Acylated Iridoid Glycosides from the Flowers of *Verbascum lasianthum* Boiss. ex Bentham

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Two new (**1** – **2**) and five known iridoid glycosides (**3** – **7**) as well as a known saponin (**8**) were isolated from the flowers of *Verbascum lasianthum* and their structures were determined as 6-*O*-(4′′-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylaucubin (**1**), 6-*O*-(4′′-*O*-*trans*-*p*-methoxycinnamoyl)-α-L-rhamnopyranosylaucubin (**2**), sinuatol (**3**), aucubin (**4**), geniposidic acid (**5**), catalpol (**6**), ajugol (**7**) and ilwensisaponin A (**8**) on the basis of 1D and 2D NMR spectral analysis.

Key words: *Verbascum lasianthum*, Scrophulariaceae, Acylated Iridoid Glycosides, Lasianthoside I, Lasianthoside II

Introduction

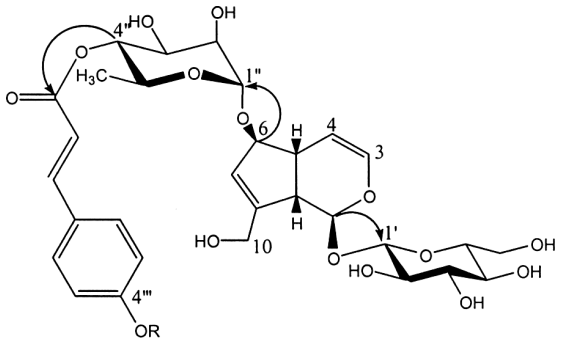
Verbascum, commonly known as "Mullein", is a biennial plant widely spread in the family Scrophulariaceae [1]. *Verbascum* flowers are boiled in milk and is applied externally for pruritic conditions in urogenital organs and boiled in cauldron for anal fistula then anus is exposed to vapors in Turkish traditional medicine [2]. During our field expeditions on Turkish folk medicine, we have recorded that the flowers of *Verbascum lasianthum* Boiss. ex Bentham is used for hemorrhoids in southwest Anatolia [3].

Verbascum species have been known to be rich in iridoid glycosides which is well known for its variety of iridoids being of value for taxonomic evaluation of this genus [4].

In a connection with a study on the constituents of the genus *Verbascum*, we investigated a number of *Verbascum* species. In previous papers, we described the isolation of nine iridoid glycosides and two phenylethanoid glycosides from the roots of *V. lasianthum* [5, 6]. In a continuation of the studies on *Verbascum lasianthum*, we report here the isolation and structure elucidation of two new acylated iridoid glycosides (**1** – **2**), five known iridoid glycosides (**3** – **7**) and a known saponin (**8**) from the flowers of the titled plant.

Results and Discussion

The methanolic extract of *V. lasianthum* was suspended in water and partitioned with $CHCl_3$. Removing the chloroform layer, the aqueous layer was fractionated over polyamide VLC followed by C_{18}-VLC, Si gel CC and reverse phase (C8)-HPLC to yield two new (**1**-**2**) and five known iridoid glycosides (**3** – **7**) in addition to a known saponin (**8**) (see Fig.). The NMR data of compounds **3** – **8** were in excellent agreement with those reported for sinuatol (**3**), aucubin (**4**), geniposidic acid (**5**), catalpol (**6**), ajugol (**7**) and ilwensisaponin A (**8**) [5, 7 – 10].



1: R=H; **2**: R=CH_3

: Selected HMBC correlations

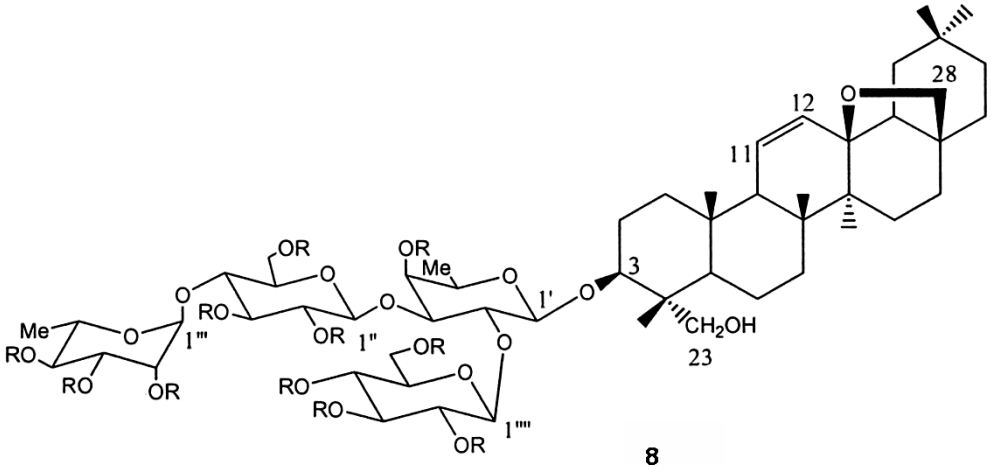
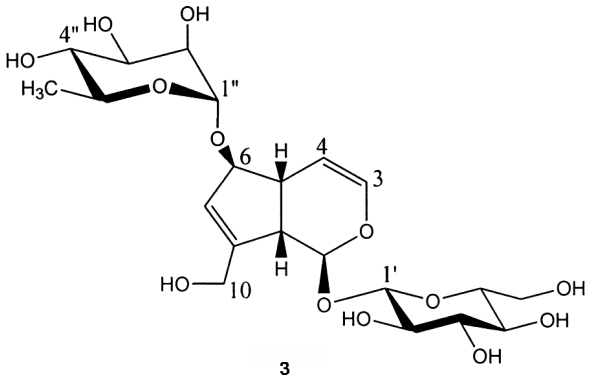
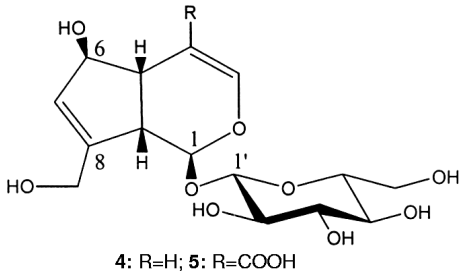
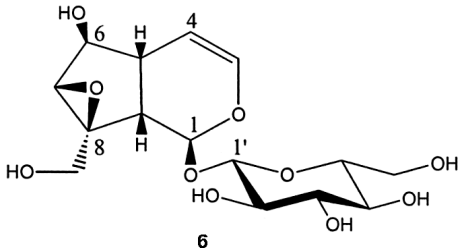
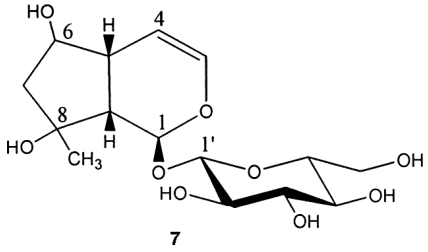


Fig. Isolated compounds from *V. lasianthum* (**1** – **8**).









Compound **1** was obtained as an amorpous powder, $[\alpha]_D^{20} - 93.3°$ (MeOH; *c* 0.1). The molecular formula of **1** was determined to be $C_{30}H_{38}O_{15}$ due to the positive-ion high resolution-ESIMS molecular ion peak at *m/z* 661.2041 $[M+Na]^+$ (calcd. 661.2047) together with 1D and 2D NMR data (see Table). The UV spectra of **1** exhibited maxima at 205, 316 nm, suggesting the presence of an iridoid enol-ether system and an aromatic acid moiety. Similarly, its IR spectra absorption bands were typical for a hydroxyl group (3430 cm^{-1}), a conjugated ester carbonyl (1708 cm^{-1}), a double bond (1645 cm^{-1}) and an aromatic ring (1604, 1546, 1360 cm^{-1}). ^1H and ^{13}C NMR data of **1** were in accordance with the presence of a C-4 non-substituted iridoid skeleton. The ^{13}C NMR and DEPT-135 spectra (see Table) showed 30 resonances, including nine for a 4-non-substituted iridoid aglycone moiety, twelve for two sugar units and the remaining nine for an aromatic acid moiety. The complete assignment of proton and carbon resonances were based on the ^1H-^1H COSY, ^{13}C-^1H COSY (see Table) and DEPT experiments. NMR data of compound **1** showed signals very similar to those of 6-*O*-α-L-rhamnopyranosylaucubin (= sinuatol) (see Table) [5, 7] with ad-

Table 1. ^{13}C and ^{1}H NMR spectral data (DMSO-d_6) and HMBC of **1** and **2** with model compound.

		1			**2**			Sinuatol [7]	
Position	Type	δ_C [ppm] (100 MHz)	δ_H [ppm] (400 MHz)	J [Hz]	δ_C [ppm] (150 MHz)	δ_H [ppm] (600 MHz)	J [Hz]	δ_C [ppm] (20 MHz)	HMBC
1	CH	96.0	4.86 *d*	6.0	96.2	4.87 *d*	6.0	96.7	C-3, C-1′
3	CH	141.1	6.30 *d*	4.8	141.3	6.33[a]		140.8	C-1, C-5
4	CH	104.7	5.04 *d*	5.6	104.9	5.01 *d*	5.4	105.8	C-9
5	CH	42.8	2.68 *m*		43.0	2.70 *m*		41.6	
6	CH	87.5	4.34 *m*		87.6	4.35 *m*		88.3	C-1″
7	CH	125.7	5.78 *br s*		125.3	5.76 *br s*		126.9	C-5, C-9
8	C	149.4			149.6			149.2	
9	CH	47.3	2.74 *d*	7.2	47.4	2.75 *m*		47.4	
10	CH_2	60.0	4.15 *dd*	6.0, 14.4	60.2	4.15 *d*	16.8	60.4	C-7, C-8
			3.97 *dd*	6.8, 14.4		3.96 *dd*	4.8, 16.2		
β-D-Glucose									
1′	CH	98.6	4.50 *d*	8.0	98.7	4.48 *d*	8.4	99.3	C-1
2′	CH	74.0	3.00[a]		74.1	2.96 *t*	8.4	73.6	C-3′, C-4′
3′	CH	77.2	3.16[a]		77.3	3.15[a]		76.5	C-4′
4′	CH	70.6	3.13[a]		70.8	3.12[a]		70.4	C-5′
5′	CH	77.7	3.02[a]		77.9	3.02[a]		77.0	C-4′, C-6′
6′	CH_2	61.5	3.65[a]		61.7	3.66 *dd*	6.0, 12.0	61.5	C-5′
			3.44 *d*	12.0		3.40 *dd*	6.0, 11.4		
α-L-Rhamnose									
1″	CH	100.1	4.95 *br s*		100.3	4.91 *br s*		100.4	
2″	CH	71.4	3.65 – 3.76[a]		71.5	3.67 – 3.74[a]		71.2	
3″	CH	68.8	3.65 – 3.76[a]		68.9	3.67 – 3.74[a]		71.0	
4″	CH	74.3	5.01 *t*	9.6	74.5	4.99 *t*	9.6	72.9	C=O
5″	CH	67.1	3.75[a]		67.2	3.74[a]		69.8	C-6″
6″	CH_3	18.0	1.06 *d*	6.4	18.3	1.05 *d*	6.0	17.4	C-5″
Acyl moiety									
1‴	C	125.6			125.8				
2‴	CH	130.7	7.64 *d*	8.4	130.8	7.65 *d*	8.4		C-4‴, C-6‴, C-α, C-β
3‴	CH	116.3	6.89 *d*	8.4	116.2	6.90 *d*	8.4		C-1‴, C-5‴
4‴	C	160.4			161.8				
5‴	CH	116.3	6.89 *d*	8.4	116.2	6.90 *d*	8.4		C-1‴, C-3‴
6‴	CH	130.7	7.64 *d*	8.4	130.8	7.65 *d*	8.4		C-2‴, C-4‴, C-α, C-β
α	CH	115.4	6.45 *d*	16.0	115.1	6.45 *d*	15.6		C-1‴, C=O
β	CH	145.3	7.60 *d*	16.0	145.0	7.60 *d*	16.2		C-2‴, C-6‴, C-α, C=O
C=O	C	166.8			166.8				
OCH_3	CH_3				56.0	3.76 *s*			C-4‴

[a] Signal patterns are unclear due to overlapping.

ditional signals arising from an aromatic acid moiety. The signals of two *trans* olefinic protons (δ_H = 6.45 and 7.60, d, J = 16.0 Hz), as well as two pairs of *ortho*-coupled aromatic protons (δ_H = 6.89 and 7.64, d, J = 8.4 Hz) in the ^{1}H NMR spectrum, showed clearly that the acyl moiety was *trans-p*-coumaroyl [11]. ^{13}C NMR and DEPT-135 spectra of **1** confirmed the presence of the *trans-p*-coumaric acid. The site of esterification was determined to be C-4″ position of the rhamnopyranosyl moiety based on the chemical shift (downfield) of C-4″ ($\Delta\delta = +1.4$, δ_C = 74.3 in **1**; δ_C = 72.9 in sinuatol) and the upfield shifts of the C-3″ ($\Delta\delta = -2.2$, δ_C = 68.8 in **1**; δ_C = 71.0 in sinuatol) and the C-5″ ($\Delta\delta = -2.7$, δ_C = 67.1 in **1**; δ_C = 69.8 in sinuatol) signals (see Table). This assumption was also supported by the HMBC correlation observed between δ_C = 166.8 (carbonyl carbon of the *trans-p*-coumaroyl group) and δ_H = 5.01 (H-4″ of rhamnose) (see Table). Consequently, compound **1** was established as 6-*O*-(4″-*O*-*trans-p*-coumaroyl)-α-L-rhamnopyranosylaucubin which was isolated for the first time from nature. We propose the trivial name lasianthoside I for this compound.

Compound **2** was also isolated as an amorphous powder, $[\alpha]_D^{20}$ − 91.9° (MeOH; *c* 0.1), with the molecular formula $C_{31}H_{40}O_{15}$ as determined by the positive-ion HR-ESIMS molecular ion peak at *m/z* 687.2069 $[M+Cl]^-$ (calcd. 687.2061), together with 1D and 2D NMR data. Its UV spectrum suggested

the presence of an iridoid enol ether system (206 nm) and an aromatic acyl moiety (316 nm). Moreover, the IR absorptions [3465 (OH), 1710 (ester C=O), 1645 (C=C-O), 1604, 1545, 1360 (aromatic ring) cm^{-1}] were in accordance with the presence of a C-4 non-substituted iridoid skeleton. The ^1H and ^{13}C NMR spectra of compound **2** closely resembled those of **1** (see Table). The only difference from compound **1** was the presence of the signal due to a methoxy group ($\delta_C = 56.0$; $\delta_H = 3.76$, s). The presence of two *trans* olefinic protons ($\delta_H = 6.45$, $J = 15.6$ Hz and 7.60, $J = 16.2$ Hz), two pairs of *ortho* coupled aromatic protons ($\delta_H = 6.90$ and 7.65, $J = 8.4$ Hz) and one aromatic methoxy group ($\delta_H = 3.76$) in the ^1H NMR spectrum, showed clearly that the acyl moiety of **2** was the *trans-p*-methoxycinnamoyl group. The site of esterification was determined to be C-4″-position of the rhamnopyranosyl moiety by the same way as in compound **1**. In conclusion, the structure of the new compound **2** was determined as 6-*O*-(4″-*O*-*trans-p*-methoxycinnamoyl)-α-L-rhamnopyranosylaucubin and the trivial name lasianthoside II was proposed.

Conclusion

Iridoids are found as natural constituents in a large number of *Verbascum* species. C-4 nonsubstituted-bicyclic H-5/H-9 β,β-*cis*-fused cyclopentanopyran ring system is the most common structural feature of these substances such as aucubin, catalpol and catalpol esters of caffeic acids, while 4-substitute iridoids, such as geniposidic acid, have been found in a limited number [4]. To our knowledge, geniposidic acid (**5**) has been isolated from *Verbascum* species for the second time. This compound has only been reported from *V. olympicum* [8], earlier.

To date, several acylated 6-*O*-α-L-rhamnopyranosylcatalpol derivatives have been reported from *Verbascum* species [4]. Our investigations on *V. lasianthum* demonstrated that rhamnopyranosylcatalpol esters are the main iridoid constituents of the roots of this species, while rhamnopyranosylaucubin esters are found in a few [5]. However, the isolation of the 6-*O*-α-L-rhamnopyranosylaucubin derivatives from the flowers of *V. lasianthum* was recorded for the first time in this study. The significance of acyl rhamnopyranosyl aucubin derivatives might be of assistance in clarifying the chemotaxonomical classification of the genus *Verbascum*.

Experimental Section

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp operating at 589 nm. The UV spectra (λ_{max}) were recorded on a Hitachi HP 8452 A spectrophotometer. The IR spectra (ν_{max}) was determined on ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectra were taken on a Bruker 400 and 600 NMR spectrometers in DMSO-d_6. ^1H and ^{13}C NMR spectra were measured and reported in ppm by using the residual solvent peak as an internal standard. Multiplicities of ^{13}C spectra were asigned by DEPT experiments. HR-ESIMS FT data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Polyamide (ICN) and reverse-phase material (C-18, sepralyte 40 μm) were used for vacuum liquid chromatography (VLC). HPLC separations were carried out on a Waters 510 model system. Column used for HPLC was reversed phase silica (Phenomenex C8 (2), 21.5 × 250 mm, 5 μm; flow rate, 6 ml/min; dedector wavelength, 237 nm). Si gel (230 – 400 mesh) (Merck) was used for column chromatography (CC). Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for TLC with with developing solvent system, $CHCl_3$-MeOH-H_2O (61 : 32 : 7). Plates were examined by UV fluorescence and sprayed with 1% vanillin in conc. H_2SO_4, followed by heating at 105 °C for 1 – 2 min.

Plant material

Verbascum lasianthum Boiss. ex Bentham was collected from Ucahirlar, Urla, Izmir (W. Anatolia), Turkey in August, 1999. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 99139).

Extraction and isolation

The air-dried and powdered flowers of *Verbascum lasianthum* (697.0 g) were extracted twice with MeOH (2 × 3000 ml) at 40 °C for 12 h. After evaporation of the combined extract *in vacuo*, 111.1 g MeOH extract was obtained. The crude extract was dissolved in water and partitioned in $CHCl_3$. The lyophilized H_2O phase (75.9 g) was fractionated over polyamide column (VLC, 250 g), eluting with H_2O, followed by increasing concentrations of MeOH to afford five main fractions (Frs. A – E). Fraction A (32.7 g) was fractionated over LiChroprep C_{18} (VLC, 150 g) using gradient H_2O-MeOH mixtures (0 – 100% MeOH) to yield compound **8** (261.3 mg) and additional seven fractions A_{2-8}. Purification of fr. A_2 (13.4 g) by silica gel column chromatography [140 g, $CHCl_3$ → $CHCl_3$-MeOH (95 : 5) → $CHCl_3$-MeOH-H_2O (70 : 30 : 3)] furnished seven fractions (A_{2a-g}). Fraction A_{2g} (7.0 g) rechromatographed on LiChroprep C_{18} (VLC, 150 g) using H_2O and MeOH-

H_2O gradients (0 – 20% MeOH) to yield compound **4** (193.8 mg), compound **5** (21.8 mg), compound **6** (30.4 mg), compound **8** (599.8 mg) and additional two fractions A_{2gI-II}. Fraction A_{2gI} (53.6 mg) was subjected to silica gel column [8 g, $CHCl_3$-MeOH (85 : 15)] to give compound **7** (9.9 mg) and compound **3** (2.8 mg). Fraction A_{2gII} (2.1 g) was likewise applied to silica gel column [25 g, $CHCl_3$-MeOH (85 : 15)] to yield compound **1** (42.0 mg) and fraction A_{2gIIa}. Fraction A_{2gIIa} (30.7 mg) was selected for further purification using HPLC [Phenomenex C8 (2), AcN-H_2O (0 – 100% AcN) over 45 min] to afford compound **2** (2.2 mg).

*6-O-(4′′-O-trans-p-coumaroyl)-α-*L*-rhamnopyranosyl-aucubin (**1**):* Amorphous powder, $[\alpha]_D^{20}$ – 93.3° (*c* 0.1, MeOH). UV/vis (CH_3OH): λ_{max}: 205, 316 nm. – IR (film): $\tilde{\nu}$ = 3430 (OH), 1708 (C=O), 1645 (C=C-O), 1604, 1546, 1360 cm^{-1}. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.06 (d, J = 6.4 Hz, 3H, 6′′-H), 2.68 (m, 1H, 5-H), 2.74 (d, J = 7.2 Hz, 1H, 9-H), 3.00 – 3.65 (br, 6H, glucose protons), 3.65 – 3.76 (br, 3H, rhamnose protons), 3.97 (dd, J = 6.8/14.4 Hz, 1H, 10b-H), 4.15 (dd, J = 6.0/14.4 Hz, 1H, 10a-H), 4.34 (m, 1H, 6-H), 4.50 (d, J = 8.0 Hz, 1H, 1′-H), 4.86 (d, J = 6.0 Hz, 1H, 1-H), 4.95 (br s, 1H, 1′′-H), 5.01 (t, J = 9.6 Hz, 1H, 4′′-H), 5.04 (d, J = 5.6 Hz, 1H, 4-H), 5.78 (br s, 1H, 7-H), 6.30 (d, J = 4.8 Hz, 1H, 3-H), 6.45 (d, J = 16.0 Hz, 1H, α-H), 6.89 (d, J = 8.4 Hz, 2H, 3′′′/5′′′-H), 7.60 (d, J = 16.0 Hz, 1H, β-H), 7.64 (d, J = 8.4 Hz, 2H, 2′′′/6′′′-H). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 18.0 (C-6′′), 42.8 (C-5), 47.3 (C-9), 60.0 (C-10), 61.5 – 77.7 (C-2′-6′), 67.1 (C-5′′), 68.8 (C-3′′), 71.4 (C-2′′), 74.3 (C-4′′), 87.5 (C-6), 96.0 (C-1), 98.6 (C-1′), 100.1 (C-1′′), 104.7 (C-4), 115.4 (C-α), 116.3 (C-3′′′/5′′′), 125.6 (C-1′′′), 125.7 (C-7), 130.7 (C-2′′′/6′′′), 141.1 (C-3), 145.3 (C-β), 149.4 (C-8), 160.4 (C-4′′′), 166.8 (C=O). HMBC: Table. HRESIFTMS *m/z*: 661.2041 $[M+Na]^+$ (calcd. *m/z* 661.2047 for $C_{30}H_{38}O_{15}Na$).

*6-O-(4′′-O-trans-p-methoxy-cinnamoyl)-α-*L*-rhamno-pyranosylaucubin (**2**):* Amorphous powder, $[\alpha]_D^{20}$ – 91.9° (*c* 0.1, MeOH). UV/vis (CH_3OH): λ_{max}: 206, 316 nm. – IR (film): $\tilde{\nu}$ = 3465 (OH), 1710 (C=O), 1645 (C=C-O), 1604, 1545, 1360 cm^{-1}. – ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.05 (d, J = 6.0 Hz, 3H, 6′′-H), 2.70 (m, 1H, 5-H), 2.75 (m, 1H, 9-H), 2.96 – 3.66 (br, 6H, glucose protons), 3.67 – 3.74 (br, 3H, rhamnose protons), 3.76 (s, 3H, Ar-OCH_3), 3.96 (dd, J = 4.8/16.2 Hz, 1H, 10b-H), 4.15 (d, J = 16.8 Hz, 1H, 10a-H), 4.35 (m, 1H, 6-H), 4.48 (d, J = 8.4 Hz, 1H, 1′-H), 4.87 (d, J = 6.0 Hz, 1H, 1-H), 4.91 (br s, 1H, 1′′-H), 4.99 (t, J = 9.6 Hz, 1H, 4′′-H), 5.01 (d, J = 5.4 Hz, 1H, 4-H), 5.76 (br s, 1H, 7-H), 6.33 (overlapped, 1H, 3-H), 6.45 (d, J = 15.6 Hz, 1H, α-H), 6.90 (d, J = 8.4 Hz, 2H, 3′′′/5′′′-H), 7.60 (d, J = 16.2 Hz, 1H, β-H), 7.65 (d, J = 8.4 Hz, 2H, 2′′′/6′′′-H). – ^{13}C NMR (150 MHz, DMSO-d_6): δ = 18.3 (C-6′′), 43.0 (C-5), 47.4 (C-9), 56.0 (OCH_3), 60.2 (C-10), 61.7 – 77.9 (C-2′-6′), 67.2 (C-5′′), 68.9 (C-3′′), 71.5 (C-2′′), 74.5 (C-4′′), 87.6 (C-6), 96.2 (C-1), 98.7 (C-1′), 100.3 (C-1′′), 104.9 (C-4), 115.1 (C-α), 116.2 (C-3′′′/5′′′), 125.3 (C-7), 125.8 (C-1′′′), 130.8 (C-2′′′/6′′′), 141.3 (C-3), 145.0 (C-β), 149.6 (C-8), 161.8 (C-4′′′), 166.8 (C=O). HMBC: Table. HRESIFTMS *m/z*: 687.2069 $[M+Cl]^-$ (calcd. *m/z* 687.2061 for $C_{31}H_{40}O_{15}Cl$).

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