SUPPORTING INFORMATION

Rare *Streptomyces* sp. polyketides as modulators of K-Ras localization

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General experimental details

Fine chemicals were purchased from Merck or Sigma Aldrich unless otherwise specified. Analytical Grade solvent was used for solvent extractions and solid phase extractions (SPE). Solvents used for HPLC and HPLC-MS purposes were of HPLC grade supplied by Labscan and filtered and degassed through a 0.45 μm polytetrafluoroethylene (PTFE) membrane prior to use. Water for HPLC purposes was filtered through an ELGA Purelab Ultra system prior to degassing. Deuterated solvents were supplied by Cambridge Isotopes (Andover, MA, USA). Commercial reagents were used without further purification.

Solid phase extractions were carried out on cartridges as specified. Prior to use each cartridge was conditioned with methanol followed by water, before equilibration with the initial solvent. Elution was carried out under applied vacuum and fractions analyzed by HPLC-DAD-MS. High performance liquid chromatography (HPLC) was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series Diode Array Detector, Polymer Laboratories PL-ELS1000 Evaporative Light Scattering Detector (ELSD) and Agilent 1100 Series fraction collector, controlled using ChemStation Agilent software. Ultra high performance liquid chromatography (UHPLC) was performed using an Agilent 1290 Infinity Series equipped with a Diode Array Detector. LC-HRMS was performed using a Dionex UltiMate 3000 HPLC coupled to a Bruker micrOTOF with an ESI probe [column Phenomenex Gemini-NX 2.1 × 150 mm, 3 μm, gradient elution 10–100% MeCN/H_2O over 10 min (with isocratic 0.025% formic acid as a modifier), 250 μL/min flow rate].

Electrospray ionisation mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes. High-resolution (HR) ESIMS measurements were obtained on a Bruker micrOTOF with an ESI probe by direct infusion in acetonitrile at 3 μL/min using sodium formate clusters as an internal calibrant. Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell at room temperature. UV-visible absorption spectra were obtained using a CARY50 UV-visible spectrophotometer in 1 cm quartz cells.

Nuclear magnetic resonance (NMR) experiments were carried out on a Bruker Avance 600 MHz spectrometer with 5 mm PASEL $^1H/D$-^{13}C Z-Gradient probe, controlled by TopSpin II software. In all cases spectra were acquired at 25 °C (unless otherwise specified) in solvents as specified in the text, with referencing to residual ^1H signals in the deuterated solvent.

Collection and taxonomy

MST-134270 was isolated from a roadside embankment soil sample collected in 1997 near Pamplona in Spain. Analysis of the 16S rRNA gene sequence showed 99% identity with the genus *Streptomyces* but resolution to a specific species are not feasible based on 16S data alone. A search of the MST HPLC database containing 50,000 cultures revealed a single replicate (MST-RA9773) displaying the same co-metabolite pattern of secondary metabolites. The strain MST-RA9773 was isolated from a soil sample collected in 1995 near Barellan, New South Wales in Australia. Analysis of the 16S rRNA gene sequence showed 99% identity with the genus *Streptomyces* but resolution to a specific species are not feasible based on 16S data alone. Sequence alignment of the two strains revealed 99% identity between the strains. Further, the sequences for both strains identified the cultures as replicates of a novel species in the genus *Streptomyces*.

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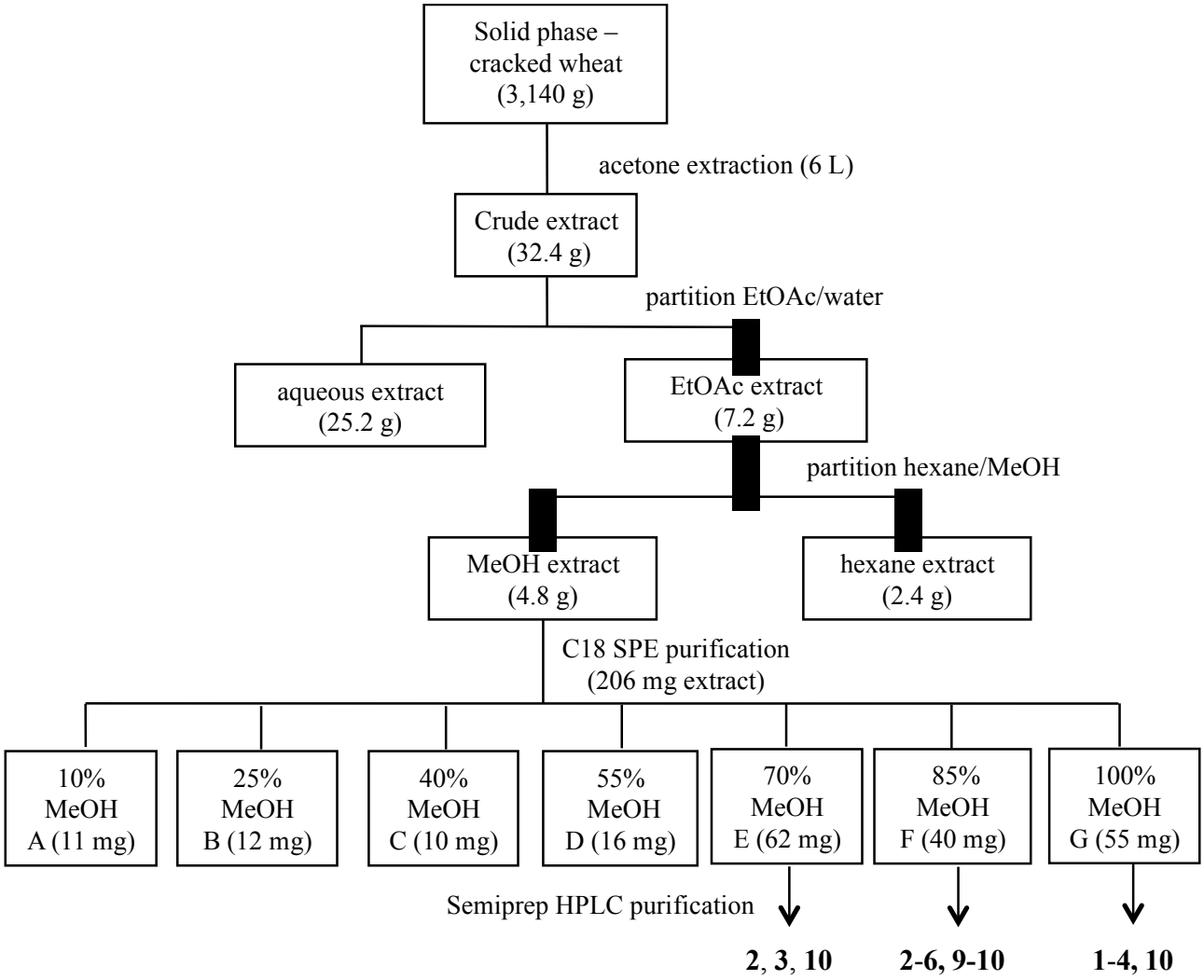


Scheme S1. Isolation scheme of MST-134270 compounds

Table S1a. NMR (600 MHz, DMSO-d_6) data for (+)-oxanthromicin (**1**)

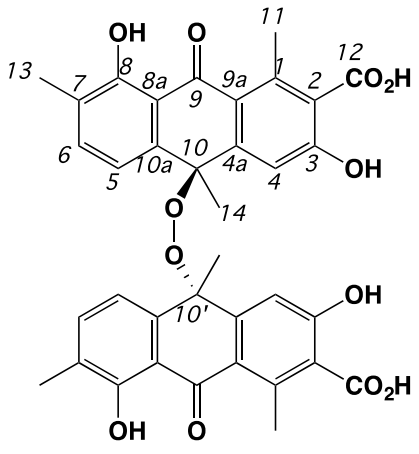
No. [A]	δ_C	δ_H (mult., *J* in Hz)	HMBC (^{1}H to ^{13}C)	COSY	ROESY
1	139.5				
2	120.0				
3	158.3				
4	112.1	7.16, s	2, 3, 4a, 9, 9a, 10, 12		14
4a	149.0				
5	116.7	6.33, d (*7.8*)	7, 8, 8a, 9, 10	6	6, 14
6	135.9	7.12, d (*7.8*)	5, 7, 8, 10a, 13	5	5, 13
7	124.9				
8	159.6				
8a	114.5				
9	189.3				
9a	127.2				
10	79.7				
10a	141.2				
11	20.6	2.69, s	1, 2, 9, 9a		8-OH
12	168.8				
13	15.2	2.18, s	6, 7, 8		6, 8-OH
14	33.0	1.44, s	4a, 10, 10a		4, 5
8-OH		13.46, s	7, 8, 8a		11, 13

[A]Only a single symmetric unit tabulated.

Table S1b. NMR data of (+)-oxanthromicin (**1**) and (−)-oxanthromicin[B]

No. [A]	δ_C (125 MHz)[C]	δ_C (25.2 MHz)[C]	δ_H (mult., *J* in Hz) (600 MHz)[D]	δ_H (mult., *J* in Hz) (80 MHz)[D]
1	139.5	139.4		
2	120.0	119.9		
3	158.3	158.2		
4	112.1	112.1	6.65, s	6.7, s
4a	149.0	148.9		
5	116.7	116.7	6.53, d (7.8)	6.55, d (8)
6	135.9	135.8	7.25, d (7.8)	7.25, d (8)
7	124.9	124.8		
8	159.6	159.5		
8a	114.5	114.5		
9	189.3	189.3		
9a	127.2	127.1		
10	79.7	79.7		
10a	141.2	141.1		
11	20.6	20.5	2.75, s	2.85, s
12	168.8	168.7		
13	15.2	15.1	2.20, s	2.24, s
14	33.0	32.9	1.21, s	1.2, s

[A]Only a single symmetric unit tabulated. [B]Wright, J.J. et al, *J. Chem. Commun.* **1984**, 473. [C] solvent DMSO-d_6. [D]solvent CD_3OD

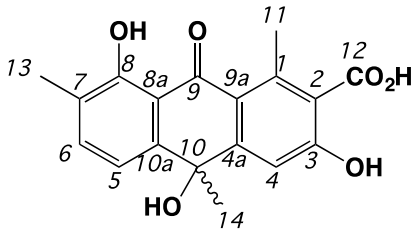


1

Table S2. NMR (600 MHz, DMSO-d_6) data for (±)-*hemi*-oxanthromicin A (**2**)

No.	δ_C	δ_H, mult. (*J* in Hz)	HMBC (^{1}H to ^{13}C)	COSY	ROESY
1	139.1				
2	118.8				
3	158.2				
4	110.4	7.38, s	2, 3, 4a, 9, 9a, 10, 12		14, 10-OH
4a	155.3				
5	114.9	7.22, d (*7.8*)	6, 7, 8, 8a, 9, 10	6	6, 14, 10-OH
6	136.2	7.44, d (*7.8*)	5, 8, 10, 10a, 13	5	5, 13
7	123.9				
8	159.5				
8a	114.1				
9	189.5				
9a	126.2				
10	69.5				
10a	147.9				
11	20.1	2.63, s	1, 2, 9a		
12	168.8				
13	15.1	2.18, s	6, 7, 8		6
14	39.5	1.43, s	4a, 10, 10a		4, 5
8-OH		13.22, s	6, 7, 8, 8a, 9		
10-OH		6.11, s	4a, 10, 14		4, 5

Assignments supported by HSQC data

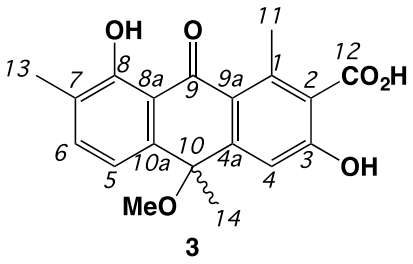


2

Table S3. NMR (600 MHz, DMSO-d_6) data for (±)-*hemi*-oxanthromicin B (**3**)

No.	δ_C	δ_H (mult., *J* in Hz)	HMBC (^{1}H to ^{13}C)	COSY	ROESY
1	140.2				
2	120.3				
3	159.5				
4	110.5	7.14, s	2, 3, 4a, 9, 9a, 10, 12		14, 10-OMe
4a	150.3				
5	115.4	7.05, d (*7.8*)	7, 8, 8a, 9,10	6	6, 14, 10-OMe
6	136.7	7.52, d (*7.8*)	8,10a, 13	5	5, 13
7	124.6				
8	160.2				
8a	115.4				
9	189.1				
9a	126.7				
10	75.9				
10a	142.5				
11	20.4	2.67, s	1, 2, 9a		
12	168.7				
13	15.1	2.20, s	6, 7, 8		6
14	37.7	1.52, s	4, 10, 10a		4, 5
8-OH		13.47, s	7, 8, 8a		
10-OMe	51.8	2.81, s	10		4, 5

Assignments supported by HSQC data



3

Table S4. NMR (600 MHz, DMSO-d_6) data for (±)-*spiro*-oxanthromicin A (**4**)

No.	δ_C	δ_H (mult., *J* in Hz)	HMBC (^{1}H to ^{13}C)	COSY	ROESY
1	140.2[a]				
2	119.0				
3	158.6[a]				
4	106.8	7.38, s	2, 3, 9, 9a, 10, 12		14
4a	139.4				
5	128.3				
6	137.8	6.83, s	7, 8, 10a, 13, 10'		13, 4', 5'
7	126.1[b]				
8	159.7				
8a	112.4				
9	190.3				
9a	127.2[a]				
10	126.1[b]				
10a	129.9				
11	20.8	2.77, s	1, 2, 9a		8-OH
12	168.6				
13	15.2	2.04, s	6, 7, 8		6, 8-OH
14	128.8	6.96, dd (*4.8, 4.8*)	10, 10a, 10'	14'	4, 14'
1'	139.8[a]				
2'	119.0				
3'	[c]				
4'	113.9	6.64, s	2', 9', 9a', 10'		6, 14'
4a'	154.6				
5'	118.6	6.48, d (*7.9*)	7', 8a', 9', 10'	6'	6, 14'
6'	136.4	7.29, d (*7.9*)	8', 10a', 13'	5'	13'
7'	123.5				
8'	159.9				
8a'	114.7				
9'	189.7				
9a'	126.2[a,b]				
10'	45.5				
10a'	148.2				
11'	20.7	2.71, s	1', 2', 9a'		8'-OH
12'	168.8				
13'	15.0	2.16, s	6', 7', 8'		6', 8'-OH
14'	47.1	3.13, m	5, 10, 4a', 10', 10a', 14	14	4', 5', 14
8-OH		14.1	7, 8, 8a		11, 13
8'-OH		13.7	7', 8', 8a'		11', 13'

[a] assignment from HMBC correlations; [b] assignments interchangeable; [c] signal not detected; assignments by HSQC data

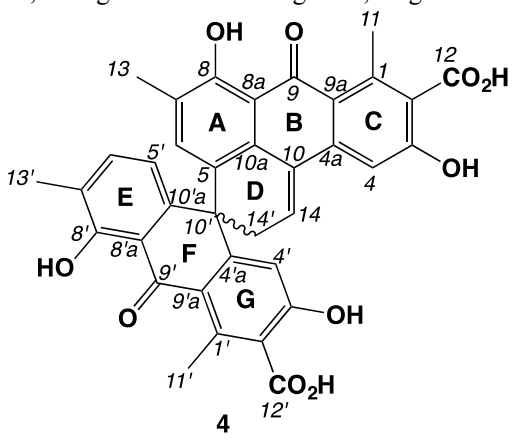


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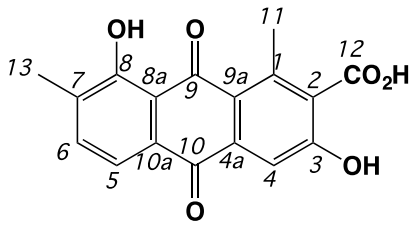
Table 5. NMR (600 MHz, DMSO-d_6) data for oxanthroquinone (**9**)

No.	δ_C	δ_H, mult. (*J* in Hz)	HMBC (^{1}H to ^{13}C)	COSY
1	140.5			
2	122.5			
3	158.6			
4	112.0	7.53, s	2, 9a, 10	
4a	136.5			
5	118.0	7.56, d (*7.8*)	7, 8a, 10	6
6	136.4	7.62, d (*7.8*)	8, 10a, 13	5
7	134.6			
8	160.0			
8a	115.7			
9	189.7			
9a	130.2			
10	181.7			
10a	131.4			
11	20.0	2.76, s	1, 2, 9a	
12	168.3			
13	15.9	2.29, s	6, 7, 8a	
8-OH		13.22, s	7, 8, 8a	

Assignments supported by HSQC data



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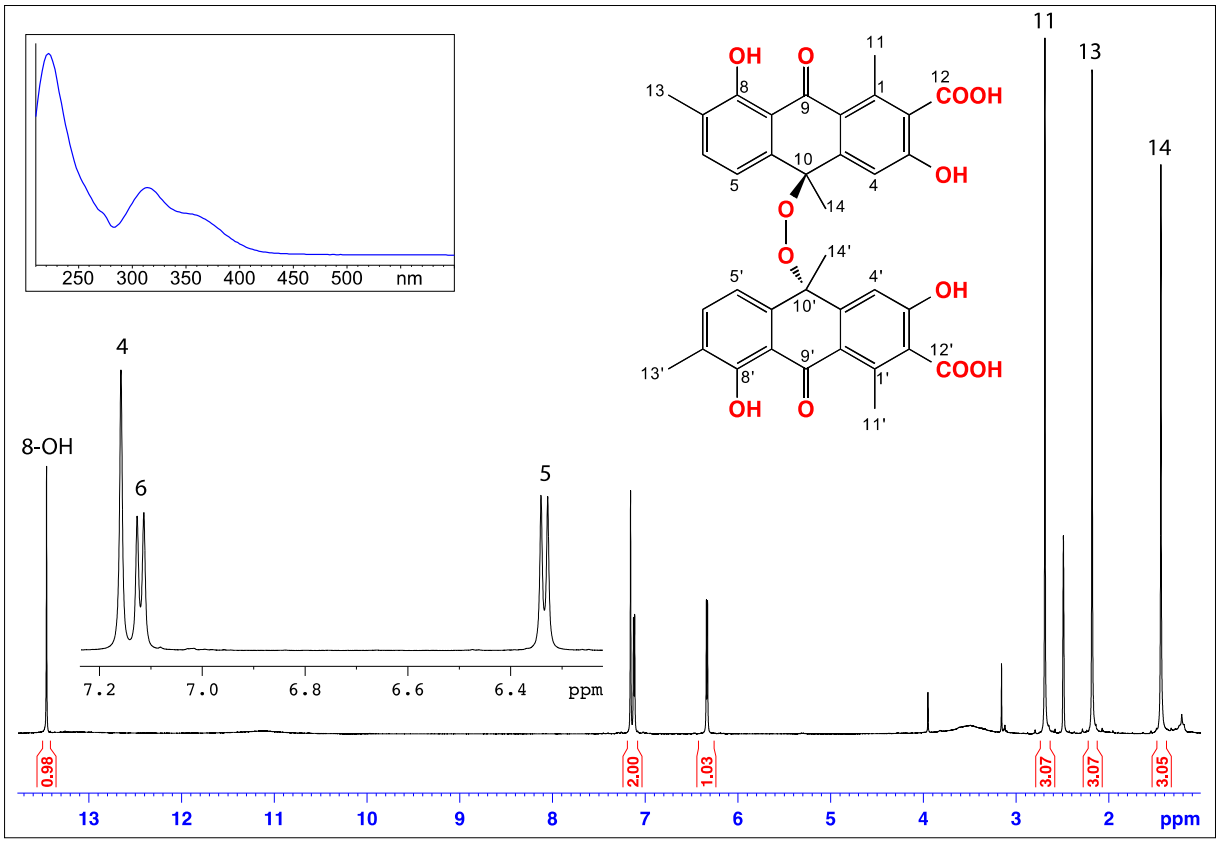

Figure S1a. ^{1}H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of (+)-oxanthromicin (**1**)

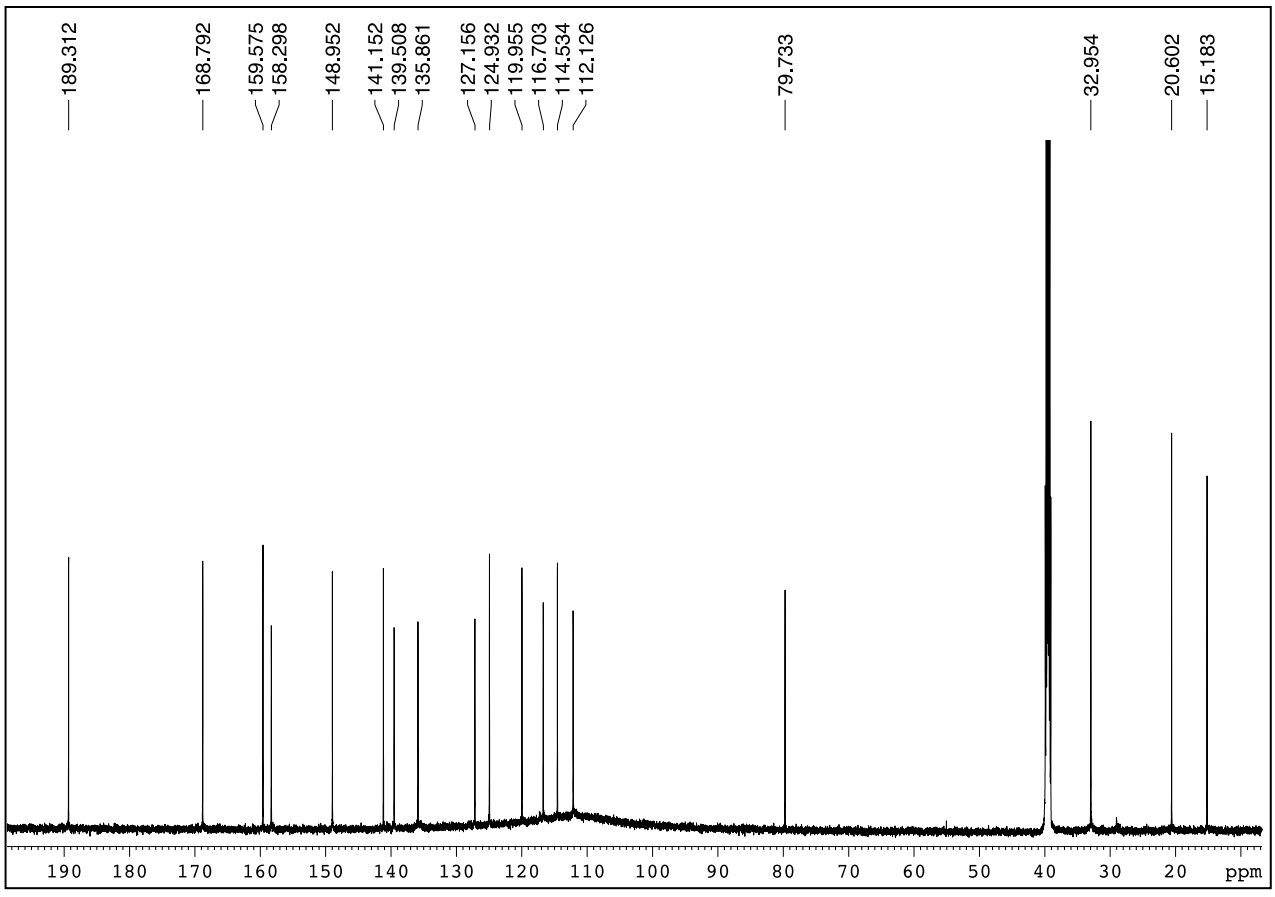

Figure S1b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of (+)-oxanthromicin (**1**)

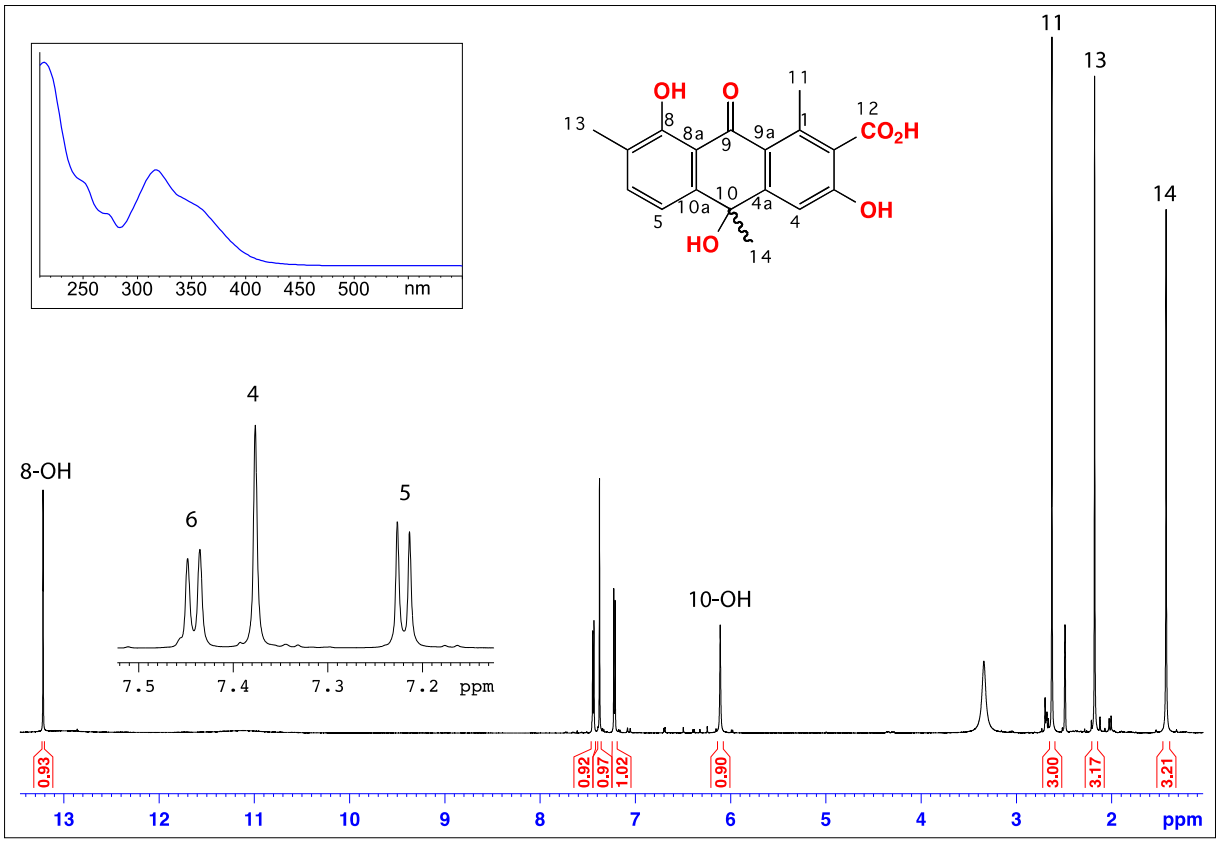

Figure S2a. [1]H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of (±)-*hemi*-oxanthromicin A (**2**)

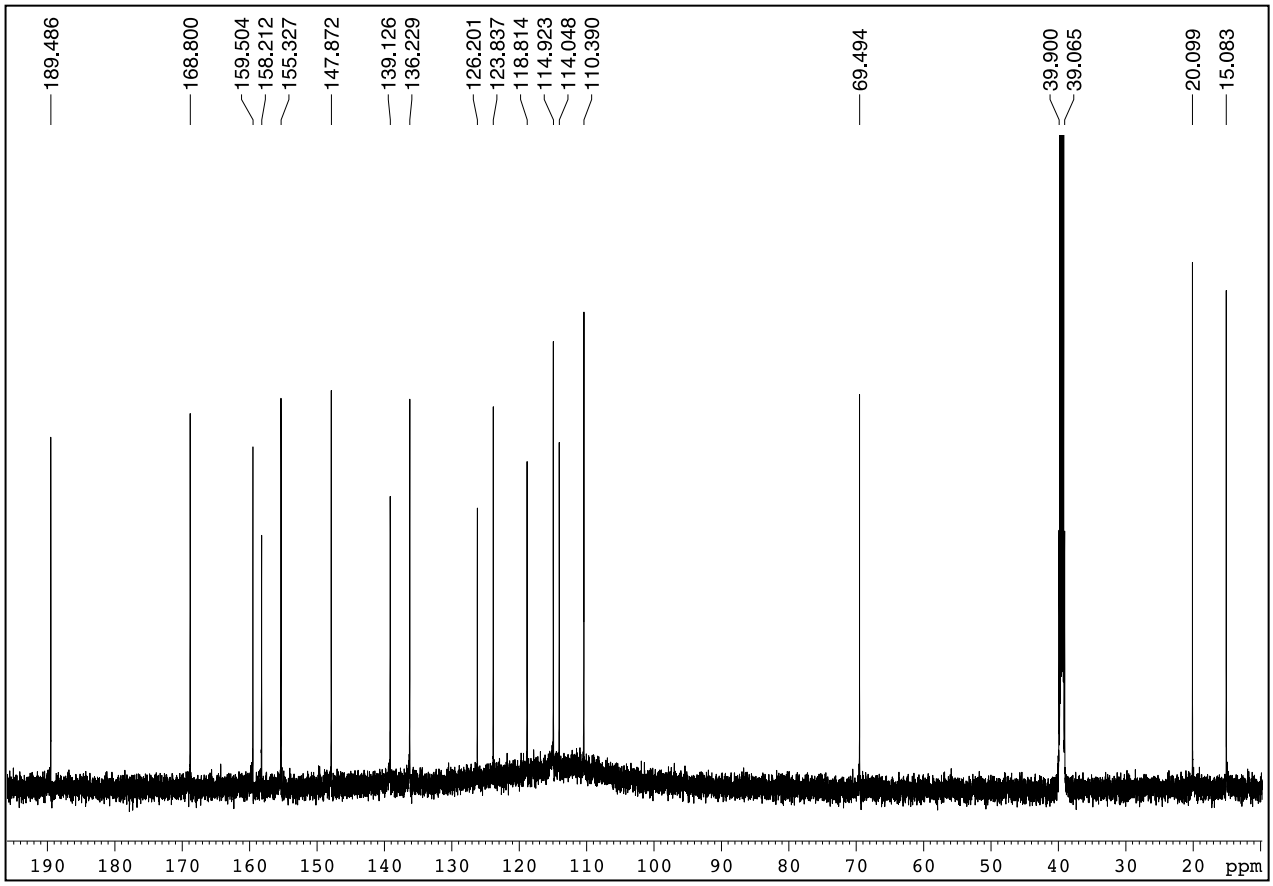

Figure S2b. [13]C NMR (150 MHz, DMSO-d_6) spectrum of (±)-*hemi*-oxanthromicin A (**2**)

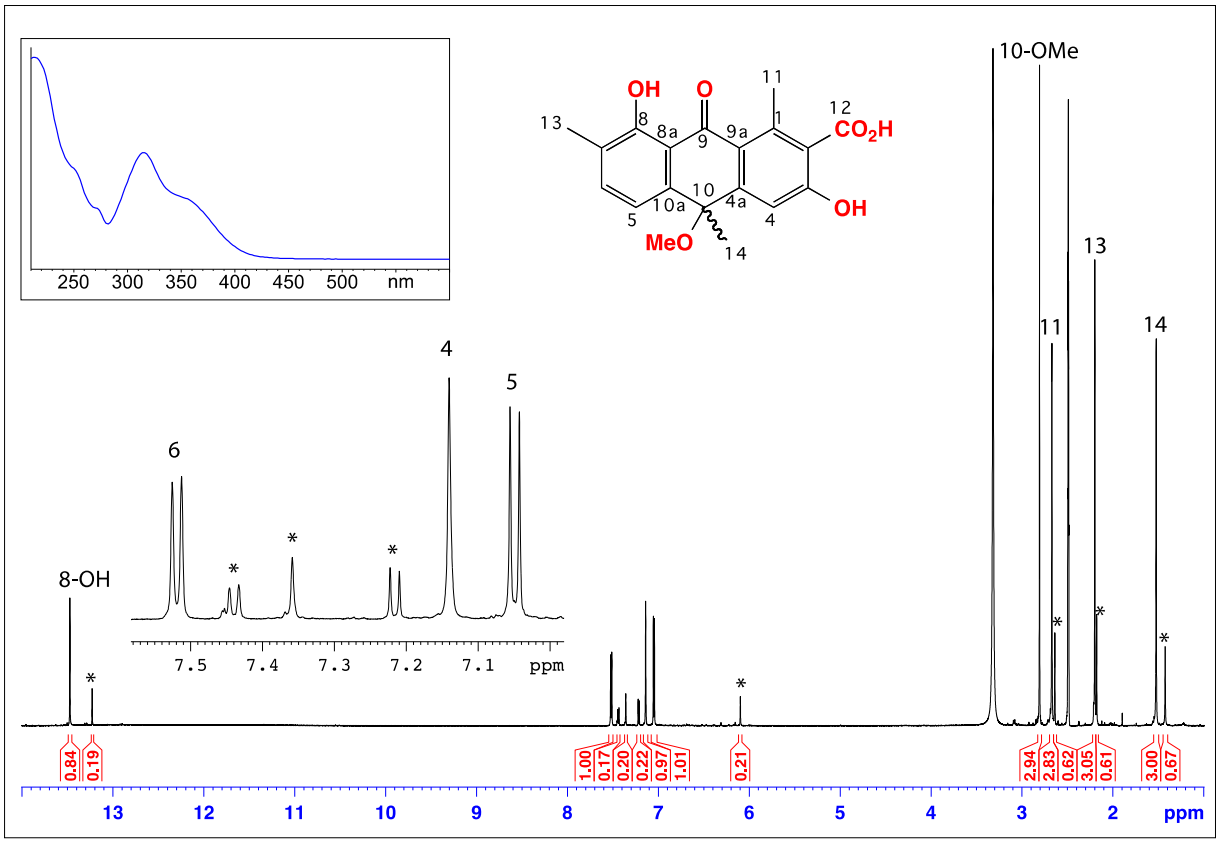

Figure S3a. [1]H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of (±)-*hemi*-oxanthromicin B (**3**) (* signals from (±)-*hemi*-oxanthromicin A)

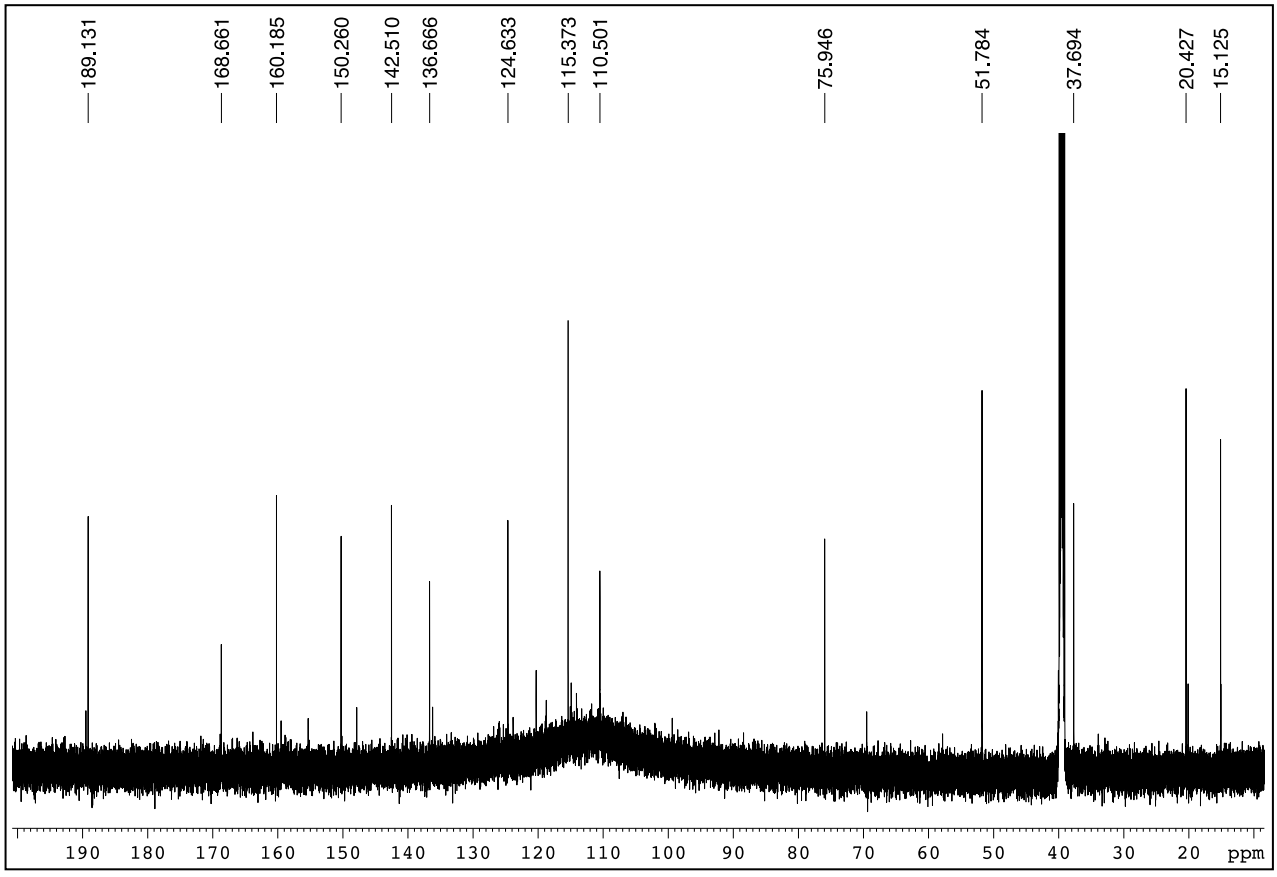

Figure S3b. [13]C NMR (150 MHz, DMSO-d_6) spectrum of (±)-*hemi*-oxanthromicin B (**3**)

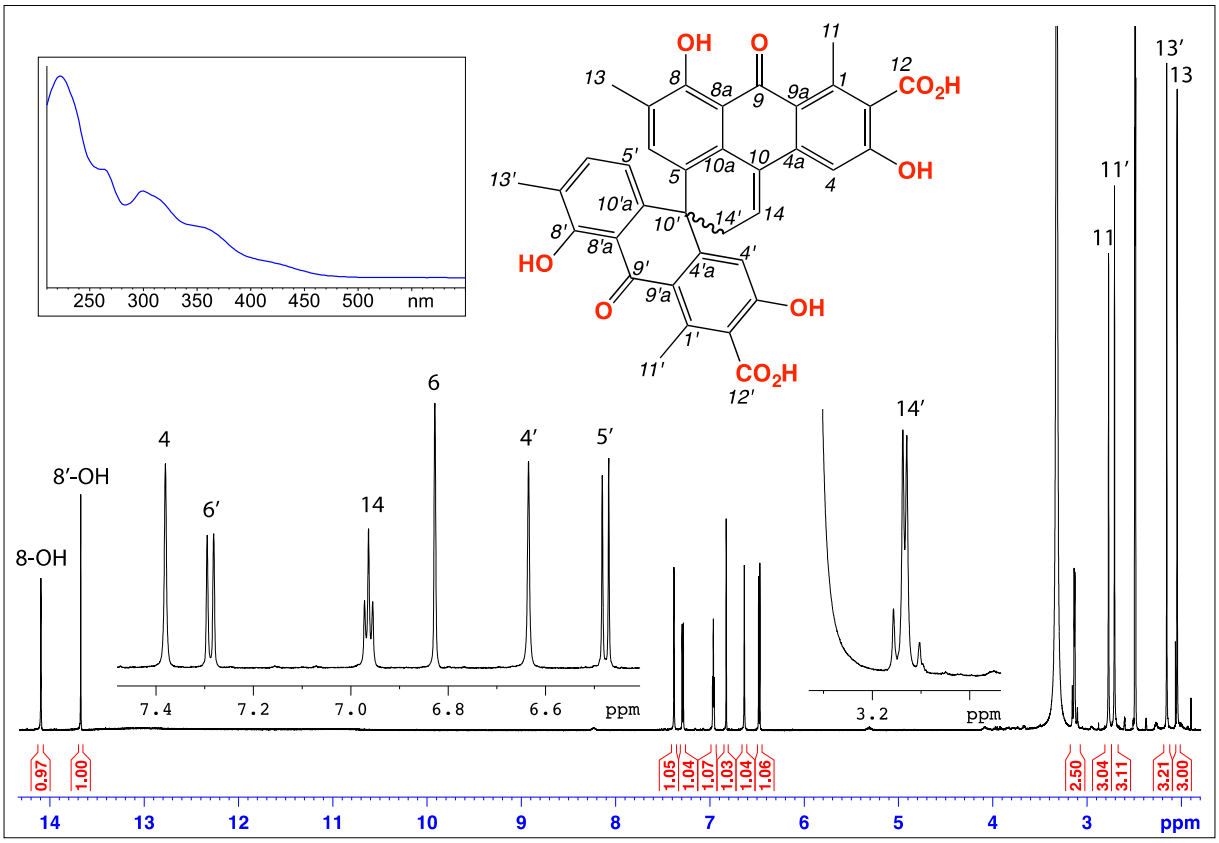

Figure S4a. [1]H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of (±)-*spiro*-oxanthromicin A (**4**)

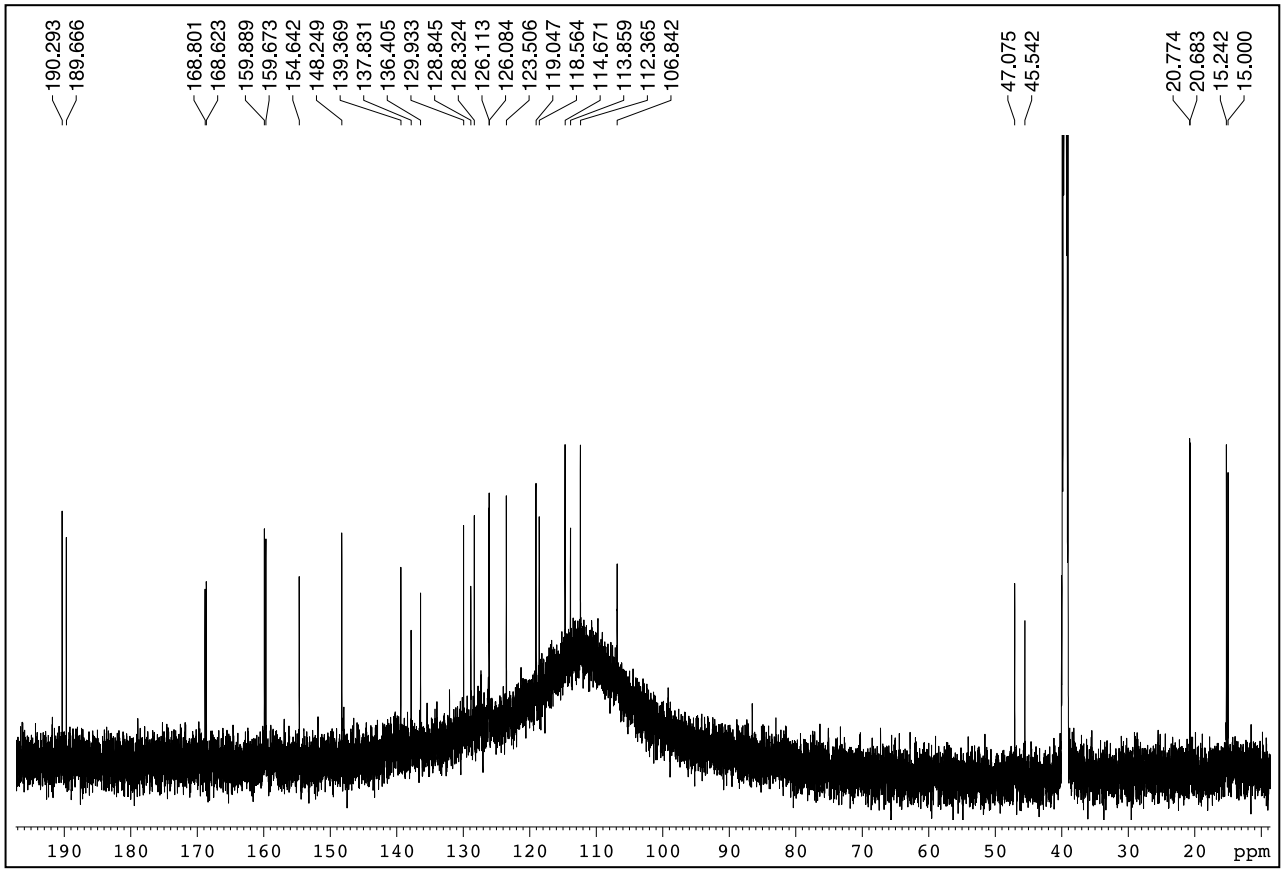

Figure S4b. [13]C NMR (150 MHz, DMSO-d_6) spectrum of (±)-*spiro*-oxanthromicin A (**4**)

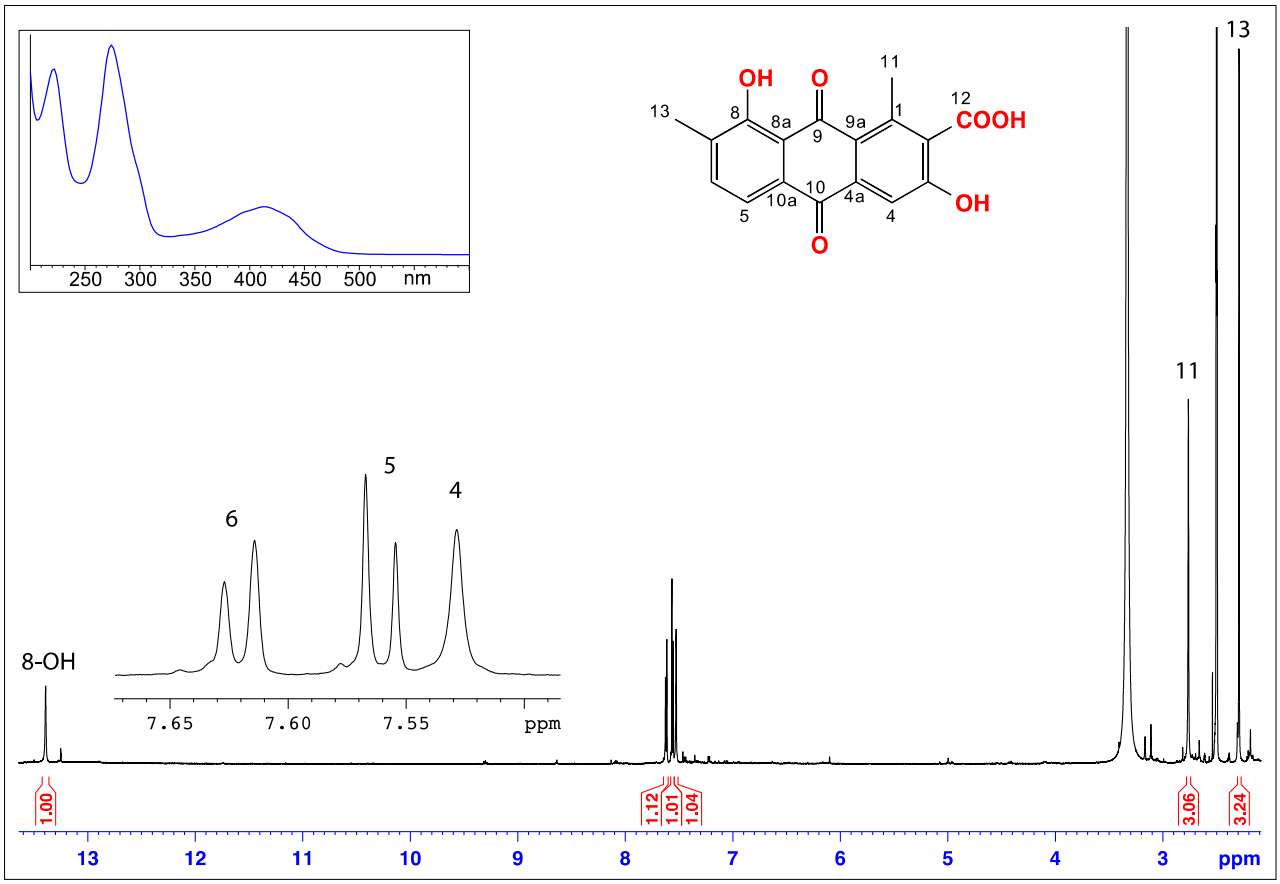

Figure S5a. [1]H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of oxanthroquinone (**9**)

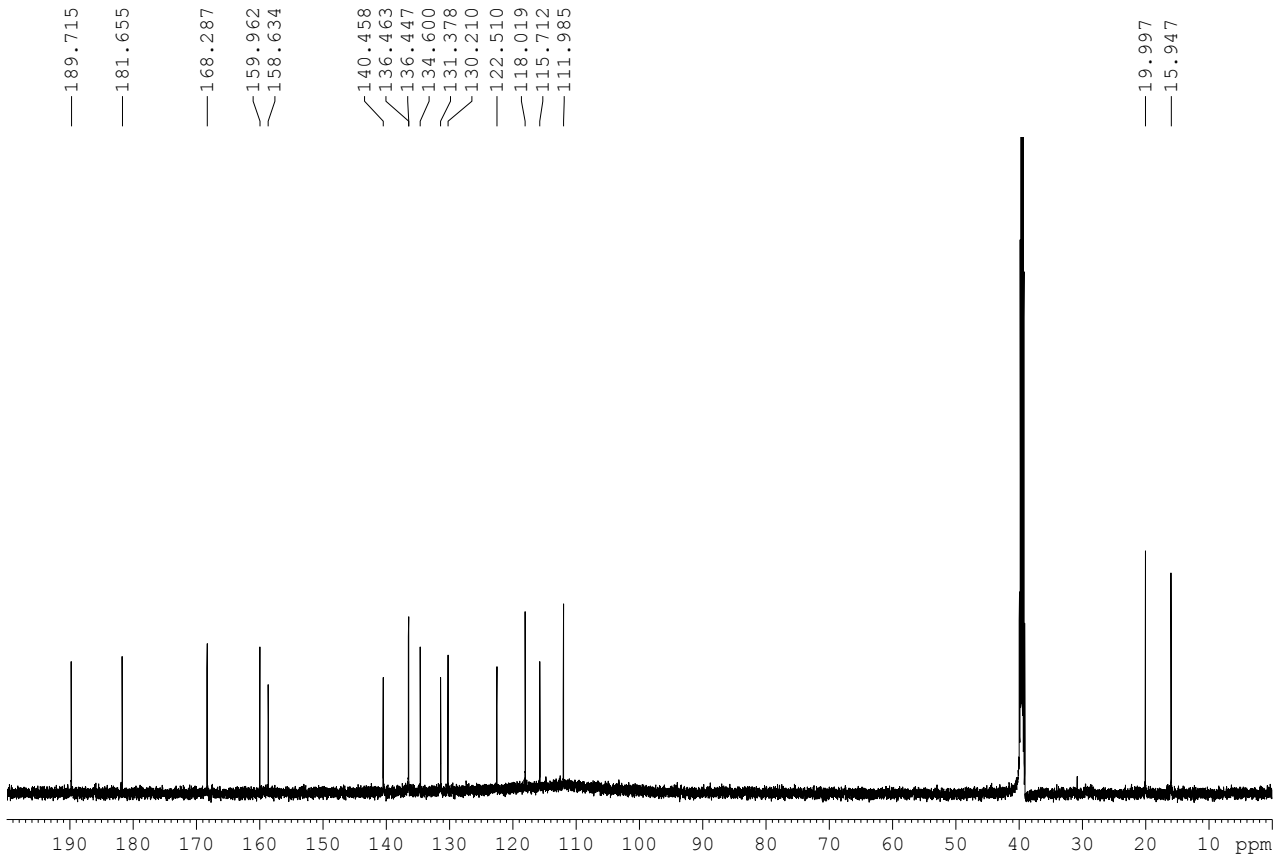

Figure S5b. [13]C NMR (150 MHz, DMSO-d_6) spectrum of oxanthroquinone (**9**)

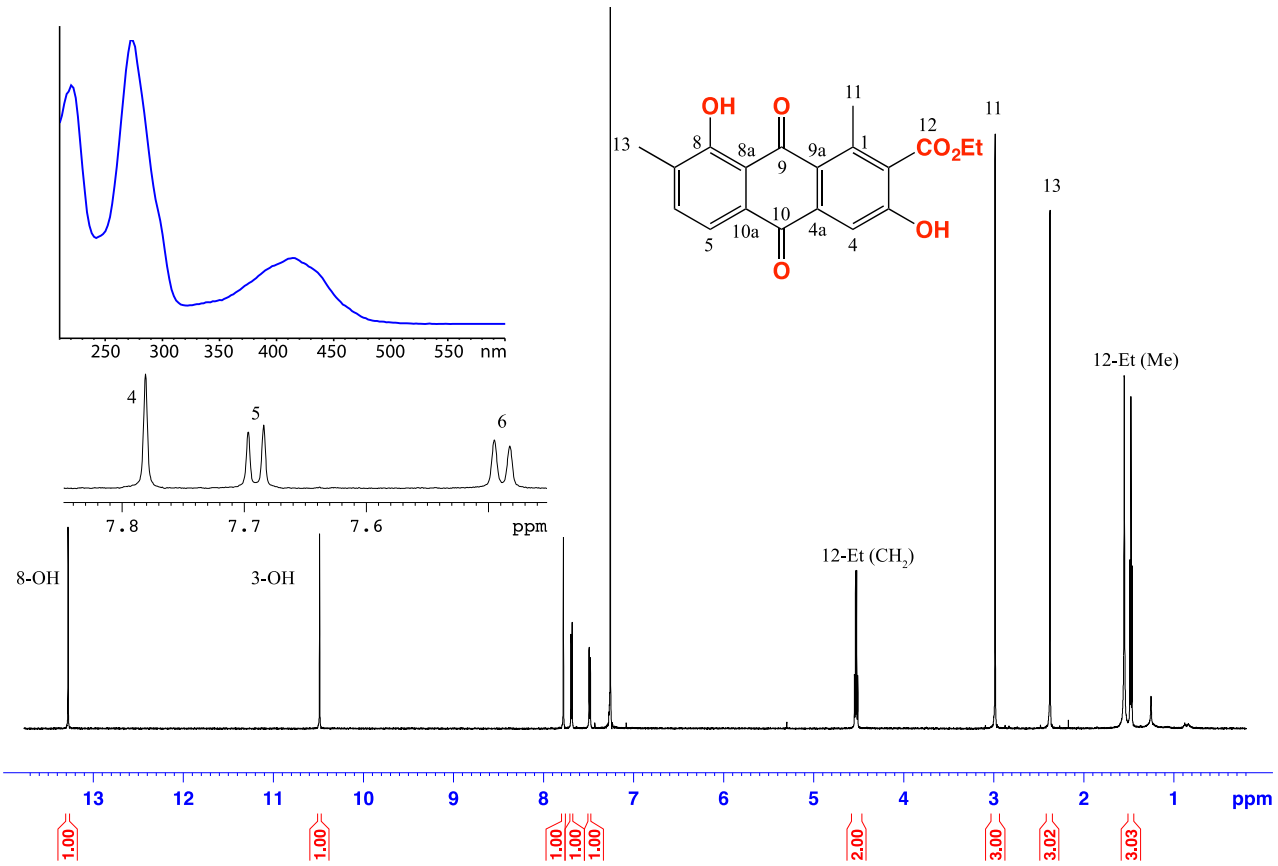

Figure S6a. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis (MeOH) spectra of oxanthroquinone ethyl ester (**11**)

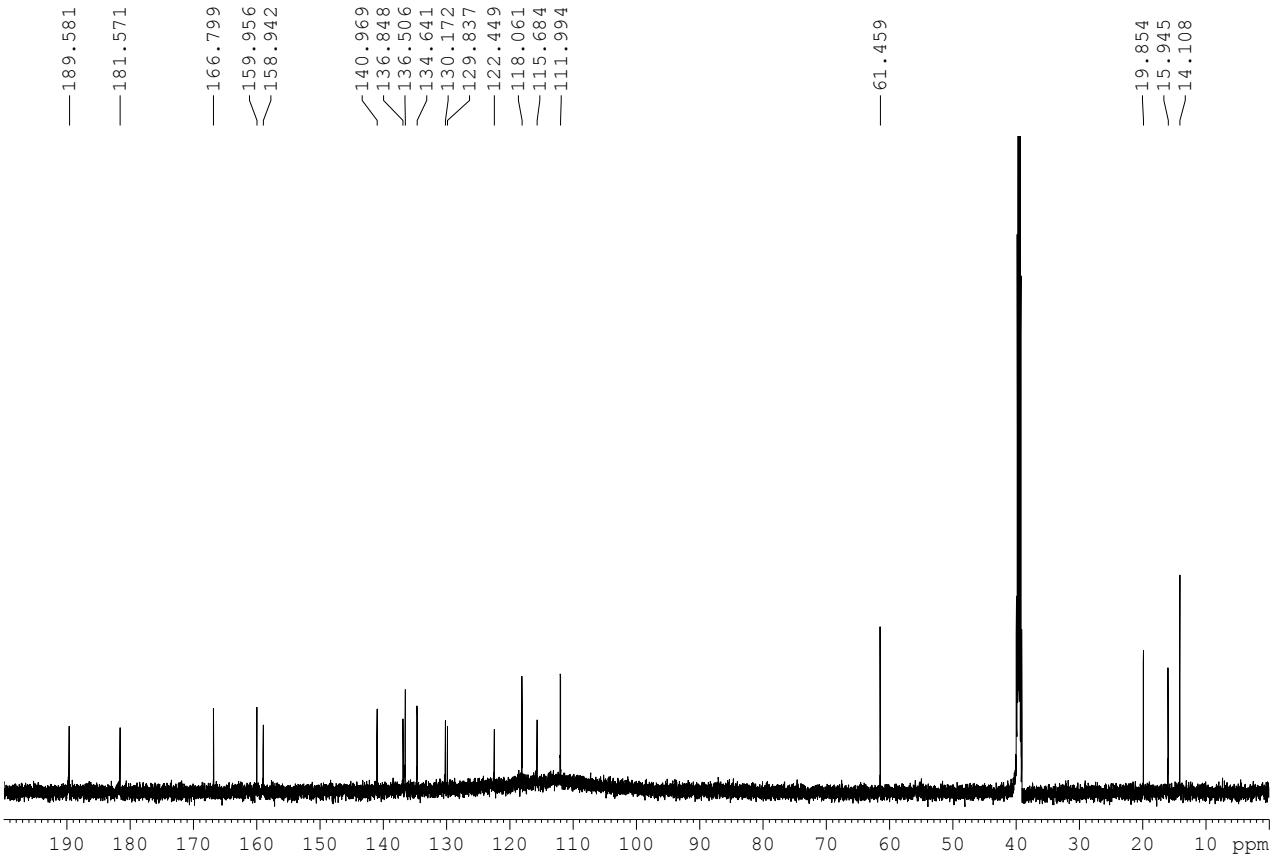

Figure S6b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oxanthroquinone ethyl ester (**11**)

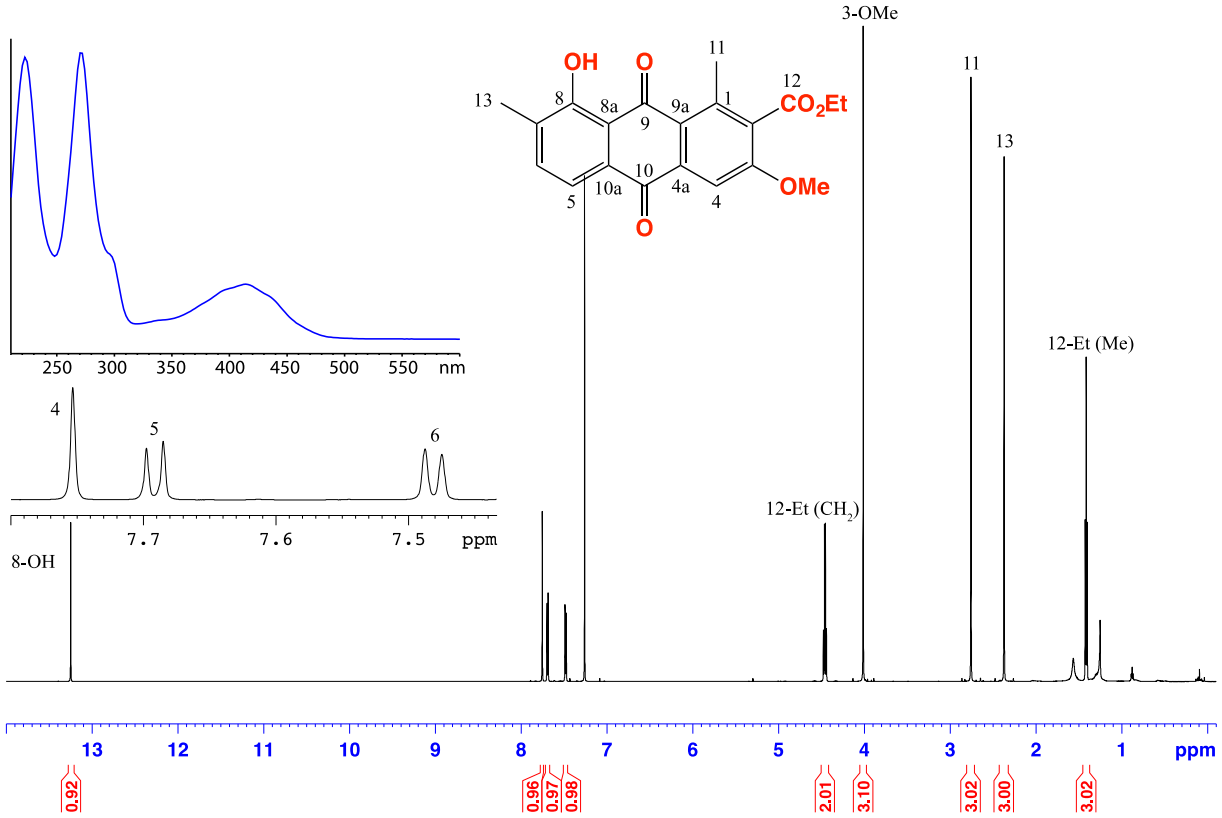

Figure S7a. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis (MeOH) spectra of 3-*O*-methyl-oxanthroquinone ethyl ester (**14**)

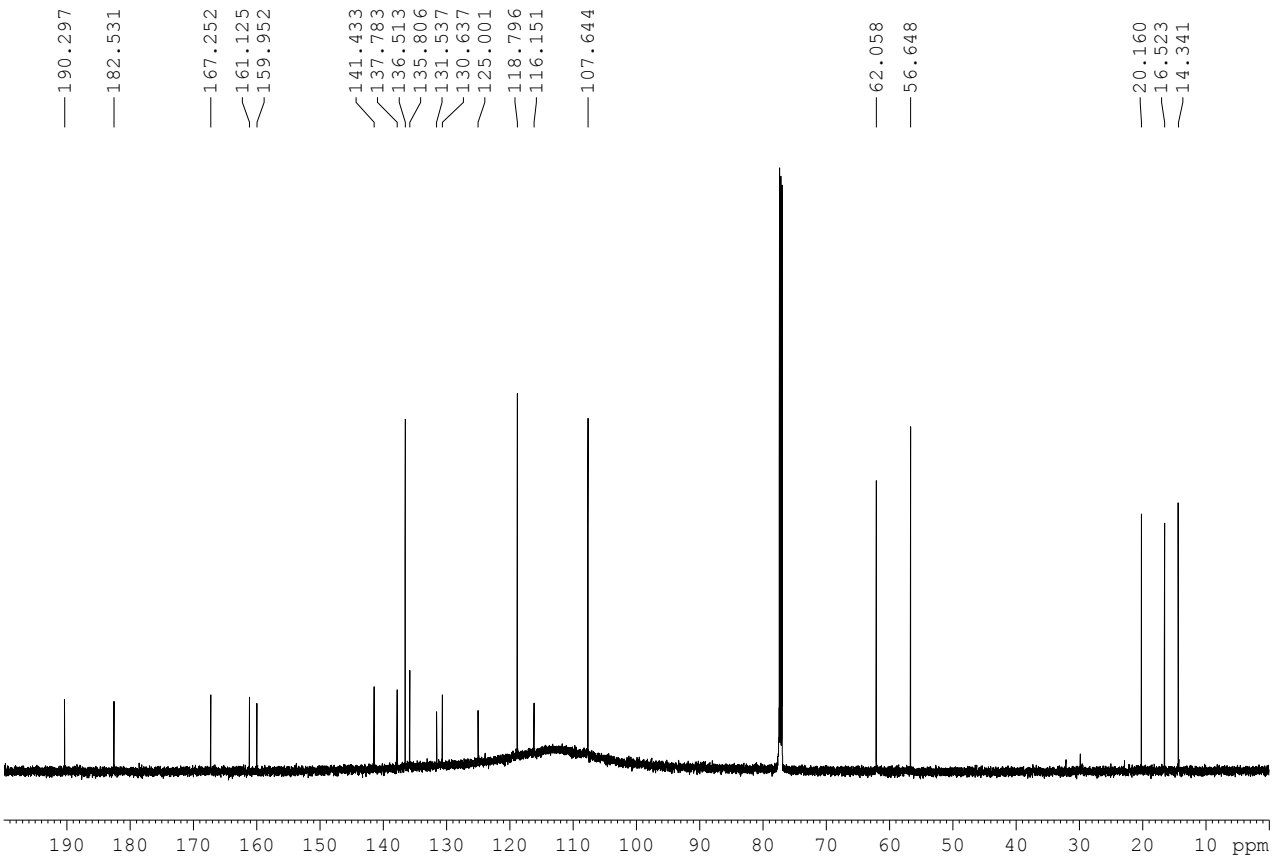

Figure S7b. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of 3-*O*-methyl-oxanthroquinone ethyl ester (**14**)

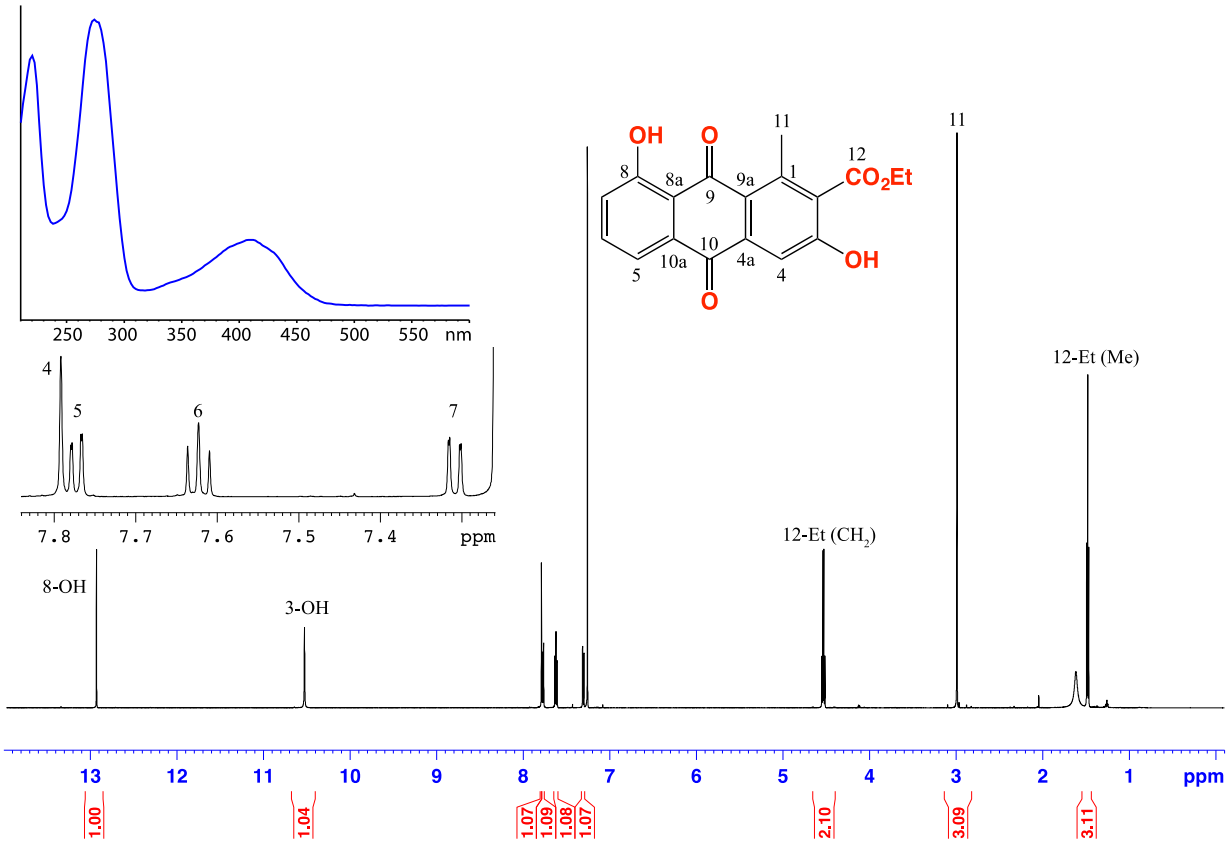

Figure S8a. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis (MeOH) spectra of 7-desmethyl-oxanthroquinone ethyl ester (**12**)

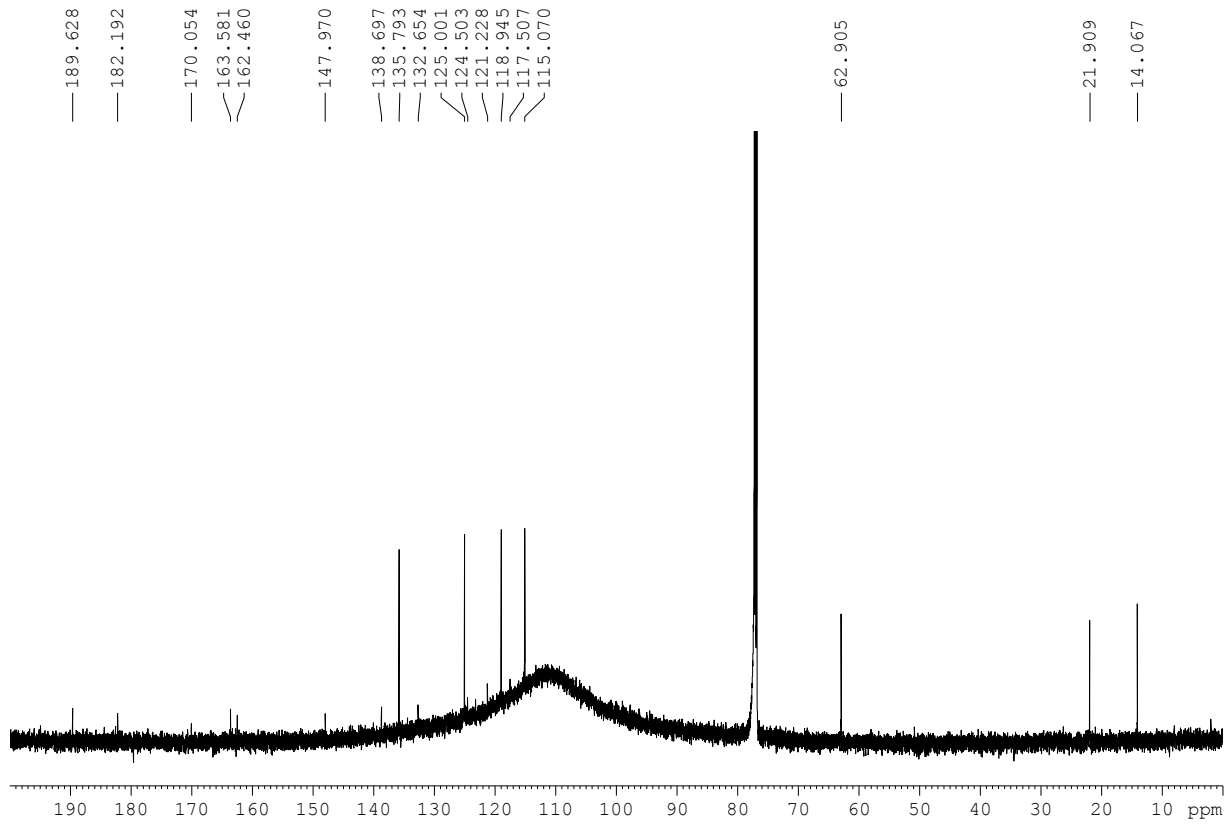

Figure S8b. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of 7-desmethyl-oxanthroquinone ethyl ester (**12**)

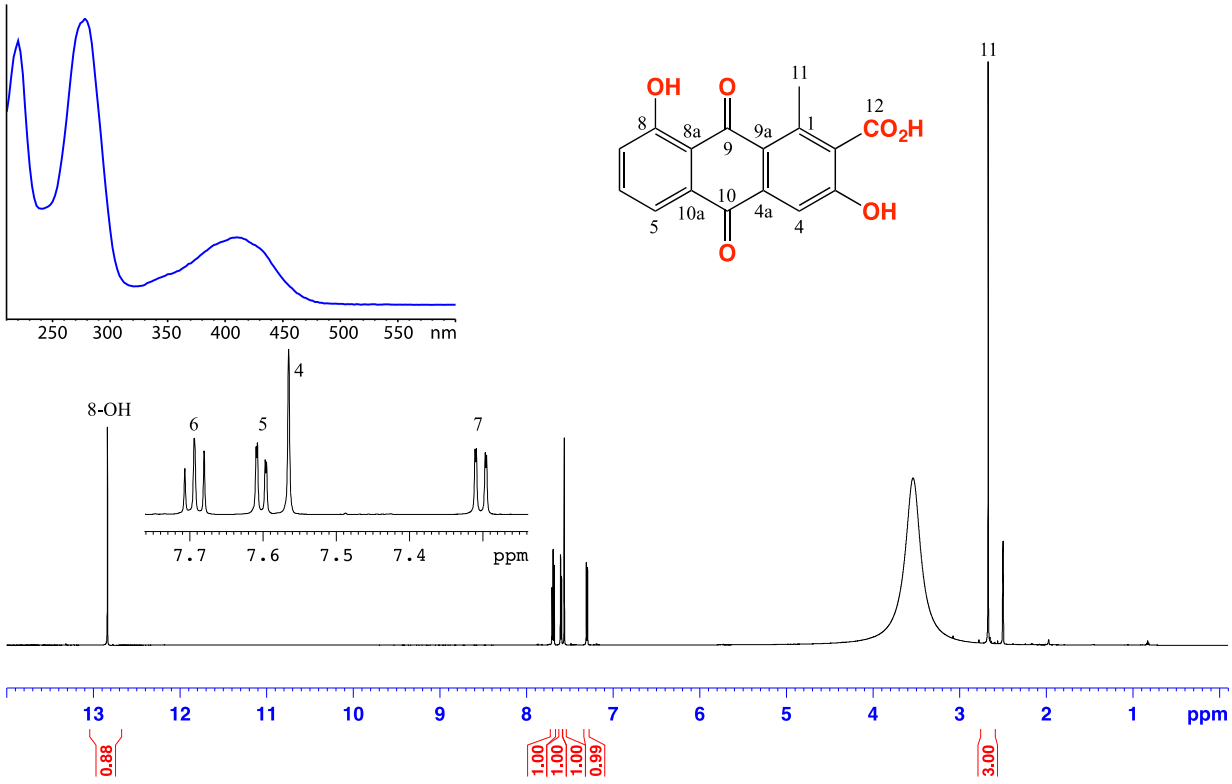

Figure S9a. [1]H NMR (600 MHz, DMSO-d_6) and UV-vis (MeOH) spectra of 7-desmethyl-oxanthroquinone (**13**)

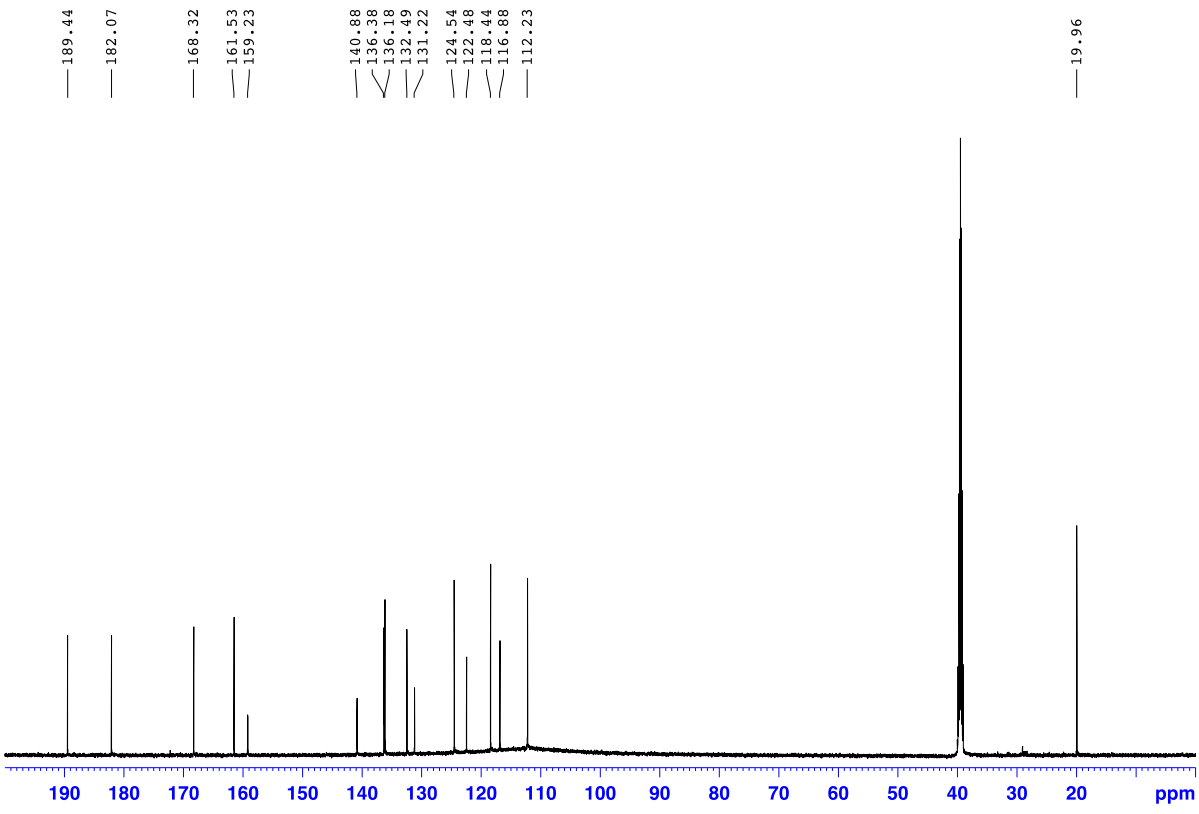

Figure S9b. [13]C NMR (150 MHz, DMSO-d_6) spectrum of 7-desmethyl-oxanthroquinone (**13**)

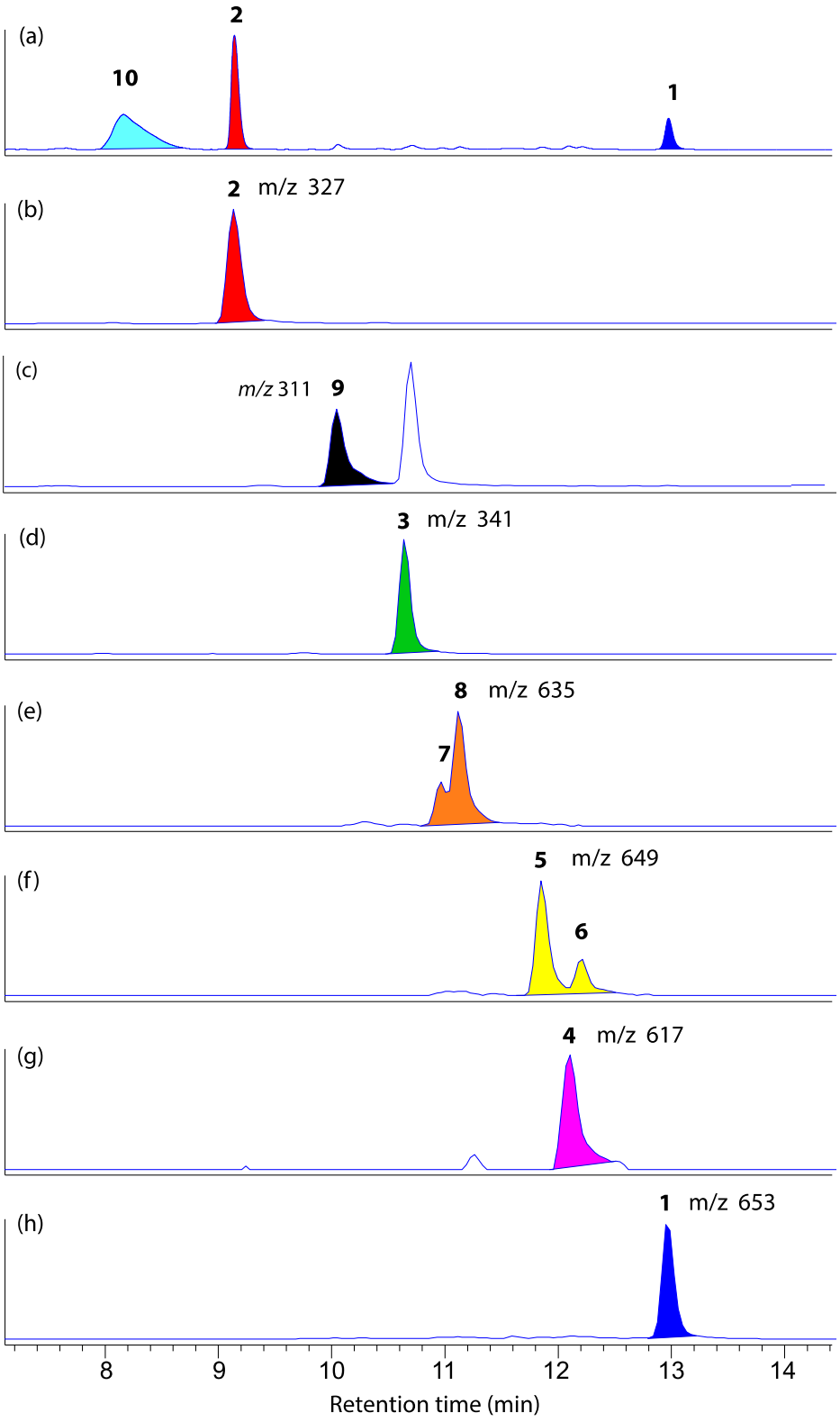

Figure S10. (a) HPLC chromatogram (210 nm) of the crude MeOH fraction [Agilent Zorbax SB-C_8, 5 µm, 4.6 × 150 mm, 15 min gradient elution at 1 mL/min, 10–100% MeCN/H_2O with an isocratic 0.01% formic acid modifier] and extracted ions ESI(–)MS chromatograms of (b) (±)-*hemi*-oxanthromicin A (**2**); (c) oxanthroquinone (**9**), (d) (±)-*hemi*-oxanthromicin B (**3**); (e) (±)-*spiro*-oxanthromicin C1/C2 (**7**/**8**); (f) (±)-*spiro*-oxanthromicin B1/B2 (**5**/**6**); (g) (±)-*spiro*-oxanthromicin A (**4**) and (h) (+)-oxanthromicin (**1**).

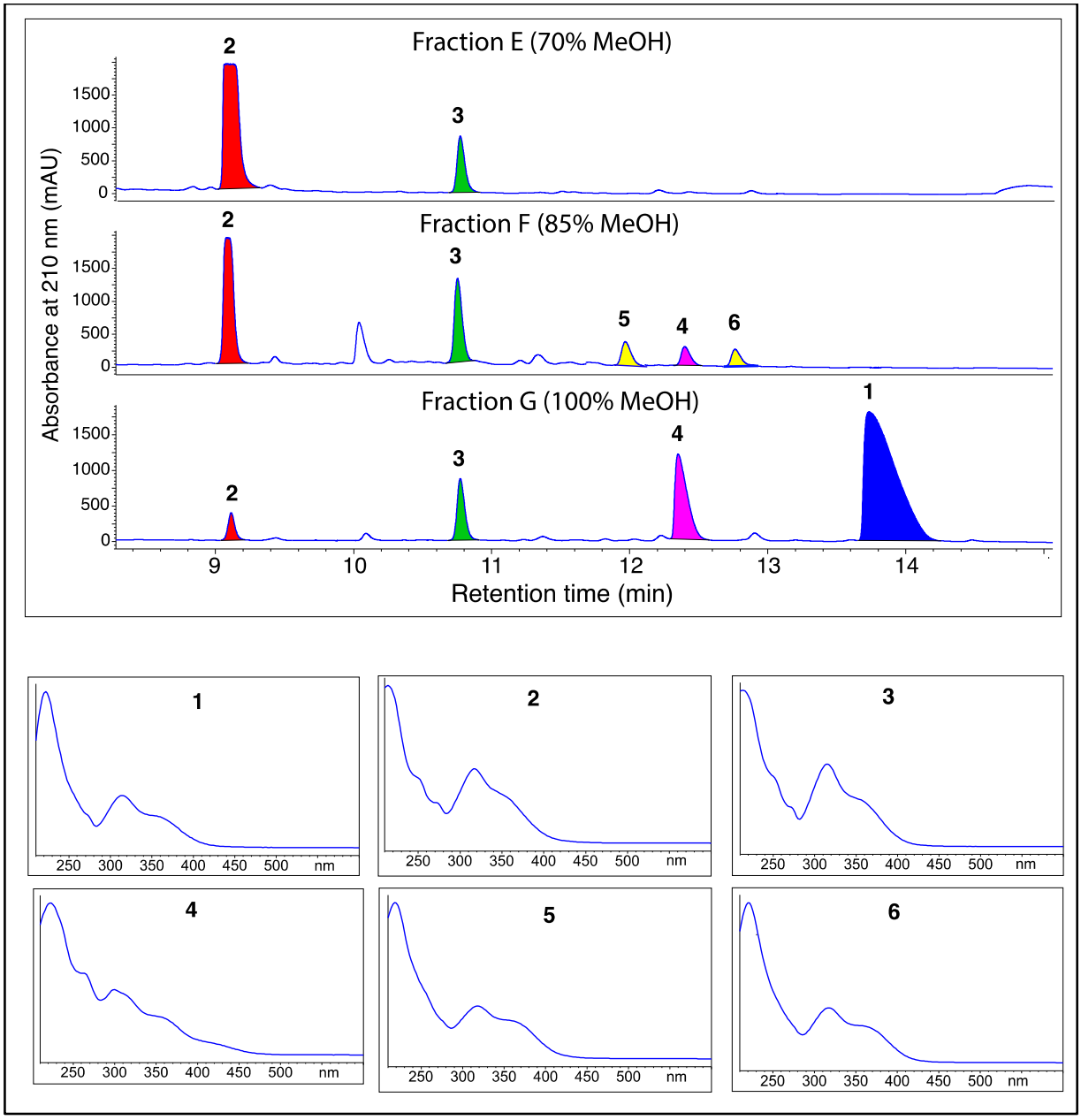

Figure S11. HPLC chromatograms of SPE fractions E–G [Agilent Zorbax XDB-C_8, 5 µm, 4.6 × 150 mm, 15 min gradient elution at 1 mL/min, 10–100% MeCN/H_2O with an isocratic 0.01% formic acid modifier] and UV-vis spectra of (+)-oxanthromicin (**1**); (±)-*hemi*-oxanthromicin A (**2**); (±)-*hemi*-oxanthromicin B (**3**); (±)-*spiro*-oxanthromicin (**4**); (±)-*spiro*-oxanthromicin B1(**5**); (±)-*spiro*-oxanthromicin B2 (**6**)

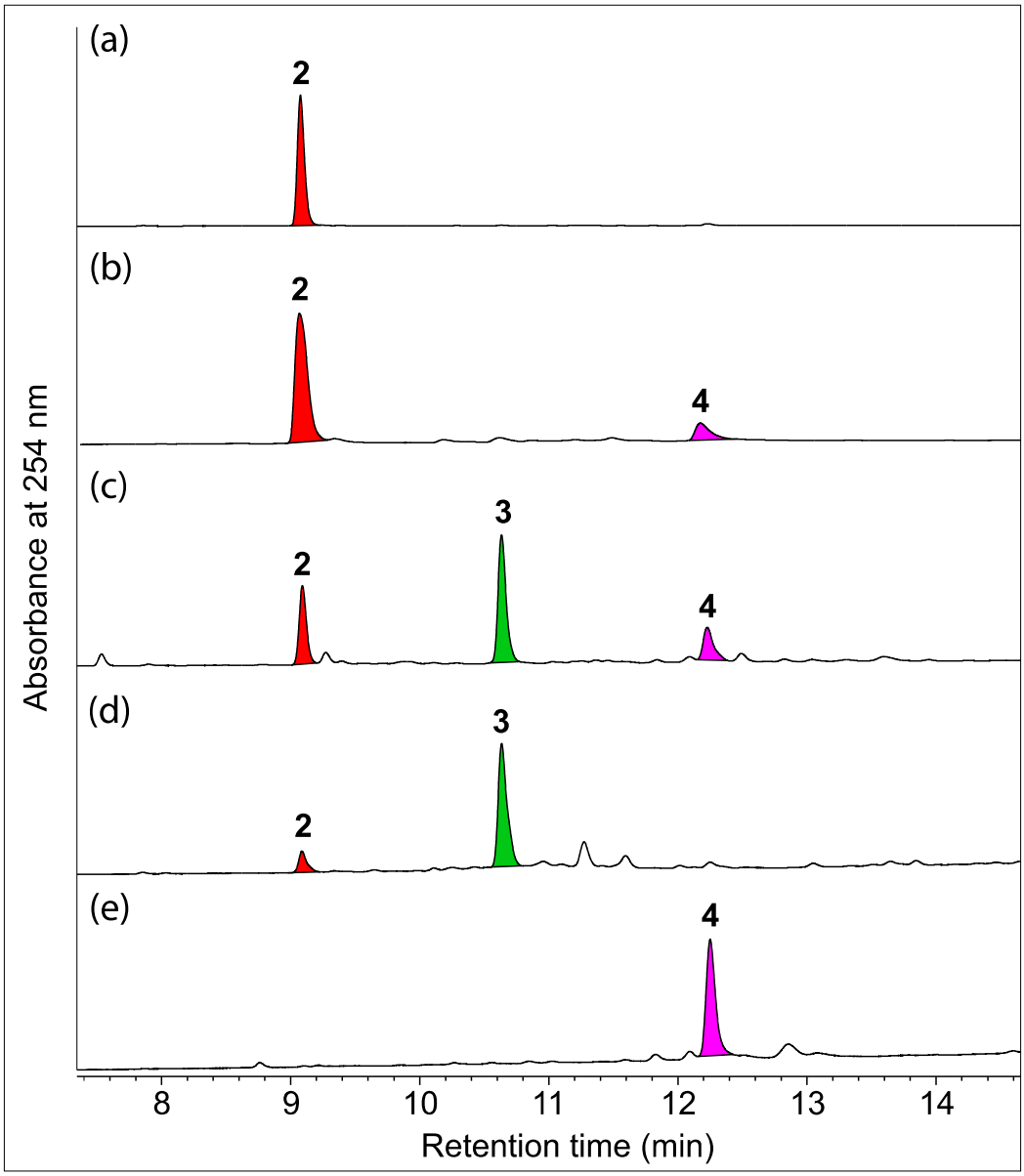

Figure S12. HPLC-DAD [Agilent Zorbax SB-C_8, 5 μm, 4.6 × 150 mm, 15 min gradient elution at 1 mL/min, 10–100% MeCN/H_2O with an isocratic 0.01% formic acid modifier] chromatograms (a) authentic **2**; (b) **2** after 24 h in 0.1% TFA/MeCN at 40 °C; (c) **2** after 24 h in 0.1% TFA/MeOH at 40 °C; (d) authentic **3** and (e) authentic **4**.

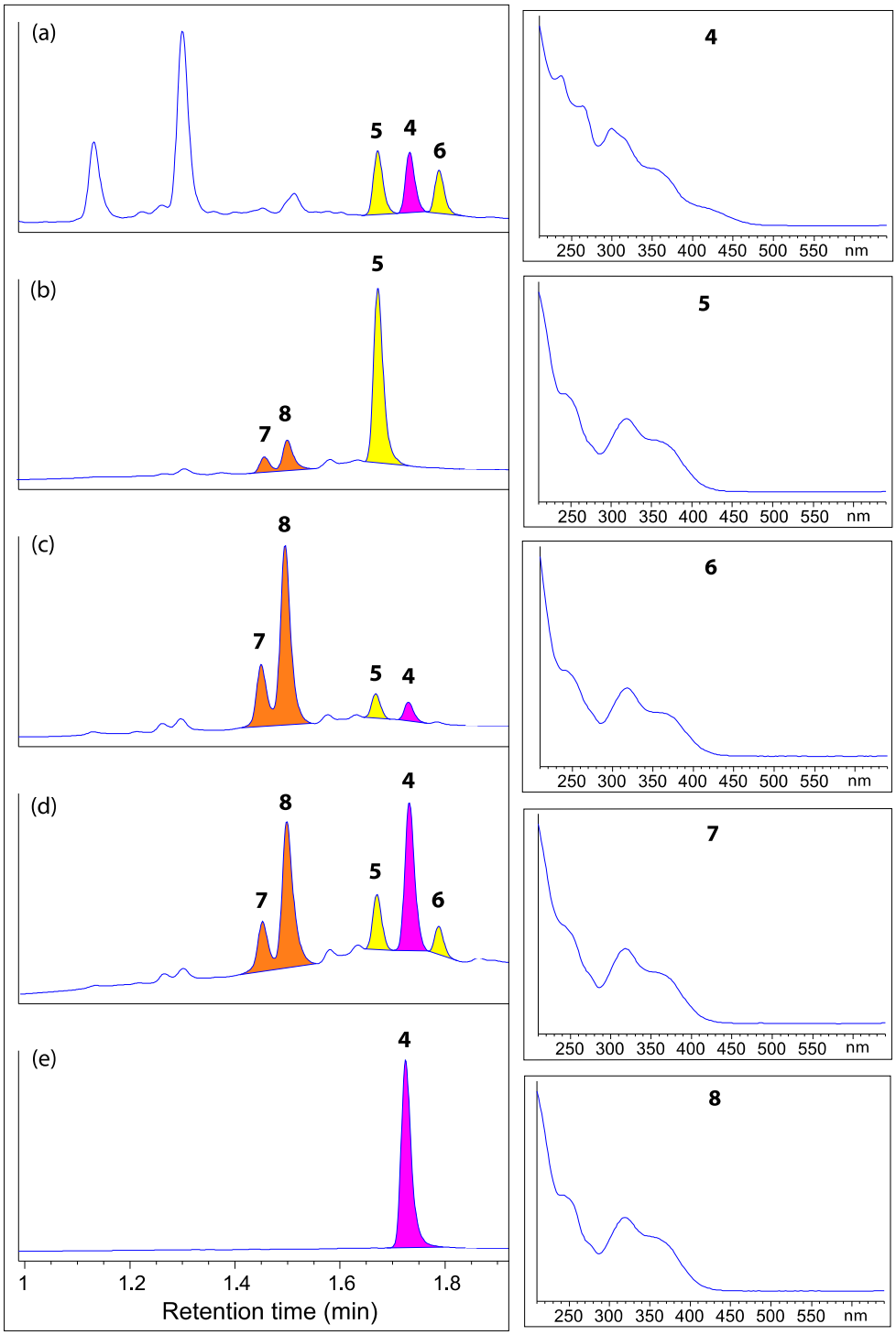

Figure S13. HPLC chromatograms [Agilent Zorbax SB-C_8 RRHD, 1.8 μm, 2.1 × 50 mm, 2.5 min gradient elution at 0.417 mL/min, 60–100% MeCN/H_2O with an isocratic 0.01% TFA modifier, detection at 210 nm] of (a) SPE fraction F; (±)-*spiro*-oxanthromicin B1 (**5**) at different times after HPLC purification (b) 30 min; (c) 3 h; (d) dried under N_2 and redissolved in MeOH, and (e) authentic standard of (±)-*spiro*-oxanthromicin (**4**) and UV spectra of spectra of compounds **4**–**8**.

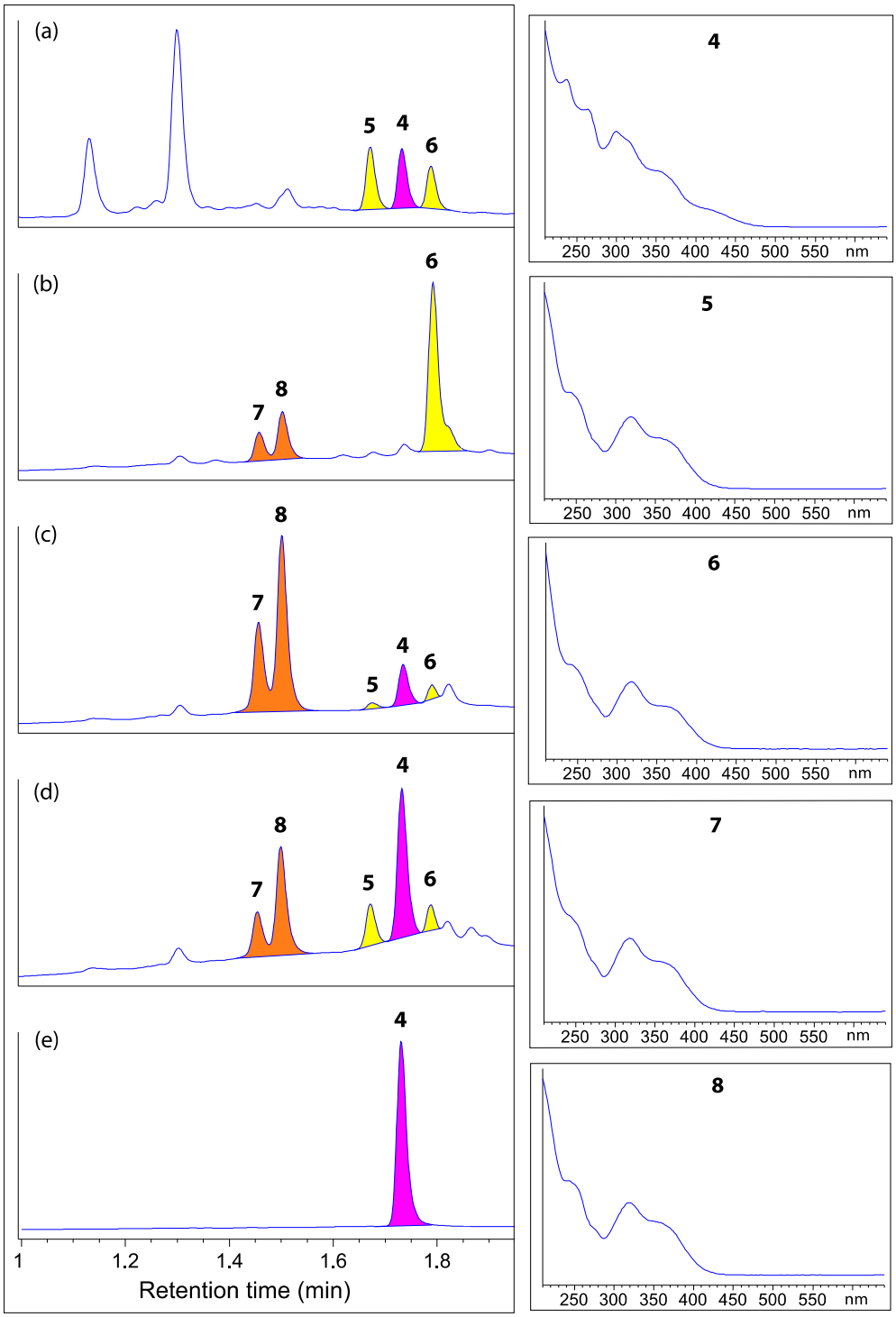

Figure S14. HPLC chromatograms [Agilent Zorbax SB-C_8 RRHD, 1.8 µm, 2.1 × 50 mm, 2.5 min gradient elution at 0.417 mL/min, 60–100% MeCN/H_2O with an isocratic 0.01% TFA modifier, detection at 210 nm] of (a) SPE fraction F; (±)-*spiro*-oxanthromicin B2 (**6**) at different times after HPLC purification (b) 30 min; (c) 3 h; (d) dried under N_2 and redissolved in MeOH, and (e) authentic standard of (±)-*spiro*-oxanthromicin (**4**) and UV spectra of compounds **4**–**8**.