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New Rearranged Limonoids from *Walsura cochinchinensis*

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†Electronic supplementary information (ESI) available: 1D and selected 2D NMR spectra of all compounds and the key X-ray crystallographic parameters of compounds **1** and **10**. See DOI: xxxx.

Abstract

Sixteen new limonoids, walsucochinoids C–R (**1**–**16**) incorporating a rearranged carbon skeleton, were isolated from the twigs and leaves of *Walsura cochinchinensis*. Their structures were established by detailed interpretation of spectroscopic data with those of **1** and **10** being secured by single-crystal X-ray diffraction experiments. Bioassays revealed that walsucochinoids D (**2**) and E (**3**) were mild mouse and human 11β-HSD1 inhibitors with IC_{50} values of 13.4±1.7 and 8.25±0.69 μM, respectively.

Introduction

Plants of the genus *Walsura* (family Meliaceae) are rich sources of bioactive triterpenyl and phenolic derivatives with diverse structures.[1–5] As an important part of our research for chemical therapies from natural sources, the previous studies of two native Chinese *Walsura* species returned biologically active nortriterpenoids with new carbon frameworks, such as the antimalarial walsuronoid A[6] and the neuroprotective walsucochins A and B.[7] Encouraged by these exciting discoveries, we recently carried out a further project aiming to search 11β-HSD1 (11β-hydroxysteroid dehydrogenase type 1) inhibitors from the *Walsura* plants. This program revealed walsucochinoids A and B as two novel limonoids with a rearranged skeleton from the non-active fraction,[8] while a focused analysis of those active fractions yielded 13 conventional triterpenoids and limonoids with some of them showing decent inhibition against both human and mouse 11β-HSD1.[9] An extensive fractionation of the remaining fractions returned 16 more limonoids with the rearranged walsucochinoid scaffold, namely, walsucochinoids C–R (**1**–**16**, Fig. 1), whose structures were assigned on the basis of spectroscopic methods including X-ray crystallography. Interestingly, our biological tests also established that walsucochinoids D (**2**) and E (**3**) moderately inhibited mouse and human 11β-HSD1, respectively. Herein, the isolation, structural elucidation and biological studies of this rare family of compounds are to be presented in this paper.

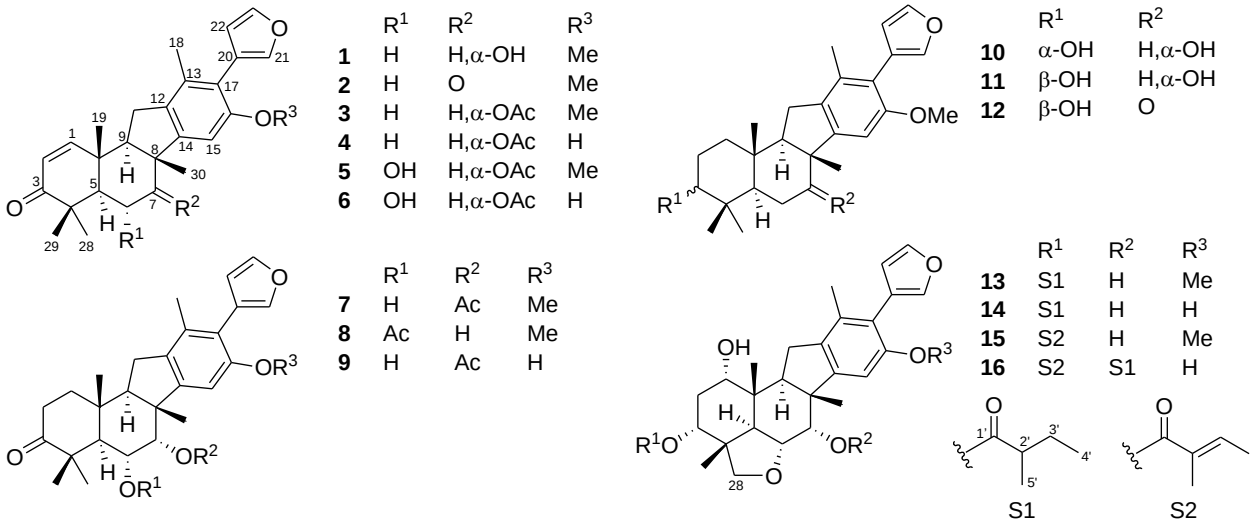

Fig. 1 Structures of walsucochinoids C–R (**1**–**16**)

Results and discussion

Compounds 1–9 bearing a 3-ketone

Compound **1** was obtained as colorless crystals, mp 255–257 ℃. The HRESI(–)MS spectrum displayed a *quasi* molecular ion peak at *m/z* 465.2282 ([M + HCO_2]$^-$, calcd 465.2277) corresponding to a molecular formula of $C_{27}H_{32}O_4$. The IR spectrum showed the presences of hydroxyl (3433 cm^{-1}), conjugated carbonyl (1655 cm^{-1}) and phenyl (1610, 1589 and 1504 cm^{-1}) groups. Analysis of the NMR data (Tables 1 and 2), with the aid of DEPT and HSQC experiments, revealed resonances of an α,β-unsaturated ketone (δ_H 7.16 and 5.89; δ_C 125.8, 205.5 and 158.8), a β-substituted furanyl residue (δ_H 7.50, 7.39 and 6.42; δ_C 113.0, 120.1, 141.2 and 142.2), a pentasubstituted benzene (δ_H 6.57; δ_C 101.0, 119.9, 133.7, 135.6, 149.0 and 157.2) bearing a methyl (δ_H 2.17; δ_C 17.7) and a methoxyl (δ_H 3.75; δ_C 56.2), an oxygenated methine (δ_H 4.42; δ_C 70.2), and four tertiary methyls (δ_H 1.15, 1.16, 1.20 and 1.32; δ_C 19.2, 21.3, 23.7 and 27.5). These observations indicated that **1** was a limonoid with the rare walsucochinoid backbone.[8] Examination of HMBC data (Fig. 2) confirmed the above-mentioned conclusion and also established the locations of 7-OH, 16-OMe and the conjugated carbonyl moiety in ring-A.

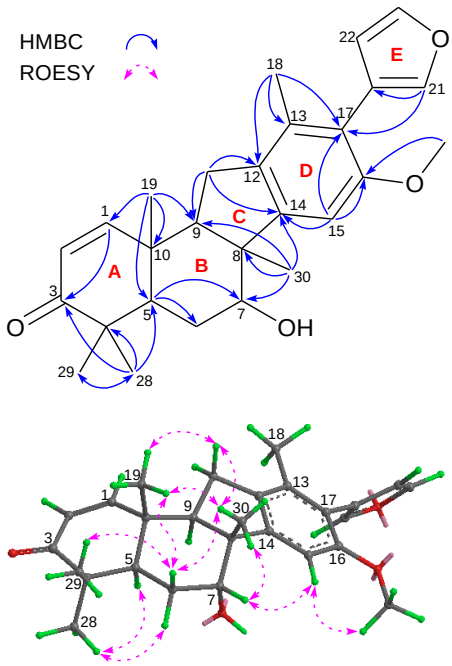

Fig. 2 Key 2D NMR correlations of walsucochinoid C (**1**).

The relative configuration of **1** was characterized by interpretation of ^{1}H–^{1}H couplings and

NOESY data (Fig. 2). The strong NOESY correlations of H-6β/H_3-19, H_3-19/H_3-30 and H_3-30/H-6β suggested that H-6β, Me-19 and Me-30 were axially bonded and they were assigned to be β-oriented as with walsucochinoid A.[8] Consequently, the magnitudes of $J_{5,6\beta}$ (13.2 Hz) and $J_{6\beta,7}$ (2.7 Hz) supported an axial H-5 and an equatorial H-7, respectively, which corroborated that H-5 was α-positioned and H-7 was β-directed. In addition, as H-11β was considered to take a *pseudo* axial position based on the strong NOE interactions of H-11β with both H_3-19 and H_3-30, the large $J_{9,11\beta}$ value (12.3 Hz) indicated that H-9 was also axially located and thus α-oriented. The relative structure of **1** was finally unambiguously confirmed by X-ray crystallography which further allowed the establishment of the absolute configuration of **1** (Fig. 3) as 5*R*, 7*R*, 8*R*, 9*R* and 10*R* [Flack parameter: 0.0(2)].[10] We hereby name it walsucochinoid C after walsucochinoids A and B,[8] and all the following new analogues with this scaffold are thus to be named sequentially.

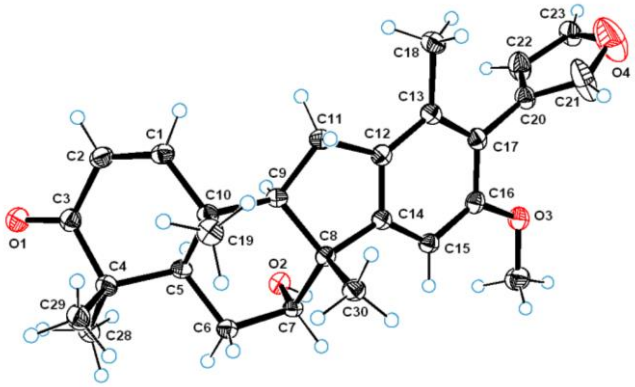

Fig. 3 X-ray structure of walsucochinoid C (**1**).

Walsucochinoids D (**2**) and E (**3**) were assigned molecular formulae of $C_{27}H_{30}O_4$ and $C_{29}H_{34}O_5$ via HRESI(+)MS data both showing $[2M + Na]^+$ ions at *m/z* 859.4182 and 947.4720, indicative of didehydro and acetylated analogues of **1**, respectively. Analyses of their NMR data (Tables 1 and 2) demonstrated this hypothesis with diagnostic resonances of a ketone (δ_C 210.0, C-7) in **2** replacing the oxymethine (δ_H 4.42; δ_C 70.2, CH-7) in **1** and of an additional acetyl group (δ_H 1.93; δ_C 21.4 and 170.9) which caused a marked deshielding on H-7 ($\Delta\delta_H$ 1.22) in **3**. These structural changes were further corroborated by the HMBC correlations from H_3-30 (δ_H 1.43) to C-7 in **2** and from H-7 to the acetyl carbonyl (δ_C 170.9) in **3**. The relative configurations of **2** and **3** were assigned to be identical with that of **1** on the basis of their similar ^1H–^1H coupling patterns and examination of ROESY data. The structures of **2** and **3** were thereby elucidated as shown.

Table 1 ^{1}H NMR data ($CDCl_3$, 400 MHz) for compounds **1**–**6**.

No.	**1**	**2**	**3**	**4**	**5**	**6**
1	7.16 (d, 10.0)	7.19 (d, 10.0)	7.17 (d, 10.0)	7.15 (d, 10.0)	7.14 (d, 9.9)	7.12 (d, 9.8)
2	5.89 (d, 10.0)	5.96 (d, 10.0)	5.91 (d, 10.0)	5.90 (d, 10.0)	5.97 (d, 9.9)	5.95 (d, 9.8)
5	2.42 (dd, 13.2, 2.7)	2.26[a] (dd, 14.3, 2.8)	2.27 (dd, 13.3, 2.2)	2.22 (dd, 13.2, 2.2)	2.23 (d, 11.6)	2.22 (d, 11.5)
6α	1.90 (ddd, 14.4, 2.7, 2.7)	2.43 (dd, 14.3, 2.8)	1.90 (ddd, 14.7, 3.3, 2.2)	1.92[c] (m)		
6β	2.07 (ddd, 14.4, 13.2, 2.7)	3.02 (dd, 14.3, 14.3)	2.14[b] (ddd, 14.7, 13.3, 2.2)	2.10[d] (m)	4.54 (ddd, 11.6, 5.9, 2.8)	4.54 (ddd, 11.5, 4.5, 2.7)
7	4.42 (brs)		5.64 (brs)	5.52 (brs)	5.70 (d, 2.8)	5.65 (d, 2.7)
9	2.47 (dd, 12.3, 6.3)	2.29[a] (dd, 11.9, 6.6)	2.44 (dd, 12.2, 6.4)	2.43 (dd, 12.2, 6.3)	2.45 (dd, 12.2, 6.3)	2.44 (dd, 12.2, 6.3)
11α	2.84 (dd, 13.7, 6.3)	2.89 (dd, 13.9, 6.6)	2.84 (dd, 13.8, 6.4)	2.81 (dd, 13.8, 6.3)	2.85 (dd, 13.8, 6.3)	2.82 (dd, 13.8, 6.3)
11β	2.69 (dd, 13.7, 12.3)	2.82 (dd, 13.9, 11.9)	2.70 (dd, 13.8, 12.2)	2.68 (dd, 13.8, 12.2)	2.66 (dd, 13.8, 12.2)	2.63 (dd, 13.8, 12.2)
15	6.57 (s)	7.24 (s)	6.48 (s)	6.46 (s)	6.49 (s)	6.47 (s)
18	2.17 (3H, s)	2.17 (3H, s)	2.17[b] (3H, s)	2.10[d] (3H, s)	2.17 (3H, s)	2.10 (3H, s)
19	1.32 (3H, s)	1.53 (3H, s)	1.34 (3H, s)	1.32 (3H, s)	1.27 (3H, s)	1.26 (3H, s)
21	7.39 (dd, 1.6, 0.7)	7.39 (brs)	7.39 (dd, 1.6, 0.7)	7.46 (brs)	7.40 (dd, 1.6, 0.7)	7.46 (dd, 1.6, 0.8)
22	6.42 (dd, 1.6, 0.7)	6.43 (brs)	6.43 (dd, 1.6, 0.7)	6.42 (brs)	6.44 (dd, 1.6, 0.7)	6.42 (dd, 1.6, 0.8)
23	7.50 (dd, 1.6, 1.6)	7.50 (brs)	7.49 (dd, 1.6, 1.6)	7.60 (dd, 1.5, 1.5)	7.50 (dd, 1.6, 1.6)	7.60 (dd, 1.6, 1.6)
28	1.20 (3H, s)	1.19 (3H, s)	1.14 (3H, s)	1.124[e] (3H, s)	1.43 (3H, s)	1.35 (3H, s)
29	1.15 (3H, s)	1.19 (3H, s)	1.14 (3H, s)	1.119[e] (3H, s)	1.36 (3H, s)	1.42 (3H, s)
30	1.16 (3H, s)	1.43 (3H, s)	1.20 (3H, s)	1.17 (3H, s)	1.23 (3H, s)	1.20 (3H, s)
16-OH				5.20 (s)		5.25 (s)
OMe	3.75 (3H, s)	3.80 (3H, s)	3.68 (3H, s)		3.69 (3H, s)	
OAc			1.93 (3H, s)	1.94[c] (3H, s)	2.01 (3H, s)	2.02[f] (3H, s)
6-OH					1.66 (d, 5.9)	2.02[f] (d, 4.5)

[a–f] Overlapping signals.

Walsucochinoids F (**4**) and G (**5**) had molecular formulae of $C_{28}H_{32}O_5$ and $C_{29}H_{34}O_6$ as supported by the HRESI(–)MS ions at *m/z* 493.2229 and 523.2337 (both $[M + HCO_2]^-$), suggestive of demethyl and oxygenated congeners of **3**, respectively. Analyses of their NMR data (Tables 1 and 2) confirmed this assumption with characteristic signals of 16-OMe (δ_H 3.68; δ_C 56.0) in **3** being substituted by a phenol group (δ_H 5.20) in **4** and those of CH_2-6 (δ_H 1.90 and 2.54; δ_C 25.0) in **3** being replaced by an oxymethine (δ_H 4.54; δ_C 69.1) in **5**. These structural variations were also authenticated by the shielded C-16 resonance ($\Delta\delta_C$ –4.0) of **4** compared to that of **3** and the alteration of the double doublet H-7 signal (J = 3.3, 2.2 Hz) in **3** to a doublet (J = 2.8 Hz) counterpart in **5**. By comparing the proton couplings of **4** and **5** with those of **3**, their relative configurations at C-5, C-7, C-8, C-9 and C-10 were determined to be the same as those in **3** with the new C-6 chiral center in **5** being assigned as drawn via the $J_{5,6}$ (11.6 Hz, diaxial relationship) and

$J_{6,7}$ (2.8 Hz, axial-equatorial relationship) values, which was also confirmed by ROESY data. The structures of **4** and **5** were hence characterized.

Table 2 ^{13}CNMR data ($CDCl_3$, 125 MHz) for compounds **1**–**12**

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	158.8	156.8	158.5	158.4	157.2	157.4	38.1	38.0	38.1	32.9	38.4	37.8
2	125.8	126.5	125.8	125.8	126.4	126.6	32.8	32.7	32.8	25.0	27.2	27.1
3	205.5	203.9	204.9	205.0	205.8	206.1	218.8	217.8	218.9	76.8	79.1	78.7
4	44.8	45.3	44.6	44.6	45.8	46.0	47.0	46.6	47.1	37.2	38.6	39.4
5	46.4	54.8	47.7	47.7	51.7	51.6	53.2	49.3	53.0	41.8	48.1	57.0
6	26.4	36.9	25.0	24.8	69.1	69.4	69.4	74.1	69.5	25.7	25.7	36.7
7	70.2	210.0	71.6	71.8	76.0	76.2	76.2	71.7	76.1	71.4	71.0	211.9
8	53.0	58.8	51.4	51.0	51.1	50.9	51.2	51.8	50.8	52.5	52.4	58.5
9	50.5	57.9	52.0	51.9	50.6	50.7	54.4	52.4	54.3	55.6	55.9	62.9
10	39.7	39.1	39.5	39.5	39.0	39.1	36.3	36.8	36.2	37.0	36.9	36.4
11	26.3	26.2	26.3	26.1	26.1	26.0	26.5	26.6	26.4	26.6	26.7	26.4
12	133.7	130.9	132.1	131.6	131.7	131.7	132.7	133.6	132.2	134.3	134.6	131.9
13	135.6	134.3	134.7	134.4	134.6	134.6	134.7	135.3	134.4	135.0	135.4	134.2
14	149.0	146.9	149.6	150.7	149.1	150.4	149.2	148.8	150.3	150.1	149.5	147.4
15	101.0	104.7	101.4	105.0	101.2	105.0	101.6	101.4	105.1	101.8	101.3	104.9
16	157.2	157.0	156.9	152.9	156.7	153.0	156.8	157.1	152.9	156.7	156.9	156.7
17	119.9	120.0	119.3	116.2	119.3	116.5	119.4	119.9	116.4	119.2	119.6	119.6
18	17.7	17.6	17.7	17.5	17.6	17.5	17.7	17.7	17.5	17.8	17.7	17.6
19	19.2	19.0	19.0	18.9	20.6	20.7	16.7	16.6	16.6	15.9	16.1	16.0
20	120.1	120.1	120.1	119.0	119.8	119.0	120.1	120.2	119.0	120.4	120.2	120.3
21	141.2	141.3	141.3	141.3	141.1	141.3	141.3	141.2	141.3	141.1	141.2	141.2
22	113.0	113.0	113.0	112.5	112.8	112.5	113.0	113.0	112.5	113.0	113.0	113.1
23	142.2	142.2	142.1	144.2	142.0	144.2	142.1	142.1	144.2	142.1	142.1	142.1
28	27.5	27.2	27.3	27.3	31.8	31.9	31.6	31.2	31.6	28.5	28.0	27.8
29	21.3	20.8	21.1	21.1	20.0	20.1	19.3	19.7	19.3	21.9	15.4	15.0
30	23.7	23.6	23.5	23.6	23.2	23.5	22.5	22.1	22.7	23.9	23.7	23.4
OMe	56.2	56.2	56.0		55.9		56.1	56.1		56.0	56.2	56.2
OAc			170.9	170.8	171.7	172.5	172.2	170.2	172.5			
			21.4	21.3	21.2	21.3	21.5	21.9	21.4			

Walsucochinoids H (**6**) and I (**7**) exhibited *quasi* molecular ions at *m/z* 509.2181 and 525.2497 (both $[M + HCO_2]^-$) in HRESI(–)MS analyses, consistent with molecular formulae of $C_{28}H_{32}O_6$ and $C_{29}H_{36}O_6$, and supportive of demethyl and dihydro derivatives of **5**, respectively. The NMR data of **6** (Tables 1 and 2) were highly comparable with those of **5** while only displaying signals of an aromatic hydroxyl (δ_H 5.25, 16-OH) instead of the 16-OMe (δ_H 3.69; δ_C 55.9) in the latter, and few NMR changes around C-16 due to altered substitution. In contrast to those of **5**, the NMR data

(Tables 2 and 3) of **7** revealed differences only at ring-A exhibiting the presence of two sp^3 methylenes (δ_C 38.1 and 32.8, C-1 and C-2) and a free ketone (δ_C 218.8, C-3) rather than the α,β-conjugated carbonyl fragment (δ_C 126.4, 157.2 and 205.8) in the former. The relative configurations of **6** and **7** were established as shown via excellent NMR comparisons with **5** at all stereocenters, and were further validated by ROESY experiments. Compounds **6** and **7** were thus elucidated to be the 16-*O*-demethyl and the 1,2-dihydro derivatives of **5**, respectively.

Table 3 ^{1}H NMR data ($CDCl_3$, 400 MHz) for compounds **7**–**12**

No.	**7**	**8**	**9**	**10**	**11**	**12**
1α	1.87 (2H, m)	1.89 (2H, m)	1.85 (2H, m)	1.29[c] (m)	1.25 (m)	1.21 (m)
1β				1.23[c] (m)	1.64 (ddd, 13.1, 3.3, 3.3)	1.76 (m)
2α	2.78 (m)	2.77 (m)	2.77 (m)	1.54 (m)	1.71 (2H, m)	1.77 (2H, m)
2β	2.40 (m)	2.42 (m)	2.39 (m)	2.00[d] (m)		
3				3.43 (dd, 2.6, 2.6)	3.29 (dd, 9.5, 6.6)	3.28 (dd, 7.4, 7.4)
5	2.10 (d, 11.2)	2.56[a] (d, 11.9)	2.09[b] (d, 11.3)	1.94[d] (dd, 13.0, 1.7)	1.48 (dd, 10.2, 5.7)	1.41 (dd, 3.0, 14.2)
6α				1.74 (brd, 13.0)	1.91 (2H, m)	2.41 (dd, 14.2, 3.0)
6β	4.42 (ddd, 11.2, 5.6, 3.1)	5.46 (dd, 11.9, 2.7)	4.41 (ddd, 11.3, 4.0, 3.0)	1.87 (ddd, 13.0, 13.0, 2.1)		2.85 (dd, 14.2, 14.2)
7	5.68 (d, 3.1)	4.50 (d, 2.7)	5.63 (d, 3.0)	4.33 (brs)	4.35 (brs)	
9	2.27 (dd, 12.0, 6.8)	2.48 (dd, 12.0, 6.5)	2.28 (dd, 11.9, 6.8)	2.23 (dd, 11.8, 6.9)	2.16 (dd, 11.9, 6.8)	1.96 (dd, 10.2, 7.9)
11α	2.63 (dd, 14.0, 6.8)	2.65 (dd, 13.7, 6.5)	2.60 (dd, 14.1, 6.8)	2.55 (dd, 14.0, 6.9)	2.58 (dd, 14.1, 6.8)	2.63 (2H, m)
11β	2.54 (dd, 14.0, 12.0)	2.57[a] (dd, 13.7, 12.0)	2.52 (dd, 14.1, 11.9)	2.47 (dd, 14.0, 11.8)	2.51 (dd, 14.1, 11.9)	
15	6.49 (s)	6.55 (s)	6.46 (s)	6.58 (s)	6.56 (s)	7.22 (s)
18	2.14 (3H, s)	2.14 (3H, s)	2.08[b] (3H, s)	2.15 (3H, s)	2.14 (3H, s)	2.13 (3H, s)
19	0.98 (3H, s)	1.01 (3H, s)	0.97 (3H, s)	1.03 (3H, s)	1.05 (3H, s)	1.26 (3H, s)
21	7.39 (brs)	7.38 (dd, 1.6, 0.7)	7.45 (brs)	7.38 (brs)	7.39 (brs)	7.38 (brs)
22	6.43 (brs)	6.42 (d, 1.6, 0.7)	6.41 (brs)	6.43 (brd, 1.6)	6.43 (brd, 1.6)	6.42 (dd, 1.6, 0.7)
23	7.49 (dd, 1.5, 1.5)	7.49 (dd, 1.6, 1.6)	7.60 (dd, 1.6, 1.6)	7.50 (dd, 1.6, 1.6)	7.49 (dd, 1.6, 1.6)	7.49 (dd, 1.6, 1.6)
28	1.33 (3H, s)	1.29 (3H, s)	1.32 (3H, s)	0.95 (3H, s)	1.01 (3H, s)	1.00 (3H, s)
29	1.35 (3H, s)	1.20 (3H, s)	1.34 (3H, s)	0.89 (3H, s)	0.85 (3H, s)	0.90 (3H, s)
30	1.17 (3H, s)	1.13 (3H, s)	1.15 (3H, s)	1.08 (3H, s)	1.10 (3H, s)	1.36 (3H, s)
6-OH	1.79 (d, 5.6)		1.95 (d, 4.0)			
16-OH			5.21 (s)			
OMe	3.68 (3H, s)	3.74 (3H, s)		3.76 (3H, s)	3.75 (3H, s)	3.79 (3H, s)
OAc	2.01 (3H, s)	2.21 (3H, s)	2.03 (3H, s)			

[a–d] Overlapping signals.

Compounds 10–12 bearing a 3-OH

Walsucochinoids L (**10**) was assigned a molecular formula of $C_{27}H_{36}O_4$ as suggested by HRESI(–)MS analysis at *m/z* 469.2599 ($[M + HCO_2]^-$, calcd 469.2590) indicating a tetrahydro

homologue of **1**. Analysis of the NMR data (Tables 2 and 3) of **10** validated this deduction revealing same structural features such as the β-substituted furan ring and the benzene residue as in **1**, except for a $-(CH_2)_2CH(OH)-$ fragment (C_1, C_2 and C_3) in place of the α,β-unsaturated ketone in **1**. Examination of HMBC data (Fig. 4) further demonstrated **10** as the tetrahydro derivative of **1**. The relative configuration of **10** was elucidated as shown on the basis of NMR comparison with **1** and ROESY data, while the new chiral center at C-3 was assigned via the coupling constants of H-3 with H_2-2 ($J_{2\alpha,3}$ = $J_{2\beta,3}$ = 2.6 Hz). An X-ray diffraction experiment confirmed the aforementioned structural elucidation (Fig. 5) with configurations at all stereocenters well matching those established by NMR data.

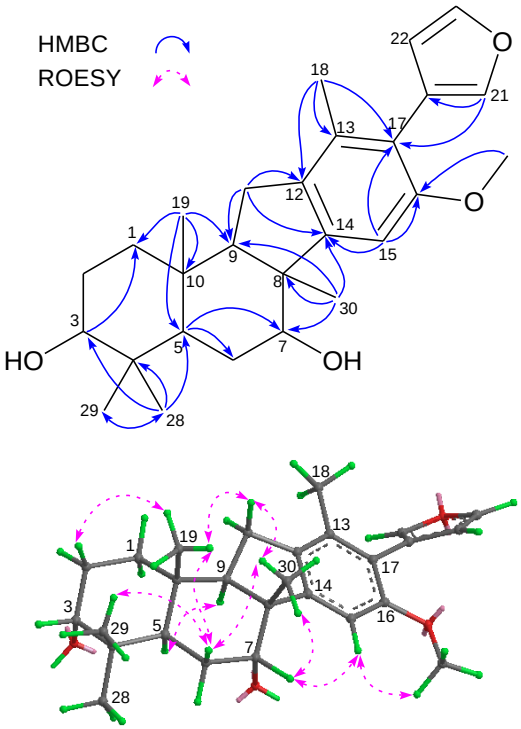

Fig. 4 Key 2D NMR correlations of walsucochinoid L (**10**).

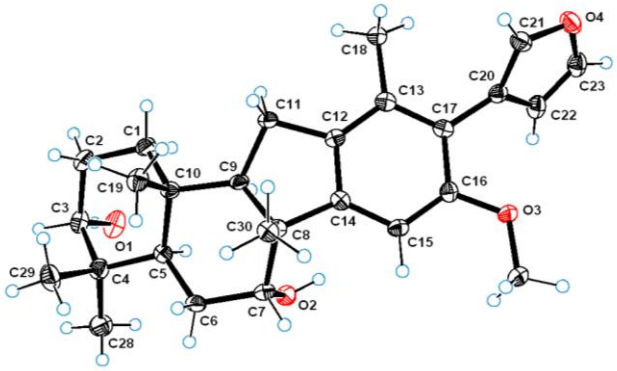

Fig. 5 X-ray structure of walsucochinoid L (**10**).

The molecular formulae of walsucochinoids M (**11**) and N (**12**) were determined to be $C_{27}H_{36}O_4$ and $C_{27}H_{34}O_4$ via the HRESI(+)MS ions at *m/z* 871.5129 and 867.4821 (both $[2M + Na]^+$) suggesting isomeric and didehydro analogues of **10**, respectively. The NMR data (Tables 2 and 3) of **11** exhibited excellent resemblances as those of **10** revealing only minor differences from C-1 to C-4, which suggested a reversed substitution mode at the C-3 stereocenter as supported by the coupling pattern of H-3 (dd, J = 9.5, 6.6 Hz) in **11** versus that (dd, J = 2.6, 2.6 Hz) in **10**. Analysis of the NMR data (Tables 2 and 3) of **12** established that it was only different from **11** in the presence of a 7-ketone functionality (δ_C 211.9) instead of the oxymethine (δ_H, 4.35; δ_C 71.0) in the latter, which was corroborated by the correlation from H_3-30 to a carbonyl signal in the HMBC spectrum of **12**. The relative configurations of **11** and **12** were characterized as depicted via comparisons with **10** and were confirmed by ROESY data. Therefore, limonoid **11** was identified to be the 3-epimer of **10** and **12** as the 7-oxo derivative of **11**.

Compounds 13–16 bearing a 6α,28-ether bridge

HRESI(+)MS analyses of walsucochinoids O (**13**) and P (**14**) revealed sodiated molecular ions at *m/z* 561.2831 and 547.2665 corresponding to molecular formulae of $C_{32}H_{42}O_7$ and $C_{31}H_{40}O_7$, and suggestive of methylated and isomeric congeners of walsuchochinoid A,[8] respectively. By comparing the NMR data (Table 4) of **13** with those of walsuchochinoid A,[8] it was evident that **13** displayed all the same structural features except for a 2-methylbutyryloxy residue (δ_C 175.1, 41.6, 26.8, 16.4, and 11.8) at C-3 replacing the isobutyryloxy group (δ_C 175.4, 34.2, 19.1, and 19.0) in the latter. The acquisition of HMBC and ROESY data (Fig. 6) furnished extra evidence for the establishment of the structure of **13** as drawn incorporating a 6α,28-ether bridge. In contrast to **13**, limonoid **14** showed highly comparable NMR data (Table 4) with the only difference being the appearance of an exchangeable phenol signal (δ_H 5.04) rather than resonances of the methoxy group (δ_H 3.77; δ_C 56.1) in the former. The relative configurations at all chiral centers in **14** were established to be identical with those of walsucochinoid A[8] as supported by the same proton coupling patterns, and this was confirmed by ROESY experiment. The structures of **13** and **14** were thus clearly characterized.

Table 4 NMR data ($CDCl_3$) for compounds **13**–**16**

No.	**13**		**14**		**15**		**16***	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	72.9	3.63[a] (m)	73.0	3.62[e] (m)	72.9	3.65[i] (m)	72.8	3.68 (brd, 8.3)
2α	30.2	2.02 (ddd, 16.0, 2.6, 2.3)	30.2	2.01 (ddd, 16.2, 2.9, 2.5)	30.2	2.05 (ddd, 16.1, 2.8, 2.2)	30.2	2.05[l] (ddd, 16.0, 2.6, 2.4),
2β		2.38[b] (ddd, 16.0, 3.6, 2.6)		2.38[f] (ddd, 16.2, 3.3, 2.9)		2.40[j] (ddd, 16.1, 3.4, 2.8)		2.42 (ddd, 16.0, 3.1, 2.6)
3	73.6	5.14 (dd, 2.6, 2.6)	73.6	5.14 (dd, 2.9, 2.9)	73.5	5.21 (dd, 2.8, 2.8)	73.7	5.20 (dd, 2.6, 2.6)
4	42.3		42.3		42.5		42.2	
5	40.5	2.52[c] (d, 12.2)	40.4	2.53[g] (d, 12.2)	40.6	2.57[k] (d, 12.2)	42.1	2.50[m] (d, 12.4)
6	75.9	4.36 (dd, 12.2, 2.8)	75.9	4.34 (dd, 12.2, 2.9)	75.9	4.36 (dd, 12.2, 2.7)	74.1	4.41 (dd, 12.4, 3.0)
7	70.1	4.54 (d, 2.8)	70.1	4.50 (d, 2.9)	70.1	4.54 (d, 2.7)	70.2	5.88 (d, 3.0)
8	53.7		53.6		53.8		53.3	
9	48.2	2.95 (dd, 12.2, 6.4)	48.1	2.93 (dd, 12.3, 6.3)	48.0	2.99 (dd, 12.2, 6.3)	49.5	2.90 (dd, 12.1, 6.3)
10	39.4		39.4		39.4		39.3	
11α	25.3	2.69 (dd, 13.8, 6.4)	25.1	2.66 (dd, 13.7, 6.3)	25.3	2.69 (dd, 13.8, 6.3)	25.2	2.64(dd, 13.8, 6.3)
11β		2.54[c] (dd, 13.8, 12.2)		2.50[g] (dd, 13.7, 12.3)		2.53[k] (dd, 13.8, 12.2)		2.52[m] (dd, 13.8, 12.1)
12	134.1		133.7		134.2		132.8	
13	135.2		134.8		135.2		134.3	
14	149.3		150.4		149.4		150.1	
15	101.7	6.66 (s)	105.4	6.70 (s)	101.8	6.67 (s)	105.4	6.51 (s)
16	156.8		152.6		156.9		152.5	
17	119.5		116.2		119.6		115.9	
18	17.6	2.13 (3H, s)	17.4	2.06 (3H, s)	17.6	2.13 (3H, s)	17.5	2.07[l](3H, s)
19	16.6	1.134[d] (3H, s)	16.6	1.129[h] (3H, s)	16.4	1.14 (3H, s)	16.1	1.14 (3H, s)
20	120.5		119.2		120.5		119.2	
21	141.1	7.36 (brs)	141.3	7.43 (brs)	141.2	7.36 (brs)	141.2	7.42 (brs)
22	113.1	6.41 (brd, 1.5)	112.6	6.39 (brs)	113.1	6.41 (brs)	112.6	6.38 (brs)
23	142.0	7.48 (dd, 1.5, 1.5)	144.3	7.59 (dd, 1.5, 1.5)	142.0	7.48 (brs)	144.1	7.58 (brs)
28α	78.2	3.57 (brd, 7.8)	78.2	3.56 (brd, 7.8)	78.4	3.57 (brd, 7.8)	78.2	3.42 (brd, 7.7)
28β		3.64[a] (d,7.8)	19.1	3.63[e] (d, 7.8)		3.64[i] (d, 7.8)		3.56 (d,7.7)
29	19.2	1.25 (3H, s)	22.9	1.24 (3H, s)	19.2	1.26 (3H, s)	18.8	1.23 (3H, s)
30	22.9	1.144[d] (3H, s)		1.121[h] (3H, s)	23.0	1.14 (3H, s)	23.2	1.19 (3H, s)
1-OH		2.50[c] (d, 9.5)		2.51[g] (d, 9.6)		2.44[j] (d, 9.1)		
7-OH		2.17 (s)		2.14 (s)		2.16 (s)		
16-OH				5.04 (s)				4.94 (s)
OMe	56.1	3.77 (3H, s)			56.2	3.77 (3H, s)		
3-OR[1]								
1′	175.1		175.2		166.7		166.5	
2′	41.6	2.34[b] (m)	41.6	2.34[f] (m)	128.1		128.2	
3′	26.8	1.45 (m), 1.64 (m)	26.8	1.45 (m), 1.64 (m)	138.8	6.78 (brq, 7.1)	138.4	6.79 (brq, 7.0)
4′	11.8	0.89 (3H, t, 7.4)	11.8	0.90 (3H, t, 7.4)	12.4	1.75 (3H, brd, 7.1)	12.4	1.76 (brd, 7.0)
5′	16.4	1.128[d] (3H, d, 6.8)	16.4	1.125[h] (3H, d, 6.8)	14.8	1.80 (3H, brs)	14.6	1.82 (3H, s)

[a–m] Overlapping signals.

* NMR data of 7-OR[2] in **16**: δ_C 175.9 (C-1″), 41.8 (C-2″), 26.7 (C-3″), 17.4 (C-5″), and 11.7 (C-4″); δ_H 2.25 (m, H-2″), 1.58 (m, H-3″a), 1.31 (m, H-3″b), 0.98 (d, *J* = 7.0, H-5″), and 0.77 (t, *J* = 7.0, H-4″).

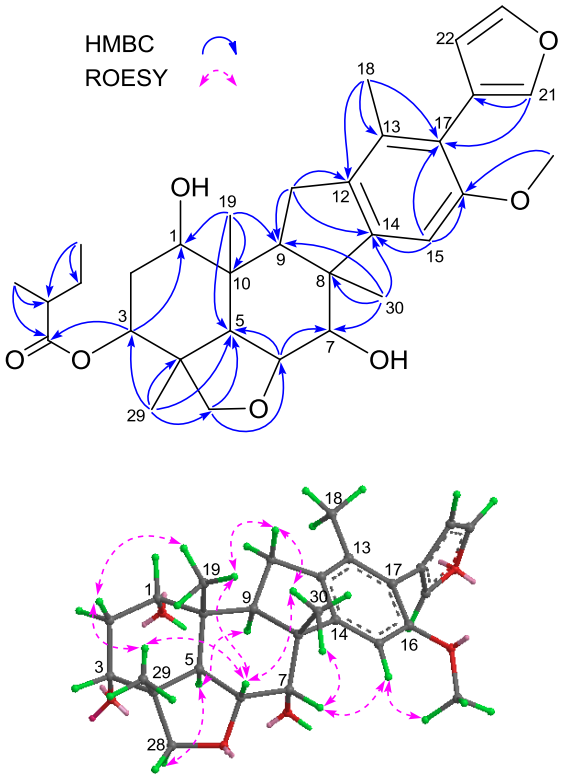

Fig. 6 Key 2D NMR correlations of walsucochinoid O (**13**) (Note: to avoid unclarity from atom overlapping, the 2-methylbutyryl group was simplified to "R" group in the 3D structure).

Walsucochinoids Q (**15**) and R (**16**) exhibited sodiated (559.2676) and protonated (607.3271) molecular ion peaks in their HRESI(+)MS spectra indicative of molecular formulae of $C_{32}H_{40}O_7$ and $C_{36}H_{46}O_8$, respectively. The NMR data (Table 4) of **15** were in agreement with a closely related homologue of **13** with the sole replacement of the 2-methylbutyryloxy moiety at C-3 in the latter by a tiglyloxy group in **15** as supported by the HMBC crosspeak from H-3 (δ_H 5.21) to the tiglyloxy carbonyl (δ_C 166.7). Compared to **15**, the NMR data (Table 4) of **16** revealed characteristic signals for an additional 2-methylbutyryloxy residue at C-7 (δ_C 70.2) apart from the presence of an aromatic hydroxyl (δ_H 4.94, 16-OH) instead of the methoxyl group in the former, which was further corroborated by the HMBC correlations from H-7 (δ_H 5.88) to the new ester carbonyl (δ_C 175.9) and from 16-OH to C-16 (δ_C 152.5), respectively. High resemblances between the remaining NMR data of **13** and **15**/**16** suggested common structural features and identical relative configurations for them, and these assignments were favored by ROESY data. Compounds **15** and **16** were hereby

identified as shown.

Compounds **1**–**16** were tested for their inhibition against human and mouse 11β-HSD1 activities using scintillation proximity assay (SPA).[11] While walsucochinoid D (**2**) showed selective inhibition against mouse 11β-HSD1 with an IC_{50} value of 13.4±1.7 μM, walsucochinoid F (**3**) only exhibited inhibitory effect on human 11β-HSD1 with an IC_{50} value of 8.25±0.69 μM.

Experimental

General experimental details

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. Melting points were measured on a SGM X-4 apparatus (Shanghai Precision & Scientific Instrument Co., Ltd.). UV data were acquired on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer using KBr disks. NMR experiments were preformed in $CDCl_3$ on a Bruker AM-400 spectrometer referenced to solvent peaks (δ_H 7.26; δ_C 77.16). ESIMS and HR-ESIMS analyses were carried out on Bruker Daltonics Esquire3000plus and Waters-Micromass Q-TOF Ultima Global mass spectrometers, respectively. Semi-preparative HPLC was performed on a Waters 1525 binary pump system equipped with a Waters 2489 detector (210 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.), C18 reversed-phase (RP-18) silica gel (150–200 mesh, Merck), CHP20P MCI gel (75–150 μm, Mitsubishi Chemical Industries, Ltd.), D101-macroporous absorption resin (Shanghai Hualing Resin Co., Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.) were used for TLC detection. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co., Ltd.), and solvents used for HPLC were of HPLC grade (J & K Scientific Ltd.).

Plant material

As previously reported.[9]

Extraction and isolation

The air-dried powder of leaves and twigs of *W. cochinchinensis* (11 kg) was extracted with 95%

EtOH at room temperature to give a crude extract (280 g) which was partitioned between H_2O and EtOAc. The EtOAc soluble partition (130 g) was fractionated on a column of macroporous resin eluted with 30%, 80% and 100% MeOH/H_2O. The 80% MeOH elution (90 g) was separated by a MCI gel column (MeOH/H_2O, 4:6 to 9:1) to return seven fractions (A–G), the fourth fraction (D, 20 g) of which was subjected to CC eluted with petroleum ether/acetone (100:1 to 1:2) to yield 14 subfractions (D1–D14). Fraction D9 was separated over a column of RP-18 silica gel (MeOH/H_2O, 5:5 to 9:1) to furnish five fractions (D9a–D9e), and the first fraction (D9a) was subjected to CC eluted with CH_3Cl/MeOH (300:1 to 60:1) to give five further fractions (D9a1–D9a5). Subfraction D9a2 was purified by semi-preparative HPLC (3.0 mL/min, 75% MeOH/H_2O isocratic elution) to return compounds **5** (28 mg), **7** (21 mg) and **16** (7 mg). D9b was purified by silica gel CC ($CHCl_3$/MeOH, 500:1 to 150:1) and HPLC to yield **12** (3 mg), **13** (19 mg), **15** (9 mg) and **14** (4 mg). Fraction D8 was sequentially fractionated by RP-18 silica gel (MeOH/H_2O, 5:5 to 4:1) and silica gel (petroleum ether/$CHCl_3$, 5:1 to 1:4) CC, and was finally purified by semi-preparative HPLC to afford **1** (12 mg), **2** (15 mg), **3** (49 mg), **4** (33 mg) and **8** (4 mg). Fraction D10 was extensively separated by columns of RP-18 silica gel (MeOH/H_2O, 5:5 to 4:1) and silica gel $CHCl_3$/MeOH (500:1 to 100:1), and was finally purified by HPLC to give **10** (100 mg), **6** (15 mg), **11** (12 mg) and **9** (16 mg).

Characterization of new compounds

Walsucochinoid C (1). Colorless crystals; mp 255–257 ℃; $[\alpha]_D^{20}$ 27.3 (*c* 0.11 in MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.56), 288 (3.62) nm; IR (KBr disk) ν_{max} 3433, 2964, 2927, 1655, 1610, 1589, 1504, 1464, 1427, 1381, 1319, 1213, 1153, 1092, 1041, 970, 872 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 421.2 $[M + H]^+$, 863.6 $[2M + Na]^+$; ESI(–)MS *m/z* 465.5 $[M + HCO_2]^-$; HRESI(–)MS *m/z* 465.2282 $[M + HCO_2]^-$ ($C_{28}H_{33}O_6$, calcd 465.2277).

Walsucochinoid D (2). White powder; $[\alpha]_D^{20}$ –41.7 (*c* 0.18 in $CHCl_3$); UV (MeOH) λ_{max} (log ε) 289 (3.36) nm; IR (KBr disk) ν_{max} 2966, 2937, 1714, 1674, 1577, 1460, 1421, 1371, 1325, 1269, 1242, 1155, 1088, 1063, 872, 820 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 419.3 $[M + H]^+$, 859.5 $[2M + Na]^+$; HRESI(+)MS *m/z* 859.4182 $[2M + Na]^+$ ($C_{54}H_{60}O_8Na$, calcd 859.4186).

Walsucochinoid E (3). White powder; $[\alpha]_D^{20}$ 35.9 (*c* 0.145 in MeOH); UV (MeOH) λ_{max} (log ε)

205 (4.61), 287 (3.40) nm; IR (KBr disk) ν_{max} 2972, 2939, 1734, 1670, 1662, 1589, 1464, 1425, 1375, 1317, 1246, 1211, 1161, 1090, 1032, 872 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 463.3 $[M + H]^+$, 485.3 $[M + Na]^+$; HRESI(+)MS *m/z* 947.4720 $[2M + Na]^+$ ($C_{58}H_{68}O_{10}Na$, calcd 947.4710).

Walsucochinoid F (4). White powder; $[\alpha]_D^{20}$ 30.3 (*c* 0.195 in MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.50), 290 (3.25) nm; IR (KBr disk) ν_{max} 3464, 2976, 1718, 1662, 1504, 1454, 1441, 1379, 1317, 1261, 1174, 1034, 872 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 449.2 $[M + H]^+$, 471.2 $[M + Na]^+$; ESI(–)MS *m/z* 447.4 $[M - H]^-$; HRESI(–)MS *m/z* 493.2229 $[M + HCO_2]^-$ ($C_{29}H_{33}O_7$, calcd 493.2226).

Walsucochinoid G (5). White powder; $[\alpha]_D^{20}$ 124.0 (*c* 0.10 in MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.42), 288 (3.71) nm; IR (KBr disk) ν_{max} 3450, 2968, 2937, 1734, 1670, 1604, 1593, 1506, 1464, 1427, 1383, 1323, 1240, 1161, 1095, 1034, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 479.3 $[M + H]^+$, 501.2 $[M + Na]^+$. 979.4 $[2M + Na]^+$; ESI(–)MS *m/z* 523.4 $[M + HCO_2]^-$; HRESI(–)MS *m/z* 523.2337 $[M + HCO_2]^-$ ($C_{30}H_{35}O_8$, calcd 523.2332).

Walsucochinoid H (6). White powder; $[\alpha]_D^{20}$ 64.8 (*c* 0.105 in MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.86), 288 (3.37) nm; IR (KBr disk) ν_{max} 3440, 2968, 2933, 1732, 1676, 1591, 1504, 1448, 1379, 1319, 1248, 1174, 1159, 1093, 1036, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 1 and 2; ESI(+)MS *m/z* 487.3 $[M + Na]^+$, 951.6 $[2M + Na]^+$; ESI(–)MS *m/z* 463.3 $[M - H]^-$; HRESI(–)MS *m/z* 509.2181 $[M + HCO_2]^-$ ($C_{29}H_{33}O_8$, calcd 509.2175).

Walsucochinoid I (7). White powder; $[\alpha]_D^{20}$ 69.2 (*c* 0.12 in MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.71), 287 (3.69) nm; IR (KBr disk) ν_{max} 3450, 2937, 1734, 1705, 1604, 1591, 1462, 1379, 1238, 1092, 1032, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 503.3 $[M + Na]^+$; ESI(–)MS *m/z* 525.5 $[M + HCO_2]^-$; HRESI(–)MS *m/z* 525.2497 $[M + HCO_2]^-$ ($C_{30}H_{37}O_8$, calcd 525.2488).

Walsucochinoid J (8). White powder; $[\alpha]_D^{20}$ 46.7 (*c* 0.03 in MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.25), 285 (3.14) nm; IR (KBr disk) ν_{max} 3440, 2931, 1739, 1720, 1707, 1604, 1462, 1427, 1383, 1311, 1242, 1095, 1030, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 481.4 $[M + H]^+$, 983.7 $[2M + Na]^+$; ESI(–)MS *m/z* 525.5 $[M + HCO_2]^-$; HRESI(–)MS *m/z* 525.2496 $[M + HCO_2]^-$ ($C_{30}H_{37}O_8$, calcd 525.2488).

Walsucochinoid K (9). White powder; $[\alpha]_D^{20}$ 92.7 (*c* 0.11 in MeOH); UV (MeOH) λ_{max} (log ε)

204 (4.66), 288 (3.59) nm; IR (KBr disk) ν_{max} 3444, 2964, 2933, 1730, 1699, 1506, 1460, 1381, 1317, 1250, 1173, 1034, 874 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 489.3 $[M + Na]^+$, 955.5 $[2M + Na]^+$; ESI(–)MS *m/z* 465.2 $[M - H]^-$; HRESI(–)MS *m/z* 511.2347 $[M + HCO_2]^-$ ($C_{29}H_{35}O_8$, calcd 511.2332).

Walsucochinoid L (10). Colorless crystals; mp 259–261 ℃; $[\alpha]_D^{20}$ –67.5 (*c* 0.20 in MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.43), 287 (3.50) nm; IR (KBr disk) ν_{max} 3614, 3537, 3481, 2931, 2873, 1591, 1506, 1456, 1423, 1383, 1304, 1211, 1153, 1090, 1032, 972, 872 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 425.3 $[M + H]^+$, 871.7 $[2M + Na]^+$; ESI(–)MS *m/z* 469.5 $[M + HCO_2]^-$, 847.6 $[2M - H]^-$; HRESI(–)MS *m/z* 469.2599 $[M + HCO_2]^-$ ($C_{28}H_{37}O_6$, calcd 469.2590).

Walsucochinoid M (11). White powder; $[\alpha]_D^{20}$ –41.0 (*c* 0.105 in MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.18), 287 (3.11) nm; IR (KBr disk) ν_{max} 3438, 2933, 2870, 1603, 1589, 1506, 1462, 1423, 1381, 1315, 1213, 1157, 1090, 1024, 874, 793 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 425.3 $[M + H]^+$, 871.7 $[2M + Na]^+$; HRESI(+)MS *m/z* 871.5129 $[2M + Na]^+$ ($C_{54}H_{72}O_8Na$, calcd 871.5125).

Walsucochinoid N (12). White powder; $[\alpha]_D^{20}$ –21.1(*c* 0.09 in MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.17), 287 (3.03) nm; IR (KBr disk) ν_{max} 3527, 3415, 2935, 2850, 1712, 1591, 1506, 1462, 1423, 1259, 1157, 1093, 1030, 874 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Tables 2 and 3; ESI(+)MS *m/z* 423.3 $[M + H]^+$, 867.6 $[2M + Na]^+$; HRESI(+)MS *m/z* 867.4821 $[2M + Na]^+$ ($C_{54}H_{68}O_8Na$, calcd 867.4812).

Walsucochinoid O (13). White powder; $[\alpha]_D^{20}$ –18.7 (*c* 0.075 in MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.68), 289 (3.56) nm; IR (KBr disk) ν_{max} 3429, 2964, 2920, 2850, 1734, 1649, 1608, 1540, 1506, 1462, 1435, 1385, 1263, 1155, 1076, 1036, 945, 874 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Table 4; ESI(+)MS *m/z* 539.4 [M + H], 561.3 $[M + Na]^+$, 1099.6 $[2M + Na]^+$; ESI(–)MS *m/z* 583.6 $[M + HCO_2]^-$; HRESI(+)MS *m/z* 561.2831 $[M + Na]^+$ ($C_{32}H_{42}O_7Na$, calcd 561.2828).

Walsucochinoid P (14). White powder; $[\alpha]_D^{20}$ –32.0 (*c* 0.05 in MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.79), 288 (3.73) nm; IR (KBr disk) ν_{max} 3435, 2966, 2935, 1730, 1618, 1506, 1460, 1385, 1313, 1244, 1159, 1036, 943, 874 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) Table 4; ESI(+)MS *m/z* 525.4 $[M + H]^+$, 1071.8 $[2M + Na]^+$; ESI(–)MS *m/z* 523.6 $[M - H]^-$, 1047.9 $[2M - H]^-$; HRESI(+)MS *m/z* 547.2665 $[M + Na]^+$ ($C_{31}H_{40}O_7Na$, calcd 547.2672).

Walsucochinoid Q (15). White powder; $[\alpha]_D^{20}$ 2.0 (*c* 0.05 in MeOH); UV (MeOH) λ_{max} (log ε)

204 (4.55), 286 (3.43) nm; IR (KBr disk) ν_{max} 3431, 2931, 1703, 1651, 1606, 1591, 1464, 1389, 1313, 1261, 1157, 1084, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Table 4; ESI(+)MS *m/z* 537.3 [M + H], 559.4 $[M + Na]^+$, 1095.6 $[2M + Na]^+$; ESI(–)MS *m/z* 581.7 $[M + HCO_2]^-$; HRESI(+)MS *m/z* 559.2676 $[M + Na]^+$ ($C_{32}H_{40}O_7Na$, calcd 559.2672).

Walsucochinoid R (16). White powder; $[\alpha]_D^{20}$ –26.1 (*c* 0.115 in MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.64), 288 (3.58) nm; IR (KBr disk) ν_{max} 3435, 2966, 2933, 2895, 1732, 1712, 1649, 1506, 1435, 1385, 1263, 1155, 1074, 1034, 874 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) Table 4; ESI(+)MS *m/z* 607.5 [M + H], 629.4 $[M + Na]^+$, 1235.8 $[2M + Na]^+$; HRESI(+)MS *m/z* 607.3271 $[M + H]^+$ ($C_{36}H_{47}O_8$, calcd 607.3271).

X-ray diffraction analysis

Walsucochinoids C (**1**) and L (**10**) were crystallized from MeOH/H_2O (50:1 and 100:1, respectively) at room temperature. The X-ray crystallographic data were obtained on a Bruker APEX-Ⅱ CCD detector employing graphite monochromated Cu-Kα radiation (λ = 1.54178 Å) at 132(2) K, and operated in the ϕ-ω scan mode. The structures were solved by direct method using SHELXS-97 (Sheldrick 2008) and refined with full-matrix least-squares calculations on F^2 using SHELXL-97 (Sheldrick 2008). All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

Crystallographic data for **1** and **10** (key parameters see Tables S1 and S2 in ESI†) have been deposited at the Cambridge Crystallographic Data Centre (Deposition Nos.: CCDC 875034 and 875035, respectively). Copies of these data can be obtained free of charge via the internet at www.ccdc.cam.ac.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44 1223-336-033; or email: deposit@ccdc.cam.ac.uk].

Bioassays

As previously reported,[9] glycyrrhetinic acid (97%, G109797, Aladdin) was used as positive control with IC_{50} values of 7.07±0.98 and 6.09±0.12 μM against mouse and human 11β-HSD1, respectively.

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