detailed analysis of 2D NMR data also led us to conclude that the chemical shifts of earlier compounds similar to bullatantriol need revision[14].

A new phenolic glycoside, 4,6-dihydroxyphenyl-1-butanone-2-β-O-glucopyranoside, along with seven known compounds were isolated from the leaves and stems of *S. sphenanthera*.

1.5 Investigation of chemical constituents from Elsholtzia bodinieri

We have investigated the aerial parts of *Elsholtzia bodinieri* and herein report the isolation of fifteen compounds, including eleven flavanones and four triterpenoids. Among them, there are two novel 18,19-*seco*-ursen-28-oic acid-3-*O*-β-D-glucopyranoside triterpenoids, bodiniosides A (**19**) and B (**20**) (Fig. 2)[15]. In addition, three new flavanones are determined as eriodictyol 7-*O*-(6″-feruloyl)-β-D-glucopyranoside, eriodictyol 7-*O*-(6″-isoferuloyl)-β-D-glucopyranoside, and luteolin 7-*O*-(6″-isoferuloyl)-β-D-glucopyranoside.

1.6 Structure elucidation of micandilactone A

Table 1 ^{1}H and ^{13}C NMR assignments and HMBC correlations of **1**[a]

No.	δ_H (mult, J, Hz)	δ_C	HMBC (^{1}H-^{13}C)
1	4.22 (d, 6.3)	81.4	3, 10, 19
2α	2.74 (d, 18.6)	35.0	1, 3, 10
2β	2.93 (dd, 6.3, 18.6)		3
3		175.2	
4		83.9	
5	2.47 (dd, 4.2, 13.4)	58.3	4, 10
6α	2.09 (m)	36.4	7, 8
6β	2.21 (overlap)		5, 7
7	4.51 (dd, 9.3, 10.1)	67.8	5, 16
8	2.99 (d, 10.1)	59.7	6, 7, 9, 15, 16, 19
9		82.2	
10		95.6	
11α	1.79 (m)	42.3	13
11β	1.98 (m)		9, 19
12α	1.67 (m)	32.6	13, 17
12β	1.98 (overlap)		14
13		49.3	13, 15, 16, 17, 18, 20, 22
14	3.31 (s)	54.1	
15		99.7	
16		207.4	
17		220.7	
18	1.58 (s)	30.8	12, 13, 14, 17
19α	2.52 (ABd, 15.8)	41.8	9
19β	2.23 (ABd, 15.8)		9, 10, 11
20		80.2	
21	1.77 (s)	18.9	17, 20, 22
22		75.5	
23	4.99 (d, 1.5)	76.8	14, 20, 22, 24
24	5.42 (dd, 1.5, 2.0)	75.2	23, 26
25	3.26 (m)	42.5	26
26		177.5	
27	1.17 (d, 7.1)	7.8	24, 25, 26
29	1.24 (s)	27.7	4, 5, 30
30	1.04 (s)	20.8	4, 5, 29
20-OH	5.90 (s)		17, 20, 21, 22
22-OH	7.56 (s)		20

[a] Data were recorded in C_5D_5N on Bruker AM-400 MHz spectrometer (^{1}H, ^{13}C) and Bruker DRX-500 MHz spectrometer (HMBC), chemical shifts (δ) were in 10^{-6}.

Micrandilactone A (**1**) (Fig. 4) crystallized as colorless prisms, has the molecular formula of $C_{29}H_{36}O_{12}$ as deduced by its HREI MS (found 576.2178, calcd 576.2207), requiring 12 degrees of unsaturation. The IR spectrum showed absorptions at 3439cm^{-1} and 1776cm^{-1}, revealing the presence of hydroxyl and γ-lactone groups[16]. The ^{1}H NMR (Table 1) spectrum exhibited signals due to four tertiary methyls and a secondary methyl. The ^{13}C NMR spectrum indicated that **1** contained two ester groups, two carbonyl groups, seven quaternary carbons including six oxygenated ones, eight methines including four oxygenated ones, five methylenes and five methyls, which suggested a highly oxygenated triterpene skeleton.

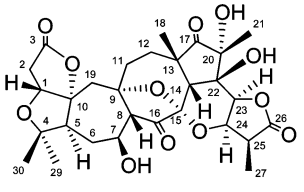

Fig. 4 Structure of micandilactone A

Interpretation of HMBC data showed the following correlations (Table 1): Me-29 (δ 1.24, s) and Me-30 (δ 1.04, s) with C-4 and C-5; H-1 (δ 4.22), H_2-2 (δ 2.74/2.93) and H-5 (δ 2.47) with C-10; H-1 and H_2-2 with an ester group at C-3; H-8 (δ 2.99) with C-9 and C-19; H_2-19 (δ 2.23/2.52) with C-9 and C-10. This, along with two proton spin-systems deduced from COSY correlations, H-1-H-2 and H-5-H-8, led to the establishment of partial structure **1a** (Fig. 5).

A methyl singlet resonance at δ 1.58 corresponding to Me-18 showed HMBC cross peaks with a quaternary carbon (C-13) and with C-12, C-14 and C-17, which required that C-12, C-14 and C-17 all be attached to the carbon (C-13) bearing the methyl group. This was confirmed by the observations of correlations between the methine at δ 3.31 (H-14, s) and C-13, C-17 and C-18. Another methyl singlet resonance at δ 1.77 (Me-21) also showed HMBC correlations with the other two oxygenated quaternary carbons (C-20 and C-22) and a ketone carbon (C-17) suggested that the quaternary carbon (C-20) bearing the methyl group (Me-21) was situated between C-17 and

C-22. Furthermore, correlations of H-14 with C-20 and C-22 established the connection of C-14 with C-22. Additional COSY correlations (H_2-11-H_2-12) not only established the attachment of C-11-C-12 but also gave rise to partial structure **1b**. The third fragment **1c**, was assigned by a continuous sequence from C-23 to C-26 and Me-27 deducing from COSY and HMBC spectra, as well as by the characteristic IR spectral γ-lactone group absorption (1776 cm^{-1})[16]. A hydroxyl group (δ 5.90) was assigned as 20-OH for its cross peaks with C-17, C-20, C-22 and Me-21. In the same manner, another hydroxyl group (δ 7.56) was proven to be located at C-22 by its correlation to C-20. Moreover, HMBC correlations observed between H_2-19 and C-9, C-11 and between H_2-11 (δ 1.79/1.98) and C-9, C-19 allowed the combination of **1a** and **1b** to afford **1d**. Further more, correlations of H-23 to C-14, C-20 and C-22 required direct connection of C-23 with C-22 and permitted fragments **1c** and **1d** to be joined to one another to get **1e** (Fig. 5).

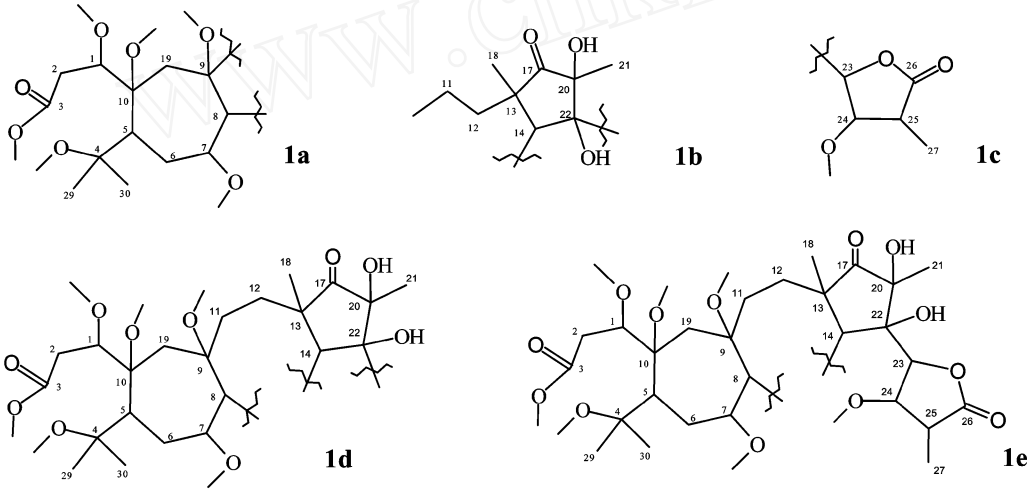

Fig. 5 Structural fragments of 1

Up to now, the above NMR spectroscopic data analysis has elucidated the constitution of partial structure **1e**. However, no additional HMBC connectivities necessary for constructing the structure of **1** were observed among the three oxygenated methines (C-1, C-7 and C-24). In addition, as C-3, C-4, C-9, C-10, C-15 and C-16 were quaternary carbons, which was not possible to determine the correct connections among these carbons.

Since it was difficult to elucidate the complete structure of **1** only by NMR spectroscopic analysis, the crystals were submitted to a single crystal X-ray diffraction. A view of the solid-state conformation was provided in Figure 6. It indicated that **1** had suffered an oxidative cleavage between C-3 and C-4, followed by lactonization to give rise to a five-membered lactone ring A and a tetrahydrofuran ring B. C-9 connected to C-15 through an oxygen bridge with the hydroxyl group located at C-7. Signal due to the angular methyl attached to C-14 (Me-28) was obviously absent in the case of **1**, which suggested that Me-28 had suffered an oxidation to form a carboxylic group, followed by the loss of CO_2. Thus the basic skeleton of **1** was elucidated as 3,4:9,10-*seco*-14-norcycloartane.

The relative stereochemistry of **1** was determined by a 2D ROESY experiment and was confirmed unequivocally by X-ray crystallographic data. Stereochemically, Me-29 was biogenetically α-oriented, Me-30 and Me-21 were β-oriented. ROESY correlations for Me-30/H-1, Me-29/H-5, H-5/H-7, 20-OH/H-24 and H-24/H-23, H-25 indicated that H-1 was β-orientation, H-5, H-7, H-23, H-24 and H-25 were the same α-orientations. Me-18 and H-14 showed mutual correlations, but no cross peaks with H-23, suggested that H-14 and Me-18 were β-orientations. H-8 was suggested to be β-oriented considering the coupling constant (1H, d, $J_{7,8}$ = 10.1Hz). The stereochemistry of the four quaternary carbons, C-9, C-10, C-15 and C-22 was deduced as *R*, *S*, *R* and *S* configuration, respectively, by X-ray diffraction study.

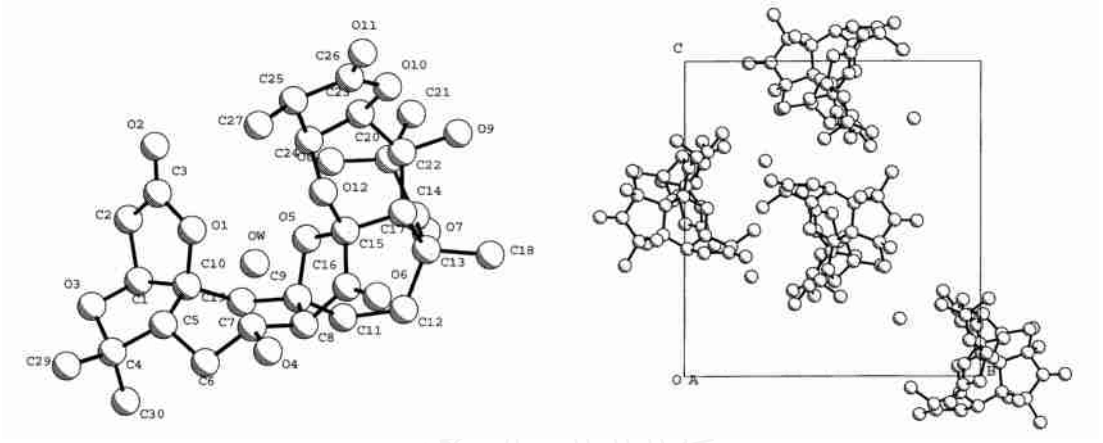

Fig. 6 X-ray structure of 1 showing relative configuration

2 Bioactive investigation

The anti-HIV activities and cytotoxicities of compounds isolated from the genus *Schisandra* were tested by microtiter syncytium formation infectivity assay, including cytotoxicity in C8166 and MT-4 cells, inhibition of syncytium formation in HIV-1_{IIIB} infected C8166 cells, and protective effect in HIV-1_{IIIB} infected MT-4 host cells. Among these compounds, SM-10 possessed cytotoxicity with CC_{50} value of 22.63 μg/mL on tested human T cell leukemia cell line C8166 at the assayed doses, demonstrated potent anti-HIV-1 activity with EC_{50} value of 0.13 μg/mL (selectivity index: 174.08), and exerted protective activity on HIV-1_{IIIB} infected MT-4 host cells with a selectivity index of 43.27. SM-26 possessed minimal cytotoxicity (CC_{50} > 200μg/mL), and the inhibitory activity on HIV-1_{IIIB} induced syncytium formation was EC_{50} = 7.71μg/mL. The selectivity index was > 25.94. In addition, SM-26 exerted its potent protective activity on HIV-1_{IIIB} infected MT-4 host cells with a selectivity index of > 425.5 at the concentration of 0.47μg/mL.

This result is encouraging and warrants further structural modification to both decrease cytotoxicity and increase antiviral inhibitory activities. The potent anti-HIV-1 activity and unique structural features of SM-10 make it as promising lead compound for the preparation of anti-HIV drug. Further anti-HIV evaluation is in progress to better define the anti-HIV potencies of SM-10. Such a study would provide valuable information on the therapeutic development of new generation for anti-HIV drug.

3 Possible biogenetic origin of schisanartane, schiartane and 18-nor-schiartane

Since sixteen novel highly oxygenated compounds with three unique skeletons isolated from three *Schisandra* species have not previously been encountered in nature, we proposed the name schisanartane, schiartane and 18-nor-schiartane for the three different types of skeletons. The concurrence of these compounds among three species prompted us to ponder rationally the inherent correlation among three new skeletons and the known cycloartane. Orienting these novel compounds in a triterpenic perspective reveals the natural context among schisanartane, schiartane, 18-nor-schiartane and the known cycloartane skeletons.

Here we proposed four plausible hypotheses for the biogenesis of three new skeletons: (a) possible biogenesis of schiartane from cycloartane, (b) biogenetic origin of the new 18-nor-schiartane type, (c) plausible biogenesis of schisanartane, (d) the interrelationship and a further stereochemical inference among the schisanartanes[8].

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五种五味子属药用植物及东紫苏的化学成分和生物活性

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摘　要　对 5 种五味子属(*Schisandra*)药用植物(小花五味子 *Schisandra micrantha*,狭叶五味子 *S. lancifolia*,滇翼梗五味子 *S. henryi var. yunnanensis* var. *yunnanensis*,复瓣黄龙藤 *S. Plena*,华中五味子 *S. sphenanthera*)和唇形科植物东紫苏(*Elsholtzia bodinieri*)的化学成分进行了研究,从中共分离鉴定了 101 个化合物,26 个为新化合物. 首次从 3 种五味子属植物中发现了 3 种高度氧化的新奇骨架类型. 此外,还发现了 18,19-*seco*-乌索酸型苷和 17,20 断裂并失去 17 位侧链的羊毛甾烷型和环阿尔廷型八降三萜等新的骨架类型.

对分离得到的部分化合物进行了体外抗 HIV-1、急毒、抗炎和抗肿瘤活性实验,发现部分化合物具有抗 HIV-1 活性. 其中化合物 SM-10 和 SM-26 具有显著的抑制 HIV-1 病毒活性,选择指数分别为 174.08 和 > 25.04,且毒性较小,作为作用于病毒与细胞结合和融合靶点的小分子化合物具有重要的意义.

关键词　五味子属,东紫苏,化学成分,抗 HIV 活性,生源途径

李蓉涛:博士研究生在读期间,发表多篇论文,荣获 2005 年度中国科学院优秀博士学位论文及 2006 年度全国优秀百篇博士学位论文奖. 导师孙汉董研究员:主要从事药用和天然香料的植物资源、植物化学以及天然药物研发的基础和应用基础研究.