RSC Advances


This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

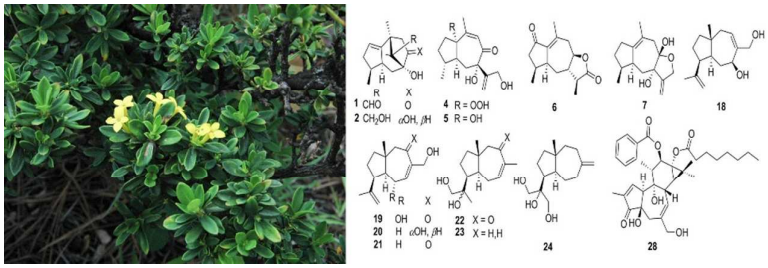
*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances




Thirteen new sesquiterpenoids were isolated from the stems of *Daphne aurantiaca* Diels.. Some compounds showed definite anti-HIV activities.

RSC Advances Accepted Manuscript

# Anti-HIV Terpenoids from *Daphne aurantiaca* Diels. Stems

Sheng-Zhuo Huang, [a] Xuan Zhang, [b] Qing-Yun Ma, [a] Yong-Tang Zheng, [b] Hao-Fu Dai,[a] Qi Wang, [a] Jun Zhou, *[,c] and You-Xing Zhao*[,a]

[a] *Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou 571101, People's Republic of China*

[b] *Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research of Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650204, People's Republic of China*

[c] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China*

* To whom correspondence should be addressed. Tel: +86-898-66989095. Fax: +86-898-66989095. E-mail: junzhou3264@126.com, zhaoyouxing@itbb.org.cn

RSC Advances Accepted Manuscript

**Abstract**

Thirteen new sesquiterpenoids, including six guaiane type auranticanols A-F (**1**, **2**, and **4**-**7**) and seven carotane type auranticanols G-M (**18**-**24**) were isolated from the stems of *Daphne aurantiaca* Diels., along with fourteen known sesquiterpenoids (**3**, **8**-**17**, **25**-**27**) and two known tigliane diterpenoids (**28**, **29**), Their structures were elucidated by extensively analyzing their MS and NMR spectroscopic data. Bioassay of anti-HIV activity indicated that compounds **11**, **14**, **19**, and **28** showed definite activities with $EC_{50}$ 2.138, 0.286, 1.773 and 0.000282 μg/mL and SI >93.545, 93.787, 10.243, and 65177.305, respectively.

Keywords: *Daphne aurantiaca*; Thymelaeceae; sesquiterpenoid; auranticanol; anti-HIV

RSC Advances Accepted Manuscript

## Introduction

Named after Greek myth, the genus *Daphne* (Thymelaeceae) was laureated for their sweet-scented flower, bark silky fiber, and officinal usage.[1, 2] Nowadays, scientists have been focusing on their diverse constituents such as diterpenoids, biflavans, lignans, and sesquiterpenoids, which possess beneficial bioactivities to human beings.[3, 4] Our previous studies on this genus indicated *Daphne* may be a potential AIDS remedy as it is rich in chemical components with strong anti-HIV activity.[5, 6] One member of the genus *Daphne* is the alpine meadows plant "*Daphne aurantiaca* Diels.", which decorates the Southwest China Hengduan Mountain highland with dazzling yellow flowers in blooming summer. Local people in Shangri-La Tibetan minority community use this plant for making religious paper, pest repeller, and traumatic injury remedy.[7, 8] Previous chemical investigation the bark of *D. aurantiaca* showed the presence of sesquiterpenoids, diterpenoids, and phenols, with the anti-inflammatory activity.[9-12] Bioactive compounds from this plant in Shanggri-La were reported, known as the three novel sesquiterpenoids daphnauranols A-C with antifeedant activities.[13] However, there is no systematic chemical analysis of this plant. To elucidate the full composition of this *D. aurantiaca* Diels., our further chemical study on their stems led to the isolation of thirteen new sesquiterpenoids: six guaiane type auranticanols A-F (**1**, **2**, and **4**-**7**) and seven carotane type auranticanols G-M (1**8**-**24**), together with sixteen known sesquiterpenoids including eleven guaiane ones as chamaejasmone D (**3**),[14] torelolone (**8**),[15] virginolide (**9**),[16] 14$\alpha$, 15$\beta$, 1(H)$\alpha$, 5(H)$\alpha$, 7(H)$\alpha$-guai-11(13)-ene-8$\beta$,12-diol (**10**),[17]

RSC Advances Accepted Manuscript

4$\alpha$,5$\alpha$,8$\alpha$,11(H)$\alpha$-2-oxo-guai-1(10)-en-12,8-olide-7$\alpha$-ol (**11**),[9]

4$\alpha$,5$\alpha$,8$\alpha$,15$\beta$,11(H)$\alpha$-2-oxo-guai-1(10)-en-12,8-olide-7$\beta$-ol (**12**),[9]

4$\alpha$,5$\beta$-guai-9(10),7(11)-diene-12,8-olide-1$\alpha$,7$\alpha$-diol (**13**),[9]

3-oxo-guai-4-ene-11$\beta$,12-diol (**14**),[9]

1$\alpha$,4$\alpha$,5$\alpha$,8$\alpha$,11(H)$\beta$-2-oxo-guai-12,8-olide-7$\beta$-ol (**15**),[9]

1$\alpha$,10$\beta$-3-oxo-guai-4,11-diene-7$\beta$-ol (**16**), [18] 5$\alpha$,7(H)$\alpha$-6-oxo-guai-1(10)-ene-4$\beta$-ol (**17**), [19] and three carotane ones dauca-3,11-dien-2$\beta$,15-diol (**25**),[9] [1*R*-(1$\alpha$,3a$\alpha$,6$\alpha$,8a$\alpha$)]-felikiol (**26**),[20] styxone B (**27**),[21] and two tigliane diterpenoids 12-*O*-benzoylphorbol-13-octanoate (**28**)[9] and phorbol 13-monoacetate (**29**)[22] (**Fig. 1**). Herein, the isolation process, structural elucidation, proposed biogenetic pathways, and anti-HIV activity assay of these compounds were described.

## Results and discussion

### Structural elucidation of 1-2, 4-7, and 18-24

Auranticanol A (**1**) was obtained as colorless oil and defined with the molecular formula $C_{15}H_{20}O_3$ from HRESIMS (*m/z* 271.1305 $[M+Na]^+$, calcd for $C_{15}H_{20}O_3Na$, 271.1310) , with six degrees of unsaturation. Its IR spectrum revealed the absorptions of hydroxyl (3433 $cm^{-1}$), and carbonyls and double bond (overlapped 1750, 1721, and 1619 $cm^{-1}$). The $^1H$ NMR spectrum (**Table 1**) of **1** exhibited signals of three methyls [$\delta_H$ 0.91 (3 H, s, H-13), 1.23 (3 H, s, H-14), and 0.90 (3 H, d, $J$ = 7.2 Hz, H-15)] and an olefinic proton [$\delta_H$ 5.39 (1 H, t, $J$ = 2.1 Hz, H-2)]. The $^{13}C$ NMR (DEPT) spectroscopic data (**Table 1**) showed three methyls, three methylenes, four methines (one olefinic and one formyl), and five quaternary carbons (one olefinic, one oxygenated, and one carbonyl). The $^1H$ and $^{13}C$ NMR data of **1** were similar to those of chamaejasmone D (**3**),[14] a rare distorted guaiane skeleton, except for the markedly different shifts at $\delta_C$ 60.5 (s, C-11), and 207.5 (d, C-12) and instead of $\delta_C$ 49.0 (s,

RSC Advances Accepted Manuscript

C-11) and 65.8 (t, C-12) in chamaejasmone D, indicating that C-12 was dehydrogenated to form aldehyde group in **1**. The HMBC (**Fig. 2**) correlations of **1** from H-12 [$\delta_H$ 9.63 (1 H, s)], H-13 [$\delta_H$ 0.91 (3 H, s)], H-14 [$\delta_H$ 1.23 (3 H, s)], and H-6 [$\delta_H$ 1.91 (1 H, dd, $J$ = 4.2, 12.2 Hz) and 1.53 (1 H, dd, $J$ = 10.2, 12.2 Hz)] to C-11 confirmed the hypothesis. The other correlations in the HMBC and $^1$H $^1$H COSY spectrum (**Fig. 2**) further verified the planar structure of **1**. The relative configuration of **1** was elucidated on the basis of ROESY experiment and the hypothesis that the same type of natural products might have the same stereochemistry for one plant origin. Compound **1** supposedly had the $\alpha$-orientations of H-4, H-5, 7-OH, and Me-14 as those of chamaejasmone D by its ROESY experiment (**Fig. 3**) and comparison of their similar $^{13}$C NMR data. Thus, the structure of **1** was assigned as shown and named auranticanol A.

Auranticanol B (**2**), obtained as colorless oil, had the molecular formula $C_{15}H_{24}O_3$ from HRESIMS (*m*/*z* 275.1641 $[M+Na]^+$, calcd for $C_{15}H_{24}O_3Na$, 275.1631) The $^{13}$C NMR (DEPT) spectroscopic data (**Table 1**) showed three methyls, four methylenes (one oxygenated), four methines (one olefinic and one oxygenated), and four quaternary carbons (one olefinic and one oxygenated). The $^1$H and $^{13}$C NMR data of **2** were similar to those of **3**, except for the remarkably different shift at $\delta_C$ 76.9 (d, C-8) in **2**, replacing $\delta_C$ 220.5 (s, C-8) in **3**, indicating that the carbonyl group in C-8 was hydrogenated to be oxygenated methylene in **2**. The HMBC (**Fig. 2**) correlation of **1** from H-8 [$\delta_H$ 4.57 (1 H, dd, $J$ = 4.0, 9.8 Hz)] to C-11 ($\delta_C$ 48.2) and $^1$H $^1$H COSY correlations of H-8 with H-9 [$\delta_H$ 2.15 (1 H, m) and 1.93 (1 H, m)] further confirmed this assignment. The relative configuration of **2**, also elucidated by the ROESY experiment (**Fig. 3**) and above biogenesis hypothesis, was determined to be the same as those of **1** and **3** with $\alpha$-orientations of H-4, H-5, 7-OH, and Me-14. The $\alpha$-orientation of 8-OH was proposed by the ROESY correlations of H-8/H-12 [$\delta_H$ 4.11 (1 H, d, $J$ = 15.4 Hz) and 3.50 (1 H, d, $J$ = 15.4 Hz)]. Therefore, the structure of compound **2** was elucidated as shown and named auranticanol B.

Auranticanols C (**4**) and D (**5**) were assigned to have the molecular formula $C_{15}H_{22}O_5$ and $C_{15}H_{22}O_4$ according to the analysis of HRESIMS (*m*/*z* 305.1370

RSC Advances Accepted Manuscript

[M+Na]$^+$, calcd for $C_{15}H_{22}O_5Na$, 305.1364) and (*m/z* 289.1418 [M+Na]+, calcd for $C_{15}H_{22}O_4Na$, 289.1415), respectively. The $^{13}C$ NMR and DEPT data of **4** and **5** (**Tables 1** and **2**) showed the carbon resonances similar to those of **10**. Compared with the $^{13}C$ NMR spectroscopic data of **10** ($\delta_C$ 39.0 d, 46.2 d, 81.2 d, 34.7 t, and 31.5 d), the C-1, C-7, C-8, C-9, and C-10 carbon signals of **4** and **5** were shifted downfield to ($\delta_C$ 106.7 s, 86.7 s, 199.1 s, 126.1 d, and 166.8 s, respectively) in **4** and ($\delta_C$ 87.1 s, 84.3 s, 206.0 s, 124.3 d, and 154.3 s, respectively) in **5**. This suggested that **4** and **5** were both derived from **10** via oxidations of C-1 and C-7 to oxygenated quaternary carbons and C-8 to a carbonyl group, and formation of a double bond between C-9 and C-10. This hypothesis was confirmed by its HMBC correlations of **4** (**Fig. 2**) from H-14 [$\delta_H$ 2.12 (3 H, d, *J* = 1.4 Hz)], H-9 [$\delta_H$ 5.91 (1 H, d, *J* = 1.4 Hz)], and H-6 [$\delta_H$ 2.63 (1 H, dd, *J* = 10.0, 13.3 Hz) and 1.69 (1 H, dd, *J* = 6.9, 13.3 Hz)] to C-1, from H-6 to C-8, and from H-9, H-12 [$\delta_H$ 5.39 (1 H, d, *J* = 1.2 Hz), 5.26 (1 H, d, *J* = 1.2 Hz)], and H-13 [$\delta_H$ 4.25 (1 H, d, *J* = 14.8 Hz) and 4.01 (1 H, d, *J* = 14.8 Hz)] to C-7. The assignment of **5** was also confirmed by similar HMBC and $^1H$ $^1H$ COSY correlations (**Fig. 2**). The only difference between **4** and **5** was the chemical group at C-1: a hydroxyl group in **5** and while a hydroperoxyl in **4**. This was confirmed by their assigned molecular formulas and chemical shifts at C-1. The similar ROESY correlations (**Fig. 3**) of **4** and **5** to **10** indicated that **4** and **5** possessed the same relative configuration which were determined to have $\alpha$-orientations of OH (OOH)-1, H-5 and OH-7 as **10** for their similar $^{13}C$ NMR data and the same biogenesis origin. The $\alpha$-orientation of Me-15 in **4** and **5** were proved by NOE of H-5 [$\delta_H$ 2.09 (1 H, m)]/H-15 [$\delta_H$ 1.15 (3 H, d, *J* = 6.4 Hz)] in **4** and H-5 [$\delta_H$ 1.97 (1 H, m)]/H-3α [$\delta_H$ 1.91 (1 H, m)] and H-3α/H-15 [$\delta_H$ 1.04 (3 H, d, *J* = 6.9 Hz)] in **5**. Thus, the structures of **4** and **5** were assigned and named auranticanols C and D, respectively.

Auranticanol E (**6**) was defined with the molecular formula $C_{15}H_{20}O_3$ from HRESIMS (*m/z* 271.1314 [M+Na]$^+$, calcd for $C_{15}H_{20}O_3Na$, 271.1310). The $^{13}C$ NMR data (**Table 2**) of **6** were similar to those of **9**, except for the markedly different shifts at $\delta_C$ 136.0 (s, C-1), 146.3 (s, C-10), 47.6 (d, C-3), 32.2 (d, C-4), 38.5 (d, C-11), and 12.6 (q, C-13) instead of the corresponding carbons at $\delta_C$ 62.6 (d, C-1), 27.6 (d, C-10),

RSC Advances Accepted Manuscript

130.4 (d, C-3), 180.9 (s, C-4), 140.7 (s, C-11), and 118.6 (t, C-13) in compound **9**, indicating that the olefinic carbons C-3, C-4, C-11 and C-13 were saturated and the C-1-C-10 were dehydrogenated to form a double bound in **6**. The HMBC (**Fig. 2**) correlations of **6** from H-3 [$\delta_H$ 2.38 (1 H, dd, $J$ = 7.5, 17.2 Hz) and 2.03 (1 H, dd, $J$ = 3.2, 17.2 Hz)] and H-14 [$\delta_H$ 2.24 (3 H, s)] to C-1, from H-5 [$\delta_H$ 2.78 (1 H, m)] and H-9 [$\delta_H$ 2.77 (1 H, m) and 2.57 (1 H, dd, $J$ = 1.8, 17.2 Hz)] to C-10, and from H-13 [$\delta_H$ 1.27 (3 H, d, $J$ = 7.9 Hz)] to C-12 ($\delta_C$ 179.7 s), together with the key $^1$H $^1$H COSY correlations H-4 [$\delta_H$ 2.35 (1 H, m)]/H-5, H-11 [$\delta_H$ 2.75 (1 H, m)]/H-13[$\delta_H$ 1.27 (3 H, d, $J$ = 7.9 Hz)] further verified the hypothesis. The other correlations in the HMBC and $^1$H $^1$H COSY spectrum further confirmed the atom connectivity in **6** (**Fig. 2**). The configuration of the skeleton in **6** was elucidated by the ROESY experiment (**Fig. 3**) and determined to possess the $\alpha$-orientations of H-5 and H-8 as **9**. The $\beta$-orientations of H-7, Me-13, and Me-15 were elucidated by NOE of H-5/H-3$\alpha$ [$\delta_H$ 2.38 (1H, dd, $J$ = 7.5, 17.2 Hz)], H-3$\beta$ [$\delta_H$ 2.03 (1H, dd, $J$ = 3.2, 17.2 Hz)]/H-15[$\delta_H$ 0.89 (3H, d, $J$ = 6.9 Hz)], H-4/H-6$\alpha$[$\delta_H$ 1.74 (1H, m)], H-6$\beta$[$\delta_H$ 1.60 (1H, m)]/H-13, H-7[$\delta_H$ 2.84 (1H, m)]/H-13, and H-8[$\delta_H$ 4.66 (1H, ddd, $J$ = 3.3, 7.6, 7.8 Hz)]/H-11. Thus, the structure of **6** was assigned as shown and named auranticanol E.

Auranticanol F (**7**) was showed with the molecular formula $C_{15}H_{22}O_3$ from HRESIMS ($m/z$ 273.1459 $[M+Na]^+$, calcd for $C_{15}H_{22}O_3Na$, 273.1466). Comparison of its 1D NMR data (**Table 2**) with those of **5** suggested that **7** had a similar skeleton of **5**. The differences were the remarkably different shifts at $\delta_C$ 144.1 (s, C-1), 103.9 (s, C-8), 42.0 (t, C-9), and 122.5 (s, C-10) in **7** instead of $\delta_C$ 87.1 (s, C-1), 206.0 (s, C-8), 124.3 (d, C-9), and 154.3 (s, C-10) in **5**, revealing that the double bound at C-9-C-10 in **5** was moved to C-1-C-10, and the carbonyl C-8 in **5** was linked to C-12 via an oxygen atom forming a hemiketal goup in **7**. The key HMBC (**Fig. 2**) correlations of **7** from H-3 [$\delta_H$ 1.34 (1 H, m), 1.68 (1 H, m)], H-9 [$\delta_H$ 2.73 (1 H, d, $J$ = 15.6 Hz) and 2.28 (1 H, d, $J$ = 15.6 Hz)] and H-14 [$\delta_H$ 1.67 (3 H, s)] to C-1 and from H-12 [$\delta_H$ 4.46 (1 H, d, $J$ = 13.2 Hz) and 4.32 (1 H, d, $J$ = 13.2 Hz)] to C-8 further supported this hypothesis. The other correlations in the HMBC and $^1$H $^1$H COSY spectrum (**Fig. 2**) further confirmed the atom connectivity in **7**. The $\alpha$-orientations of H-5 and OH-7 in

RSC Advances Accepted Manuscript

**7** were elucidated by ROESY experiment (**Fig. 3**) and determined to be the same as those of **5** for biosynthesis origin. And the $\beta$-orientations of OH-8 and Me-15 were elucidated by key NOE of H-15 [$\delta_H$ 0.93 (3 H, d, $J$ = 6.9 Hz)]/H-6$\beta$ [$\delta_H$ 1.72 (1H, m)], H-6$\alpha$ [$\delta_H$ 1.37 (1H, dd, $J$ = 9.6, 12.0 Hz)]/H-12 [$\delta_H$ 4.32 (1H, d, $J$ = 13.2 Hz)], and H-6$\beta$/H-12. Thus, the structure of **7** was assigned as shown and named auranticanol F.

Auranticanol G (**18**) was defined with the molecular formula $C_{15}H_{24}O_2$ from HRESIMS ($m/z$ 259.1673 $[M+Na]^+$, calcd for $C_{15}H_{24}O_2Na$, 259.1673). The $^1H$ and $^{13}C$ NMR data (**Table 3**) of **18** were similar to those of **25**, except for the signals at $\delta_C$ 143.7 (s, C-1), 68.8 (d, C-2), and 128.0 (d, C-7) instead of $\delta_C$ 143.8 (s, C-1), 127.4 (d, C-2), and 70.0 (d, C-7) in **25**, indicating that the hydroxyl group in C-7 moved to C-2 and the double bound moved to C-1-C-7 in **18**. The HMBC (**Fig. 2**) correlations of **18** from H-2 [$\delta_H$ 4.23 (1 H, d, $J$ = 1.9, 4.1 Hz)], H-3 [1.83 (1 H, ddd, $J$ = 1.9, 4.4, 14.8 Hz) and 1.54 (1 H, ddd, $J$ = 4.1, 11.3, 14.8 Hz)], H-6 [2.13 (2 H, m)], and H-14 [$\delta_H$ 4.03 (1 H, d, $J$ = 16.0 Hz) and 3.93 (1 H, d, $J$ = 16.0 Hz)] to C-1 and from H-2 to C-4 ($\delta_C$ 46.2 d) and the $^1H$ $^1H$ COSY correlations of H-6/H-7 [$\delta_H$ 5.67 (1 H, m)] and H-2/H-3 confirmed this structural change. The other correlations in the HMBC and $^1H$ $^1H$ COSY spectrum (**Fig. 2**) further determined the atom connectivity in **18**. The relative configurations of C-4 and C-5 in **18** were determined to be the same as those of **25** based on their similar NMR data and the hypothesis that the skeleton of carotane had the same stereochemistry for probable common biogenesis. The $\alpha$-orientations of H-2 and H-10 were elucidated by NOE of H-2/H-4 [$\delta_H$ 2.53 (1 H, ddd, $J$ = 1.6, 11.3, 13.2 Hz)] and Me-13 [$\delta_H$ 1.70 (3 H, s)]/Me-15 [$\delta_H$ 0.86 (3 H, s)] (**Fig. 3**). Thus, the structure of **18** was assigned as shown and named auranticanol G.

Auranticanol H (**19**) was formulated as $C_{15}H_{22}O_3$ from HRESIMS ($m/z$ 273.1460 $[M+Na]^+$, calcd for $C_{15}H_{22}O_3Na$, 273.1466). **19** had the similar $^{13}C$ NMR data (**Table 3**) to those of **25**, and the differences were $\delta_C$ 70.0 (d, C-3) and 202.3 (s, C-7), replacing $\delta_C$ 28.2 (t, C-3) and 70.0 (d, C-7) in **25**, indicating that in **19** C-3 and C-7 were oxidized to an oxygenated methine and a carbonyl group, respectively. The HMBC correlations and $^1H$ $^1H$ COSY correlations (**Fig. 2**) of **19** confirmed the hypothesis and the atom connectivity in **19**. The relative configuration of C-4 and C-5

RSC Advances Accepted Manuscript

in **19** was also presumptively determined to be the same as those of **25** for biogenesis hypothesis. The $\alpha$-orientations of OH-3 and H-10 were elucidated by ROESY experiment. Thus, the structure of **19** was assigned as shown and named auranticanol H.

Auranticanol I (**20**) was established a molecular formula $C_{15}H_{24}O_2$ from HRESIMS (*m/z* 259.1673 $[M+Na]^+$, calcd for $C_{15}H_{24}O_2Na$, 259.1673). The $^1H$ and $^{13}C$ NMR data (**Table 3**) of **20** were closely similar to those of **25**, except for little difference of the shift at C-10 in **20**, indicating that **20** and **25** were epimers at C-10. Its HMBC and $^1H$ $^1H$ COSY correlations (**Fig. 2**) accorded with the atom connectivity in **20**. The relative configuration of C-4 and C-5 in **20** was determined to be the same as those of **19**. And the $\alpha$-orientations of OH-7 and H-10 were determined by the key ROESY (**Fig. 3**) correlations of H-15 [$\delta_H$ 0.89 (3 H, s)] with H-13 [$\delta_H$ 1.67 (3 H, s)] and H-7 [$\delta_H$ 4.55 (1 H, d, $J$ = 2.2, 8.6 Hz)]. Thus, the structure of **20** was assigned as shown and named auranticanol I.

Auranticanol J (**21**) was defined with the molecular formula $C_{15}H_{22}O_2$ from HRESIMS (*m/z* 257.1516 $[M+Na]^+$, calcd for $C_{15}H_{22}O_2Na$, 257.1517). The $^{13}C$ NMR data (**Table 4**) of **21** were similar to those of **20**, except for the remarkably different shift at $\delta_C$ 203.6 (s, C-7) instead of $\delta_C$ 70.2 (d, C-7) in **20**, indicating that the C-7 was oxidated to be a carbonyl group in **21**. The HMBC and $^1H$ $^1H$ COSY (**Fig. 2**) correlations further confirmed the above hypothesis and the atom connectivity in **21**. The relative configuration of **21** was elucidated by ROESY experiment (**Fig. 3**) and biogenetically determined to be the same as those of **19**. Thus, the structure of **21** was assigned as shown and named auranticanol J.

Auranticanol K (**22**) indicated the molecular formula $C_{15}H_{24}O_3$ from HRESIMS (*m/z* 275.1620 $[M+Na]^+$, calcd for $C_{15}H_{24}O_3Na$, 275.1623). The $^{13}C$ NMR data (**Table 4**) of **22** were also similar to those of **21**, The main differences were the signals $\delta_C$ 76.9 (s, C-11), 70.3 (t, C-12), and 21.9 (q, C-14) substituted for $\delta_C$ 145.7 (s, C-11), 113.4 (t, C-12), and 66.6 (t, C-14) in **21**, indicating that the C-11 and C-12 were oxygenated and linked hydroxyl groups and C-14 was deoxygenized in **22**. This was supported by the key HMBC (**Fig. 2**) correlations from H-13 [$\delta_H$ 1.19 (3 H, s)] to

RSC Advances Accepted Manuscript

C-10 ($\delta_C$ 47.4 d), C-11 and C-12, and from H-14 [$\delta_H$ 1.19 (3 H, s)] to C-7 ($\delta_C$ 203.1 s), C-1 ($\delta_C$ 136.5 s), and C-2($\delta_C$ 140.7 d). The configurations of C-4, C-5, and C-10 in **22** were elucidated by ROESY experiment (**Fig. 3**) and determined to be the same as those of **21** for their similar $^{13}$C NMR data. Therefore, the structure of **22** was assigned as shown and named auranticanol K.

Auranticanol L (**23**) was formulated as $C_{15}H_{26}O_2$ from HRESIMS (*m*/*z* 261.1829 $[M+Na]^+$, calcd for $C_{15}H_{26}O_2Na$, 261.1829). The $^{13}$C NMR data (**Table 4**) of **23** were similar to those of **22**, except for the carbon shift at $\delta_C$ 41.9 (t, C-7) replacing of $\delta_C$ 203.1 (s, C-7) in **22**, indicating that the carbonyl group in C-7 was deoxygenized to form the methylene in **23**. The HMBC and $^1$H $^1$H COSY correlations (**Fig. 2**) further confirmed the hypothesis and the atom connectivity in **23**. The configuration of **23** was also biogenetically determined to be the same as those of **22** by the ROESY experiment (**Fig. 3**). Thus, the structure of **23** was assigned as shown and named auranticanol L.

Auranticanol M (**24**) was defined with the molecular formula $C_{15}H_{24}O_3$ from HRESIMS (*m*/*z* 253.1808 $[M-H]^-$, calcd for $C_{15}H_{25}O_2$, 253.1803). Comparison of the similar $^{13}$C NMR data (**Table 4**) of **24** with those of **23** showed the carbon signals at $\delta_C$ 65.7 (t, C-13), 150.8 (s, C-1), 41.0 (t, C-2), and 105.3 (t, C-14) in **24** replaced those of $\delta_C$ 24.4 (q, C-13), 137.2 (s, C-1), 124.6 (t, C-2), and 28.3 (q, C-14) in **23**, indicating that the C-13 in **23** was oxygenated to a methylol in **24**, and the double bond of C-1-C-2 in **23** moved to C-1-C-14 in **24**. This deduction was proved by the HMBC and $^1$H $^1$H COSY (**Fig. 2**) correlations. The relative configuration of **24** was biogenetically elucidated to be the same as those of **23** by the ROESY experiment (**Fig. 3**) and similar NMR data of those chiral carbons. Thus, the structure of **24** was assigned as shown and named auranticanol M.

The isolates from the stems of *D. aurantiaca* mainly were divided into two types of sesquiterpenoid guaiane and carotane. The new guaiane sesquiterpenoids were plausibly derived from the guaiane skeleton of **11** and **12** via chemical reactions or transformations as hydrolysis, hydrogenation, oxidation, electrophilic addition,[23] H [1, 3] σ migration,[24] and electron migration.[25, 26] The new carotane sesquiterpenoids may

RSC Advances Accepted Manuscript

be generated from the known natural product **25** via changes as isomerization, rearrangement, oxidation, hydrogenation, H [1, 3] σ migration,[24] and electron migration.[25, 26]

**Bioactivity evaluation**

Twenty compounds (**1-7**, **11-14**, **18-24**, **28**, and **29)** were tested for anti-HIV bioactivity (**Table 5**). They were evaluated by the inhibition assay for the cytopathic effects of HIV-1 ($EC_{50}$) and cytotoxicity assay against C8166 cell line by MTT methods. Two guaiane type sesquiterpenoids **11** and **14** showed moderate activities with $EC_{50}$ 2.138 μg/mL SI >93.545 and 0.286 μg/mL SI 93.787, respectively. The carotane type sesquiterpenoid **19** also showed moderate activities with $EC_{50}$ 1.773 μg/mL and SI 10.243. The tigliane diterpenoid **28** showed better anti-HIV bioactivities than the positive control with $EC_{50}$ 0.000282 μg/mL and SI 65177.305 (positive control 3'-azido-3'-deoxythymidine $EC_{50}$ 0.001656 μg/mL and SI 593164.855). Other 16 compounds showed weak anti-HIV bioactivities with $EC_{50}$ ranged from 12.530 to 136.937 μg/mL.

**Conclusion**

There are a series of terpenoids including a number of diterpenoids and a few of sesquiterpenoids isolated from *Daphne* species. The plant *D. aurantiaca* was also in accordance with this common law as both types of terpenoids were isolated. [3] From our result, the chemical constitute specificity of this plant was more sesquiterpenoids and less diterpenoids. The genus *Daphne* exhibited obvious anti-HIV-1 activities according to previous studies, however no compound from this plant was identified to be responsible for this activity. Among the isolates tested here, three sesquiterpenoids including two guaiane and one carotane showed moderate activities with the low SI. Nevertheless, one tigliane diterpenoid showed better anti-HIV bioactivity with $EC_{50}$ value of 0.000282 μg/mL than the positive control 3'-azido-3'-deoxythymidine even though the SI was lower. The other tigliane diterpenoid without long-chain fatty acid and benzoate revealed limited activity, which suggested the esterification with organic acid especially long-chain lipophilic acid in C-12 and C-13 in tigliane diterpenoid is

RSC Advances Accepted Manuscript

required for the anti-HIV bioactivity.

**Experimental section**

**General**

The Optical rotations were obtained on a Horiba SEAP-300 polarimeter (Kyoto, Japan). Mass spectra were measured on a Bruker HCT/ Esquire (Billerica, USA) and a VG Auto Spec-3000 mass spectrometer (Manchester, UK). And UV spectra were obtained on a Hitachi UV 210A spectrophotometer (Tokyo, Japan). IR spectra were acquired on a Bio-Rad FTS-135 spectrometer (Berkeley, USA) with KBr pellets. 1D and 2D NMR spectra were measured using a Bruker AV-400 or a DRX-500 (Billerica, USA) instrument with TMS as an internal standard. Column chromatography (CC) was performed on Silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), reverse phase-18 (RP-18) (40-70 μm, Fuji Silysia Chemical Ltd., Nagoya, Japan) and hydroxypropyl Sephadex (Sephadex LH-20) (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Fractions were monitored by TLC and spots were visualized by heating after spraying with 5% $H_2SO_4$ in EtOH (B.p. 77-79 ℃).

**Plant material**

*Daphne aurantiaca* Diels. stems were obtained from Shangri-La Yunnan Province, People's Republic of China. The Voucher specimen (HUANG0005) was identified by Prof. Dr. Y. Niu (Kunming Institute of Botany, Chinese Academy of Sciences) and deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

**Extraction and isolation**

The stems of *D. aurantiaca* (4.5 kg) were crushed and extracted with 95% EtOH at 80 ℃ refluxing (3 hours, 3 × 20 L). The filtrated EtOH solution was concentrated to give the concrete (1.6 kg). The concrete was suspended in 3 L water and then extracted with EtOAc (3 × 3 L). After concentration, the EtOAc extract (303 g) was firstly subjected to silica gel (200–300 mesh) column (15 × 120 cm) eluted with $CHCl_3$/MeOH (50:1-1:1) to afford fractions A–D. Fraction A(79g) was defatted with

RSC Advances Accepted Manuscript

Sephadex LH-20 column (MeOH/$CHCl_3$ 1:1) and then separated repeatedly with a RP-18 column eluting with MeOH/$H_2O$ (1:5 - 1:0) to afford fractions A1-A7. Fractions A1-A7 were purified repeatedly by silica gel column (petroleum ether/acetone, 4:1) and Sephadex LH-20 (MeOH) column chromatography to yield **6** (56.3 mg), **9** (6.3 mg), **2** (21.3 mg), **11** (156.3 mg), **12** (143.2 mg), **14** (15.6 mg), and **15** (4.9 mg), respectively. Fraction B (110 g) was then subjected to silica gel column eluted with petroleum ether/acetone (10:1- 1:1) to give four fractions B1–B4. Fraction B1 was separated repeatedly with a RP-18 column with MeOH/$H_2O$ (1:5 - 5:1) to afford fractions B1a-B1e. Fractions B1a-B1e were separated repeatedly with silica gel column (petroleum ether/acetone, 3:1) and Sephadex LH-20 column (MeOH) to yield **16** (2.0 mg), **17** (2.4 mg), **21** (11.3 mg), **25** (2.3 mg), and **27** (1.8 mg), respectively. Fraction B2 was separated repeatedly with a RP-18 column (MeOH/$H_2O$ 1:5 - 5:1) to afford fractions B2a-B2f. Fractions B2a-B2f were purified repeatedly by silica gel column (petroleum ether/acetone 2:1) and Sephadex LH-20 (MeOH) column chromatography to yield **3** (34.5 mg), **7** (460 mg), **8** (3.3 mg), **10** (2.1 mg), and **13** (4.7 mg), respectively. Fraction B3 was separated repeatedly with a RP-18 column (MeOH/$H_2O$ 1:5 - 5:1) to afford fractions B3a-B3g. Fractions B3a-B3g were separated repeatedly with silica gel column (petroleum ether/acetone 2:1) and Sephadex LH-20 column (MeOH) to yield **18** (15.7 mg), **19** (4.2 mg), **20** (782.4 mg), **22** (5.6 mg), **23** (10.3 mg), and **26** (2.7 mg), respectively. Fraction B4 combined Fraction C (10 g) was separated repeatedly with a RP-18 column (MeOH/$H_2O$ 1:5 - 5:1) to afford fractions C1-C4. Fractions C1-C4 were separated repeatedly with silica gel column (petroleum ether/acetone 2:1) and Sephadex LH-20 column (MeOH) to yield **1** (23.4 mg), **4** (5.3 mg), **5** (13.1mg), and **24** (9.6 mg), respectively. Lastly the Fraction D (79g) was separated repeatedly with Sephadex LH-20 column (MeOH), and separated repeatedly in a RP-18 column (MeOH/$H_2O$ 1:5 - 9:1) to obtain fractions D1-D2. Fractions D1-D2 were separated repeatedly with silica gel column (petroleum ether/acetone 2:1) and Sephadex LH-20 (MeOH) column chromatography to yield **28** (22.3 mg) and **29** (35.9 mg), respectively.

RSC Advances Accepted Manuscript

**Auranticanol A (1):** colorless oil; $C_{15}H_{20}O_3$, $[\alpha]_D^{18}$ -51.26 (*c* 0.241, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 271 (100); HRESIMS *m/z* $[M+Na]^+$ 271.1305 (calcd for $C_{15}H_{20}O_3Na$, 271.1310); UV (MeOH) $\lambda_{max}$ (logε) 203 (3.69); IR (KBr) $\nu_{max}$ 3433, 2963, 2931, 2874, 1751, 1721, 1619, 1453, 1404, 1382, 1065, 963, 934 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see **Table 1**.

**Auranticanol B (2):** colorless oil; $[\alpha]_D^{20}$ -29.09 (*c* 0.115, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 275 (75); HRESIMS *m/z* $[M+Na]^+$ 275.1631 (calcd for $C_{15}H_{24}O_3Na$, 275.1623); UV (MeOH) $\lambda_{max}$ (logε) 203 (2.73), 237 (2.36); IR (KBr) $\nu_{max}$ 3405, 2959, 2919, 2876, 2839, 1628, 1450, 1374, 1161, 1106, 1046, 1021, 997, 982, 685 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see **Table 1**.

**Auranticanol C (4):** colorless oil; $[\alpha]_D^{20}$ +72.95 (*c* 0.176, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 305 (100); HRESIMS *m/z* $[M+Na]^+$ 305.1370 (calcd for $C_{15}H_{22}O_5Na$, 305.1364); UV (MeOH) $\lambda_{max}$ (logε) 204 (3.57), 224 (3.76); IR (KBr) $\nu_{max}$ 3428, 2959, 2932, 2879, 1682, 1626, 1434, 1385, 1166, 1031, 1022, 984, 916 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see **Table 1**.

**Auranticanol D (5):** colorless oil; $[\alpha]_D^{18}$ + 38.47 (*c* 0.261, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 289 (100); HRESIMS *m/z* $[M+Na]^+$ 289.1418 (calcd for $C_{15}H_{22}O_4Na$, 289.1415); UV (MeOH) $\lambda_{max}$ (logε) 202 (3.59), 235 (3.59), 307 (2.33); IR (KBr) $\nu_{max}$ 3427, 2958, 2935, 2873, 1675, 1636, 1440, 1379, 1246, 1182, 1115, 1065, 1009, 912 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see **Table 2**.

**Auranticanol E (6):** colorless oil; $[\alpha]_D^{18}$ -127.70 (*c* 0.496, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 271 (100); HRESIMS *m/z* $[M+Na]^+$ 271.1314 (calcd for $C_{15}H_{20}O_3Na$, 271.1310); UV (MeOH) $\lambda_{max}$ (logε) 221 (3.95), 251 (3.85); IR (KBr) $\nu_{max}$ 2957, 2929, 1767, 1708, 1629, 1452, 1409, 1381, 1340, 1256, 1166, 1098, 1054, 1019, 1003, 942, 889 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see **Table 2**.

**Auranticanol F (7):** colorless needle crystal, M.p. 123-124 ℃; $[\alpha]_D^{18}$ -9.12 (*c* 0.23, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 273 (100); HRESIMS *m/z* $[M+Na]^+$ 273.1459 (calcd for $C_{15}H_{22}O_3Na$, 273.1466); UV (MeOH) $\lambda_{max}$ (logε) 203 (3.93), 293 (1.93); IR

RSC Advances Accepted Manuscript

(KBr) $\nu_{max}$ 3539, 3387, 2961, 2946, 2873, 1430, 1330, 1236, 1217, 1157, 1115, 1087, 1034, 985, 895, 825, 651, 618, 572 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 2**.

**Auranticanol G (18):** colorless oil; $[\alpha]_D^{17}$ +0.36 (*c* 0.206, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 259 (85); HRESIMS *m/z* $[M+Na]^+$ 259.1673 (calcd for $C_{15}H_{24}O_2Na$, 259.1673); UV (MeOH) $\lambda_{max}$ (logε) 202 (3.66); IR (KBr) $\nu_{max}$ 3427, 2951, 2926, 2855, 1634, 1452, 1379, 1120, 1085, 890 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 3**.

**Auranticanol H (19):** colorless oil; $[\alpha]_D^{17}$ -0.54 (*c* 0.343, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 273 (85); HRESIMS *m/z* $[M+Na]^+$ 273.1460 (calcd for $C_{15}H_{22}O_3Na$, 273.1466); UV (MeOH) $\lambda_{max}$ (logε) 202(3.73), 236(3.72), 312(2.58), 492(1.26); IR (KBr) $\nu_{max}$ 3418, 2955, 2879, 1647, 1452, 1382, 1232, 1122, 1085, 1023, 994, 883 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 3**.

**Auranticanol I (20):** colorless needle crystal M.p. 74-75 ℃; $[\alpha]_D^{16}$ +19.30 (*c* 0.228, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 259 (30), $[2M+Na]^+$ 495 (100); HRESIMS *m/z* $[M+Na]^+$ 259.1673 (calcd for $C_{15}H_{24}O_2Na$, 259.1673); UV (MeOH) $\lambda_{max}$ (logε) 202 (3.88), 221 (3.99); IR (KBr) $\nu_{max}$ 3428, 2956, 2930, 2878, 1694, 1635, 1449, 1417, 1384, 1204, 1138, 1053 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 3**.

**Auranticanol J (21):** colorless oil; $[\alpha]_D^{18}$ +110.86 (*c* 0.288, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 257 (100); HRESIMS *m/z* $[M+Na]^+$ 257.1516 (calcd for $C_{15}H_{22}O_2Na$, 257.1517); UV (MeOH) $\lambda_{max}$ (logε) 202 (3.94), 222 (4.05); IR (KBr) $\nu_{max}$ 3425, 2956, 2885, 1642, 1448, 1421, 1381, 1287, 1238, 1220, 1087, 1005, 892 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 4**.

**Auranticanol K (22):** colorless oil; $[\alpha]_D^{16}$ +34.52 (*c* 0.270, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 275 (85); HRESIMS *m/z* $[M+Na]^+$ 275.1620 (calcd for $C_{15}H_{24}O_3Na$, 275.1623); UV (MeOH) $\lambda_{max}$ (logε) 201 (3.91), 222 (4.17), 240 (3.82); IR (KBr) $\nu_{max}$ 3427, 2950, 2928, 2878, 1645, 1636, 1455, 1385, 1283, 1245, 1202, 1126, 1053 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 4**.

**Auranticanol L (23):** colorless oil; $[\alpha]_D^{16}$ -21.86 (*c* 0.304, MeOH); ESIMS positive *m/z* $[M+Na]^+$ 261 (100); HRESIMS *m/z* $[M+Na]^+$ 261.1829 (calcd for $C_{15}H_{26}O_2Na$,

RSC Advances Accepted Manuscript

261.1829); UV (MeOH) $\lambda_{max}$ (logε) 202 (3.48), 237 (2.46), 299 (1.85), 362 (1.85); IR (KBr) $\nu_{max}$ 3421, 2962, 2921, 2853, 1705, 1631, 1450, 1379, 1130, 1045, 872, 809 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 4**.

**Auranticanol M (24):** colorless oil; $[\alpha]_D^{16}$ +19.40 (*c* 0.311, MeOH); ESIMS negetive *m/z* $[M+Cl]^-$ 289 (95); HRESIMS *m/z* $[M-H]^-$ 253.1808 (calcd for $C_{15}H_{25}O_2$, 253.1803); UV (MeOH) $\lambda_{max}$ (logε) 202(3.94), 221(4.10); IR (KBr) $\nu_{max}$ 3432, 2927, 2854, 1676, 1641, 1458, 1380, 1203, 1186, 1140, 1044, 886 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR data see **Table 4**.

### 3.4. Anti-HIV assay

Anti-HIV activity of compounds was evaluated by the cytopathic effects of HIV-1 ($EC_{50}$) and the cytotoxicity assay against the C8166 cell line ($IC_{50}$) with MTT methods as described in the literature and earlier researches.[5, 6, 27] AZT (3'-azido-3'-deoxythymidine) was used as a positive control. The concentration of the antiviral sample reducing HIV-1 replication by 50% ($EC_{50}$) was calculated and determined with the dose-response standard curve. The selectivity index (SI) was calculated with the ratio of $IC_{50}/EC_{50}$.

**Acknowledgement.**

This work was financed by NSFC (National Natural Science Foundation of China 31300294), Special Fund for Agro-Scientific Research in the Public Interest (201303117), National Support Science and Technology Subject (2013BAI11B04), Fundamental Scientific Research Funds for CATAS (ITBB2015ZD02), and Natural Science Foundation of Hainan Province (214039). The authors thank Dr. Y.L. Huang (Department of Epigenetics and Molecular Carcinogenesis, UT MD Anderson Cancer Center, USA), Liwen Tian (Southern Medical University, China), Zhikai Guo (The Scripps Research Institute, USA), and Dr. F. Jacques (Xishuangbanna Tropical Garden CAS, China) for proofreading of this paper.

**Supporting Information Available**

1D and 2D NMR spectra and mass spectra of the new compounds. This material is available free of charge via the Internet at…….

RSC Advances Accepted Manuscript

## References and notes


1. Y. Wang, M. G. Gilbert, B. Mathew, C. D. Brickell and L. Nevling, *Flora of China*, Science Press, Beijing, 2007, vol 13, pp.230-245.
2. C. Y. Wu, A. M. Lu, T. YanCheng, C. Z. Rui and D. Z. Li, eds., *The Families and Genera of Angiosperms in China*, Science Press, Beijing, 2003, pp.588-592.
3. W. C. Xu, J. G. Shen and J. Q. Jiang, *Chem. Biodiversity*, 2011, **8**, 1215-1233.
4. R. P. Borris, G. Blaskó and G. A. Cordell, *J. ethnopharmacol.*, 1988, **24**, 41-91.
5. S. Z. Huang, X. J. Zhang, X. Y. Li, H. Z. Jiang, Q. Y. Ma, P. C. Wang, Y. Q. Liu, J. M. Hu, Y. T. Zheng, J. Zhou and Y. X. Zhao, *Planta Med.*, 2012, **78**, 182-185.
6. S. Z. Huang, X. J. Zhang, X. Y. Li, L. M. Kong, H. Z. Jiang, Q. Y. Ma, Y. Q. Liu, J. M. Hu, Y. T. Zheng, Y. Li, J. Zhou and Y. X. Zhao, *Phytochemistry*, 2012, **75**, 99-107.
7. C. A. S. Kunming Institute of Botany, ed., *Flora of Yunnan*, Science Press, Kunming, 1997, vol 8, pp.219-231.
8. Yunnan institute of materia medica, *The annals of national medicine in Yunnan*, the nationalities publishing house of Yunnan, Kunming, 2009, pp.5-6.
9. S. Liang, Y. H. Shen, Y. Feng, J. M. Tian, X. H. Liu, Z. Xiong and W. D. Zhang, *J. Nat. Prod.*, 2010, **73**, 532-535.
10. S. Liang, S. X. Liu, H. Z. Jin, L. Shan, S. C. Yu, Y. H. Shen, H. L. Li, Q. Y. Wu, Q. Y. Sun and W. D. Zhang, *Chem. Commun.*, 2013, **49**, 6968-6970.
11. S. Liang, J. M. Tian, Y. Feng, X. H. Liu, Z. Xiong and W. D. Zhang, *Chem. Pharm. Bull.*, 2011, **59**, 653-656.
12. Y. X. Zhao, S. Z. Huang, Q. Y. Ma, W. L. Mei and H. F. Dai, *Molecules*, 2012, **17**, 10046-10051.
13. S.Z. Huang, X.N. Li, Q.Y. Ma, H.-F. Dai, L.C. Li, X.H. Cai, Y.Q. Liu, J. Zhou and Y.X. Zhao, *Tetrahedron Lett.*, 2014, **55**, 3693-3696.
14. L.P. Liu, K. Han, W. Chen, Y.Y. Zhang, L.J. Tong, T. Peng, H. Xie, J. Ding and H.B. Wang, *Bioorg. Med. Chem.*, 2014, **22**, 4198-4203.
15. D. C. Kim, J. A. Kim, B. S. Min, T.S. Jang, M.K. Na and S. H. Lee, *Helv. Chim. Acta*, 2010, **93**, 692-697.
16. W. Herz and P. Santhanam, *J. Org. Chem.*, 1967, **32**, 507-510.
17. Z. Cekan, V. Herout and F. Sorm, *Chem. Listy.*, 1957, **51**, 756-763.
18. G. Blay, B. Garcia, E. Molina and J. R. Pedro, *Tetrahedron*, 2007, **63**, 9621-9626.
19. A. Garcia-Granados, A. Molina and E. Cabrera, *Tetrahedron*, 1986, **42**, 81-87.
20. J. G. Diaz, B. M. Fraga, A. G. Gonzalez, M. G. Hernandez and A. Perales, *Phytochemistry*, 1986, **25**, 1161-1165.
21. J. Peng, S. G. Franzblau, F. Zhang and M. T. Hamann, *Tetrahedron Lett.*, 2002, **43**, 9699-9702.
22. M. Neeman and O. D. Simmons, *Can. J. Chemistry*, 1979, **57**, 2071-2072.
23. L. Fitjer, A. Malich, C. Paschke, S. Kluge, R. Gerke, B. Rissom, J. Weiser and M. Noltemeyer, *J. Am. Chem. Soc.*, 1995, **117**, 9180-9189.
24. I. G. Collado, J. R. Hanson and A. J. Macias-Sanchez, *Nat. Prod. Rep.*, 1998, **15**, 187-204.
25. P. M. Dewick, in *Medicinal Natural Products*, John Wiley & Sons, Ltd, Editon edn., 2001, pp. 7-34.
26. K. B. G. Torssell, ed., *Natural product chemistry :a mechanistic, biosynthetic, and ecological*

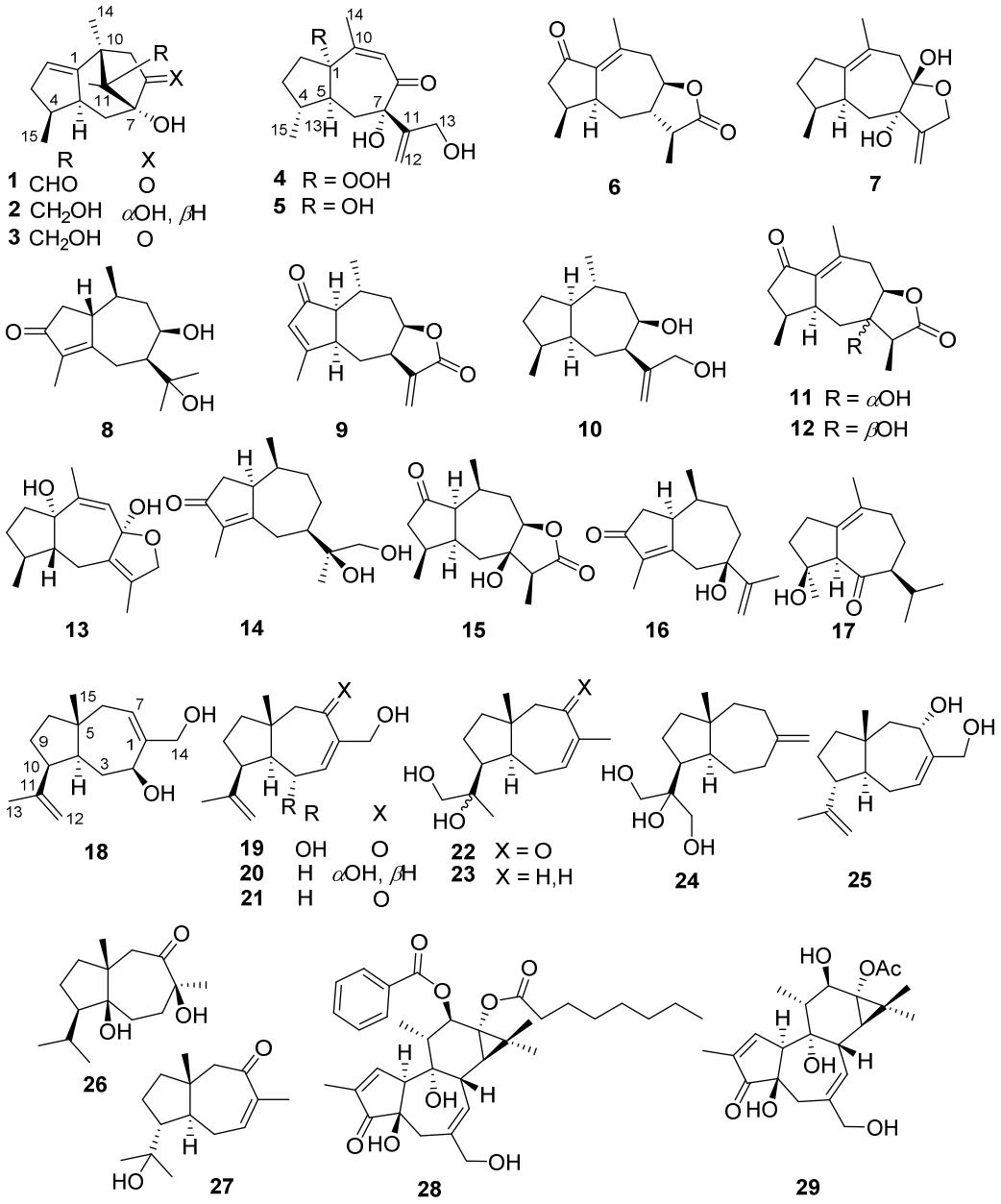

RSC Advances Accepted Manuscript

*approach*, Apotekarsocieteten, Swedish Pharmaceutical Society, Stockholm, 1997, p. 1-480.

27. S. Z. Huang, X. Zhang, Q. Y. Ma, H. Peng, Y. T. Zheng, J. M. Hu, H. F. Dai, J. Zhou and Y. X. Zhao, *Fitoterapia*, 2014, **95**, 34-41.

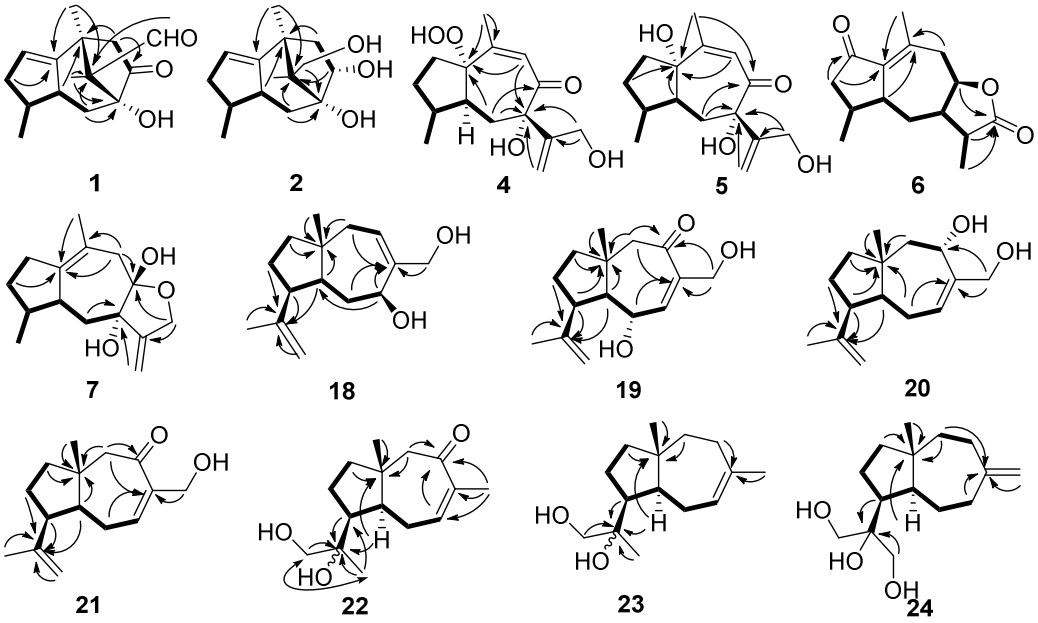
RSC Advances Accepted Manuscript

**Figure 1.** The structures of compounds **1**-**29**



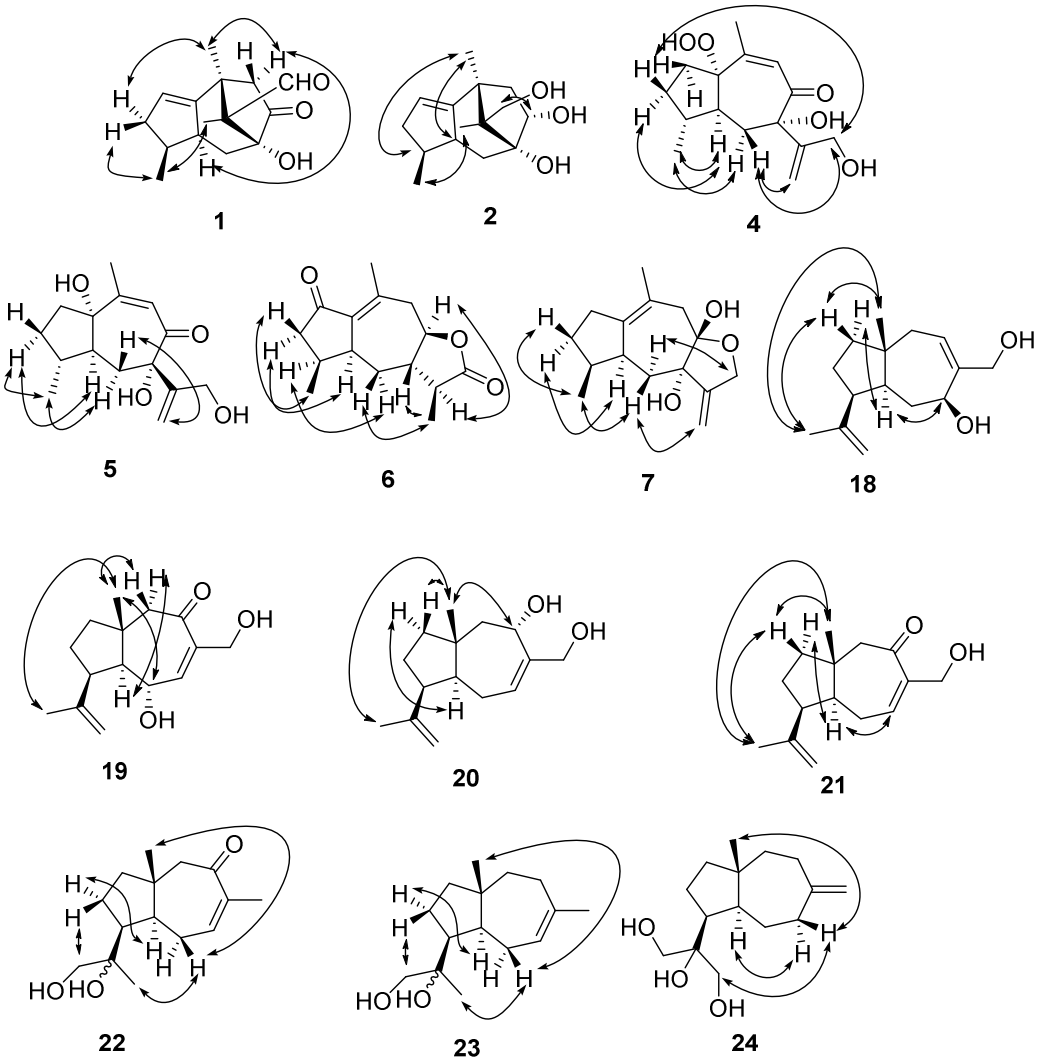

RSC Advances Accepted Manuscript

**Figure 2.** Key $^{1}H$ $^{1}H$ COSY (—) and HMBC (H→C) correlations of **1**, **2**, **4**-**7** and **18**-**24**




RSC Advances Accepted Manuscript

**Figure 3.** Key ROESY correlations of **1**, **2**, **4**-**7** and **18**-**24**




RSC Advances Accepted Manuscript

**Table 1.** $^{1}$H and $^{13}$C NMR data of compounds **1**, **2**, and **4**.

| | **1**[b] | | **2**[a] | | **4**[b] | |
|---|---|---|---|---|---|---|
| no. | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ |
| **1** | | 145.2 s | | 149.9 s | | 106.7 s |
| **2** | 5.39 (1H, t, 2.1) | 122.3 d | 5.12 (1H, t, 1.7) | 117.1 d | 3.61 (1H, ddd, 1.9, 7.3, 15.4)<br>3.57 (1H, ddd, 1.7, 6.9, 15.4) | 60.1 t |
| **3** | 2.60 (1H, ddd, 1.8, 7.1, 15.2)<br>1.99 (1H, ddd, 1.8, 1.9, 15.2) | 41.0 t | 2.59 (1H, ddd, 1.7, 7.0, 15.2)<br>1.92 (1H, ddd, 1.7, 1.9, 15.2) | 40.9 t | 1.70 (1H, m)<br>1.24 (1H, m) | 39.7 t |
| **4** | 2.46 (1H, m) | 33.2 d | 2.44 (1H, m) | 33.3 d | 1.45 (1H, m) | 32.3 d |
| **5** | 2.70 (1H, m) | 42.2 d | 3.06 (1H, m) | 41.6 d | 2.09 (1H, m) | 54.5 d |
| **6** | 1.91 (1H, dd, 4.3, 12.2)<br>1.53 (1H, dd, 10.2, 12.2) | 32.0 t | 1.72 (2H, m) | 29.4 t | 2.63 (1H, dd, 10.0, 13.3)<br>1.69 (1H, dd, 6.9, 13.3) | 37.3 t |
| **7** | | 83.0 s | | 86.3 s | | 86.7 s |
| **8** | | 217.0 s | 4.57 (1H, dd, 4.0, 9.8) | 76.9 d | | 199.1 s |
| **9** | 2.34 (1H, d, 16.9)<br>2.24 (1H, d, 16.9) | 46.3 t | 2.15 (1H, dd, 9.8, 13.2)<br>1.48 (1H, dd, 4.0, 13.2) | 42.4 t | 5.91 (1H, d, 1.4) | 126.1 d |
| **10** | | 42.7 s | | 43.9 s | | 166.8 s |
| **11** | | 60.5 s | | 48.2 s | | 147.8 s |
| **12** | 9.63 (1H, s) | 207.5 d | 4.11 (1H, d, 15.4)<br>3.50 (1H, d, 15.4) | 68.4 t | 5.39 (1H, d, 1.2)<br>5.26 (1H, d, 1.2) | 112.2 t |
| **13** | 0.91(3H, s) | 9.7 q | 0.98 (3H, s) | 13.7 q | 4.25 (1H, d, 14.8)<br>4.01 (1H, d, 14.8) | 62.8 t |
| **14** | 1.23 (3H, s) | 17.3 q | 0.95 (3H, s) | 16.3 q | 2.12 (1H, d, 1.4) | 20.1 q |
| **15** | 0.90 (3H, d, 7.2) | 17.4 q | 0.91 (3H, d, 7.2) | 17.3 q | 1.15 (3H, d, 6.4) | 18.6 q |

[a] $^{1}$H NMR data measured at 500 MHz and [b] $^{1}$H NMR data at 400 MHz in $CDCl_3$. All $^{13}$C NMR data measured at 100 MHz in $CDCl_3$.

RSC Advances Accepted Manuscript

**Table 2.** $^{1}$H and $^{13}$C NMR data of compounds **5**-**7**.

| | **5**[b] | | **6**[a] | | **7**[a] | |
|---|---|---|---|---|---|---|
| no. | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ |
| **1** | | 87.1 s | | 136.0 s | | 144.1 s |
| **2** | 2.06 (1H, m)<br>1.89 (1H, m) | 35.7 t | | 206.8 s | 2.37 (1H, m)<br>2.18 (1H, m) | 30.6 t |
| **3** | 1.91 (1H, m)<br>1.26 (1H, m) | 30.3 t | 2.38 (1H, dd, 7.5, 17.2)<br>2.03 (1H, dd, 3.2, 17.2) | 47.6 t | 1.68 (1H, m)<br>1.34 (1H, m) | 33.7 t |
| **4** | 2.74 (1H, m) | 36.1 d | 2.33 (1H, m) | 32.2 d | 2.11 (1H, m) | 39.6 d |
| **5** | 1.97 (1H, m) | 50.4 d | 2.82 (1H, m) | 40.9 d | 2.83 (1H, m) | 41.2 d |
| **6** | 1.85 (1H, dd, 5.3, 14.2)<br>1.78 (1H, dd, 11.4, 14.2) | 32.0 t | 1.74 (1H, m)<br>1.60 (1H, m) | 24.9 t | 1.72 (1H, m)<br>1.37 (1H, dd, 9.6, 12.0) | 36.9 t |
| **7** | | 84.3 s | 2.84 (1H, m) | 44.4 d | | 80.4 s |
| **8** | | 206.0 s | 4.66 (1H, ddd, 3.3, 7.6, 7.8) | 78.5 d | | 103.9 s |
| **9** | 5.80 (1H, s) | 124.3 d | 2.77 (1H, m)<br>2.58 (1H, dd, 1.8, 17.2) | 39.9 t | 2.73 (1H, d, 15.6)<br>2.28 (1H, d, 15.6) | 42.0 t |
| **10** | | 154.3 s | | 146.3 s | | 122.5 s |
| **11** | | 152.2 s | 2.75 (1H, m) | 38.5 d | | 155.7 s |
| **12** | 5.29 (1H, d, 1.3)<br>5.22 (1H, d, 1.3) | 112.2 t | | 179.7 s | 4.46 (1H, d, 13.2)<br>4.32 (1H, d, 13.2) | 67.9 t |
| **13** | 4.14 (1H, d, 15.2)<br>4.11 (1H, d, 15.2) | 62.2 t | 1.27 (3H, d, 7.9) | 12.6 q | 5.15 (1H, d, 1.2)<br>4.95 (1H, d, 1.2) | 104.4 t |
| **14** | 1.98 (1H, s) | 21.2 q | 2.24 (3H, s) | 21.9 q | 1.67 (3H, s) | 22.4 q |
| **15** | 1.04 (3H, d, 6.9) | 16.1 q | 0.89 (3H, d, 7.2) | 15.3 q | 0.93 (3H, d, 7.1) | 15.4 q |

$^{1}$H, $^{13}$C NMR data measured at 400 and 100 MHz, respectively. [a] in $CDCl_3$.,, [b] in $CD_3OD$.

RSC Advances Accepted Manuscript

**Table 3.** $^{1}$H and $^{13}$C NMR data of compounds **18**-**20**.

| | **18**[a] | | **19**[b] | | **20**[a] | |
|---|---|---|---|---|---|---|
| no. | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ |
| **1** | | 143.7 s | | 136.5 s | | 142.2 s |
| **2** | 4.23 (1H, dd, 1.9, 4.1) | 68.8 d | 6.41 (1H, d, 0.9) | 143.8 d | 5.73 (1H, dd, 7.7, 8.0) | 128.9 d |
| **3** | 1.83 (1H, ddd, 1.9, 4.4, 14.8)<br>1.54 (1H, ddd, 4.1, 11.3, 14.8) | 32.1 t | 4.37 (1H, dd, 0.9, 11.4) | 70.0 d | 2.07 (1H, m)<br>1.90 (1H, m) | 26.7 t |
| **4** | 2.53 (1H, ddd, 4.4, 11.3, 13.2) | 46.2 d | 2.38 (1H, dd, 11.2, 11.4) | 57.1 d | 1.86 (1H, m) | 50.0 d |
| **5** | | 43.8 s | | 41.3 s | | 41.8 s |
| **6** | 2.13 (2H, m) | 42.9 t | 2.63 (1H, d, 15.5)<br>2.49 (1H, d, 15.5) | 58.5 t | 2.09 (1H, dd, 2.2. 15.5)<br>1.47 (1H, dd, 8.6, 15.5) | 51.2 t |
| **7** | 5.67 (1H, m) | 128.0 d | | 202.3 s | 4.55 (1H, dd, 2.2, 8.6) | 70.0 d |
| **8** | 1.53 (1H, ddd, 4.2, 5.0, 16.4)<br>1.51 (1H, m) | 43.6 t | 1.54 (2H, m) | 42.6 t | 1.57 (1H, m)<br>1.41 (1H, m) | 42.0 t |
| **9** | 1.75 (2H, m) | 29.5 t | 1.85 (1H, m)<br>1.76 (1H, m) | 29.5 t | 1.69 (2H, m) | 28.0 t |
| **10** | 2.98 (1H, m) | 51.7 d | 3.12 (1H, m) | 48.5 d | 2.87 (1H, m) | 49.6 d |
| **11** | | 148.9 s | | 148.1 s | | 147.0 s |
| **12** | 4.78 (1H, d, 1.3)<br>4.71 (1H, d, 1.3) | 113.7 t | 4.97 (1H, d, 1.4)<br>4.95 (1H, d, 1.4) | 114.4 t | 4.79 (1H, d, 1.4)<br>4.68 (1H, d, 1.4) | 113.1 t |
| **13** | 1.70 (3H, s) | 23.3 q | 1.88 (3H, s) | 23.4 q | 1.67 (3H, s) | 23.3 q |
| **14** | 4.03 (1H, d, 16.0)<br>3.93 (1H, d, 16.0) | 69.1 t | 4.18 (1H, d, 16.0)<br>4.13 (1H, d, 16.0) | 64.2 t | 4.16 (2H, brs) | 69.8 t |
| **15** | 0.86 (3H, s) | 19.4 q | 0.98 (3H, s) | 19.4 q | 0.89 (3H, s) | 18.5 q |

[a] $^{1}$H NMR data measured at 500 MHz and [b]$^{1}$H NMR data at 400 MHz in $CDCl_3$. All $^{13}$C NMR data measured at 100 MHz in $CDCl_3$.

RSC Advances Accepted Manuscript

**Table 4.** $^{1}$H and $^{13}$C NMR data of compounds **21** - **24**.

| | **21**[b] | | **22**[c] | | **23**[a] | | **24**[c] | |
|---|---|---|---|---|---|---|---|---|
| no. | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ | $\delta_H$ multi, *J* (Hz) | $\delta_C$ |
| **1** | | 139.6 s | | 136.5 s | | 137.2 s | | 150.8 s |
| **2** | 6.50 (1H, dd, 2.1, 6.7) | 143.4 d | 6.36 (1H, dd, 7.8, 8.3) | 140.7 d | 5.40 (1H, dd, 7.7, 8.5) | 124.6 d | 1.37 (1H, m) | 41.0 t |
| | | | | | | | 1.21 (1H, m) | |
| **3** | 2.39 (1H, ddd, 3.0, 6.7, 14.8) | 30.0 t | 3.14 (1H, m) | 30.1 t | 2.48 (1H, m) | 41.0 t | 1.53 (1H, m) | 24.2 t |
| | 2.14 (1H, ddd, 2.1, 13.3, 14.8) | | 2.50 (1H, m) | | 2.01 (1H, m) | | 1.15 (1H, m) | |
| **4** | 2.25 (1H, ddd, 3.0, 13.2, 13.3) | 48.4 d | 2.37 (1H, m) | 48.9 d | 2.37 (1H, m) | 50.0 d | 1.71 (1H, m) | 49.9 d |
| **5** | | 40.4 s | - | 40.7 s | | 44.4 s | | 35.9 s |
| **6** | 2.70 (1H, d, 16.3) | 58.8 t | 2.70 (1H, d, 15.5) | 58.8 t | 2.15 (1H, m) | 30.1 t | 1.52 (1H, m) | 21.6 t |
| | 2.44 (1H, d, 16.3) | | 2.41 (1H, d, 15.5) | | 1.84 (1H, m) | | 1.31 (1H, m) | |
| **7** | | 203.6 s | | 203.1 s | 1.58 (1H, m) | 41.0 t | 1.39 (1H, m) | 41.7 t |
| | | | | | 1.16 (1H, m) | | 1.21 (1H, m) | |
| **8** | 1.56 (1H, ddd, 4.4, 5.1, 19.0) | 41.8 t | 1.54 (1H, m) | 41.6 t | 1.38 (1H, m) | 27.4 t | 1.55 (2H, m) | 23.4 t |
| | 1.41 (1H, ddd, 4.2, 11.5, 19.0) | | 1.36 (1H, m) | | 1.25 (1H, m) | | | |
| **9** | 1.74 (2H, m) | 28.6 t | 1.66 (1H, m) | 26.9 t | 1.59 (1H, m) | 26.0 t | 2.25 (1H, m) | 36.8 t |
| | | | 1.38 (1H, m) | | 1.50 (1H, m) | | 1.93 (1H, m) | |
| **10** | 2.91 (1H, ddd, 2.2, 13.2, 13.6) | 49.2 d | 2.51 (1H, m) | 47.4 d | 2.51 (1H, m) | 47.4 d | 1.58 (1H, m) | 43.0 d |
| **11** | | 145.7 s | | 76.9 s | | 75.9 s | - | 74.7 s |
| **12** | 4.87 (1H, d, 1.3) | 113.4 t | 3.39 (1H, d, 10.9) | 70.3 t | 3.55 (1H, d, 10.8) | 68.3 t | 3.68 (1H, d, 10.9) | 65.7 t |
| | 4.72 (1H, d, 1.3) | | 3.26 (1H, d, 10.9) | | 3.42 (1H, d, 10.8) | | 3.61 (1H, d, 10.9) | |
| **13** | 1.69 (3H, s) | 23.8 q | 1.19 (3H, s) | 21.2 q | 1.18 (3H, s) | 24.4 q | 3.68 (1H, d, 10.9) | 65.7 t |
| | | | | | | | 3.61 (1H, d, 10.9) | |
| **14** | 4.15 (1H, d, 12.5) | 66.6 t | 1.81 (3H, s) | 21.9 q | 1.64 (3H, s) | 28.3 q | 4.67 (1H, d, 1.9) | 105.3 t |
| | 4.09 (1H, d, 12.5) | | | | | | 4.38 (1H, d, 1.9) | |
| **15** | 0.89 (3H, s) | 19.1 q | 0.91 (3H, s) | 19.1 q | 0.78 (3H, s) | 17.3 q | 0.66 (3H, s) | 16.2 q |

[a,c] $^{1}$H NMR data measured at 400 MHz in $CDCl_3$ and [b] $^{1}$H NMR data at 500 MHz. [a] $^{13}$C NMR data measured at 100 MHz in $CDCl_3$ and [b,c] $^{13}$C NMR data 125 MHz in $CDCl_3$.

RSC Advances Accepted Manuscript

**Table 5.** Summary of anti-HIV-1 of compounds **1**-**7**, **11**-**14**, **18**-**24**, **28**, and **29**

| no. | $CC_{50}$ (μg/mL) | $EC_{50}$ (μg/mL) | SI | no. | $CC_{50}$ (μg/mL) | $EC_{50}$ (μg/mL) | SI |
|---|---|---|---|---|---|---|---|
| **1** | 155.641 | 61.511 | 2.530 | **18** | 95.424 | 23.352 | 4.086 |
| **2** | 103.979 | 49.779 | 2.089 | **19** | 18.16 | 1.773 | 10.243 |
| **3** | >200 | 136.937 | >1.461 | **20** | 26.361 | 15.446 | 1.707 |
| **4** | >200 | 80.952 | >2.471 | **21** | 15.419 | 12.530 | 1.231 |
| **5** | >200 | 77.034 | >2.596 | **22** | 163.974 | 73.895 | 2.219 |
| **6** | 175.041 | 77.000 | 2.273 | **23** | 61.010 | 60.604 | 1.007 |
| **7** | 147.926 | 69.606 | 2.125 | **24** | 151.059 | 45.484 | 3.321 |
| **11** | >200 | 2.138 | >93.545 | **28** | 18.38 | 0.000282 | 65177.305 |
| **12** | >200 | 24.246 | >8.249 | **29** | >200 | 17.808 | >11.231 |
| **13** | 62.348 | 37.932 | 1.644 | **3'-azido-3'-deox** | 982.281 | 0.001656 | 593164.855 |
| **14** | 26.823 | 0.286 | 93.787 | **ythymidine** | | | |

RSC Advances Accepted Manuscript