

Supporting information for

Unraveling the complexity of complex mixtures by combining high-resolution pharmacological, analytical and spectroscopic techniques: Antidiabetic constituents in Chinese medicinal plants

Yong Zhao,^a Kenneth Thermann Kongstad,^a Yueqiu Liu,^a Chenhua He,^{a,b} and Dan Staerk^{*,a}

^aDepartment of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

^bCollege of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

*Corresponding author. Address: Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark. Tel.: +45 35336177; fax +45 35336001.

E-mail address: ds@sund.ku.dk (D. Staerk).

Table of Contents

Table S1. Plants tested for α -glucosidase, α -amylase and PTP1B inhibitory activity at a single concentration of 50 μ g/mL.

Table S2. Identification of metabolites by HPLC-HRMS and NMR.

Figure S1. α -glucosidase (A) and PTP1B (B) inhibition curves of crude ethyl acetate extracts of *Persicaria bistorta* (L.) Samp., *Dioscorea bulbifera* L., *Boehmeria nivea* (L.) Gaudich, and *Tinospora sagittata* Gagnep.

Figure S2. UV and HRMS spectra of compound **8** obtained in the HPLC-HRMS mode.

Figure S3. 1 H-NMR spectrum of the mixture of compound **7** and **8**.

Figure S4. 1 H-NMR spectrum of compound **7** and the mixture of compound **7** and **8**.

Figure S5. HSQC spectrum of the mixture of compound **7** and **8**.

Figure S6. HMBC spectrum of the mixture of compound **7** and **8**.

Figure S7. COSY spectrum of the mixture of compound **7** and **8**.

Figure S8. UV and HRMS spectra of compound **9** obtained in the HPLC-HRMS mode.

Figure S9. 1 H-NMR spectrum of compound **9**.

Figure S10. HSQC spectrum of compound **9**.

Figure S11. HMBC spectrum of compound **9**.

Figure S12. COSY spectrum of compound **9**.

Figure S13. UV and HRMS spectra of compound **10** obtained in the HPLC-HRMS mode.

Figure S14. 1 H-NMR spectrum of compound **10**.

Figure S15. HSQC spectrum of compound **10**.

Figure S16. HMBC spectrum of compound **10**.

Figure S17. COSY spectrum of compound **10**.

Figure S18. Triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profiling of *P. bistorta*.

Figure S19. IC₅₀ curves of isolates and Acarbose (reference compound) in the α -glucosidase inhibition assays.

Figure S20. IC₅₀ curves of isolates and RK682 (reference compound) in the PTP1B inhibition assays.

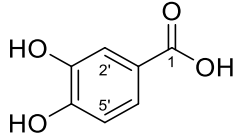
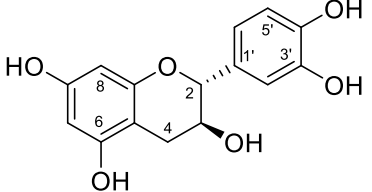
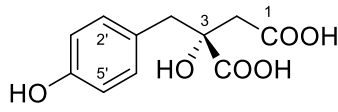
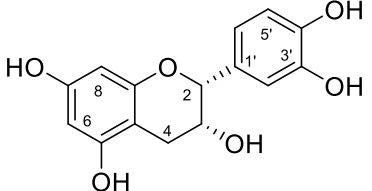
Table S1. Plants tested for α -glucosidase, α -amylase and PTP1B inhibitory activity at a single concentration of 50 μ g/mL.

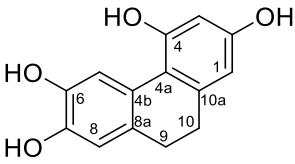
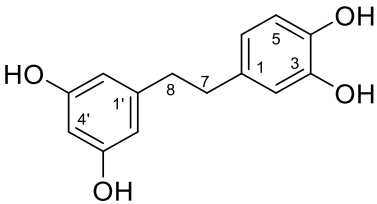
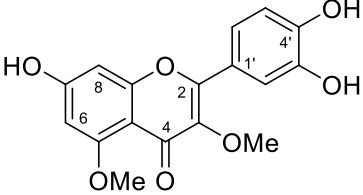
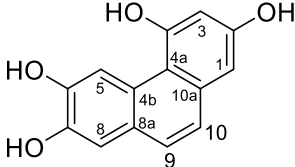
No.	Plant	Botanical family	Part used	Voucher specimen #	% inhibition at 50 μ g/mL ^a		
					AGH	AM	PTP1B
1	<i>Rhus chinensis</i> Mill.	Anacardiaceae	Root	zyc003	2.3	6.4	95.5
2	<i>Oenanthe javanica</i> (Bl.) DC.	Apiaceae	Seed	hch109	-2.5	5.8	78.0
3	<i>Oenanthe javanica</i> (Bl.) DC.	Apiaceae	Whole plant	zyc022	-1.6	4.5	92.9
4	<i>Ligusticum striatum</i> DC.	Apiaceae	Rhizome	hch114	33.2	8.4	42.9
5	<i>Rauvolfia verticillata</i> (Lour.) Baill.	Apocynaceae	Root	zyc054	20.6	6.3	28.9
6	<i>Amorphophallus rivieri</i> Durieu ex Riviere	Araceae	Tuber	zyc111	27.7	5.3	75.6
7	<i>Pinellia ternata</i> (Thunb.) Breit.	Araceae	Rhizome	zyc092	-2.1	3.6	88.1
8	<i>Ophiopogon japonicus</i> (Thunb.) Ker Gawl.	Asparagaceae	Tuber	hch104	-0.9	25.9	42.3
9	<i>Anemarrhena asphodeloides</i> Bunge.	Asparagaceae	Rhizome	hch111	51.5	8.7	82.8
10	<i>Taraxacum mongolicum</i> Hand.-Mazz.	Asteraceae	Whole plant	zyc203	4.2	6.8	52.7
11	<i>Senecio scandens</i> Buch.-Ham.	Asteraceae	Whole plant	zyc182	17.4	8.2	86.0
12	<i>Siegesbeckia orientalis</i> L.	Asteraceae	Whole plant	zyc202	18.9	7.2	89.5
13	<i>Bidens bipinnata</i> L.	Asteraceae	Whole plant	zyc184	23.3	3.2	105.8
14	<i>Bidens pilosa</i> L.	Asteraceae	Whole plant	zyc184	28.0	17.1	102.5
15	<i>Commelina communis</i> L.	Commelinaceae	Whole plant	zyc235	8.2	3.3	91.6
16	<i>Cornus officinalis</i> Siebold & Zucc.	Cornaceae	Fruit	hch112	14.2	6.0	73.3
17	<i>Sedum erythrostictum</i> Miq.	Crassulaceae	Whole plant	zyc263	61.9	10.3	87.8
18	<i>Trichosanthes kirilowii</i> Maxim.	Cucurbitaceae	Root	hch107	2.7	2.6	24.9
19	<i>Dioscorea oppositifolia</i> L.	Dioscoreaceae	Rhizome	hch103	5.7	6.2	3.8
20	<i>Dioscorea bulbifera</i> L.	Dioscoreaceae	Rhizome	zyc040	94.3	16.8	78.2
21	<i>Eucommia ulmoides</i> Oliv.	Eucommiaceae	Bark	hch113	5.8	0.8	73.1
22	<i>Phyllanthus urinaria</i> L.	Euphorbiaceae	Whole plant	zyc268	37.3	5.2	105.1
23	<i>Ocimum basilicum</i> L.	Lamiaceae	Whole plant	zyc118	53.2	7.5	78.9
24	<i>Prunella vulgaris</i> L.	Lamiaceae	Whole plant	zyc125	53.0	2.4	102.1
25	<i>Tinospora sagittata</i> Gagnep.	Menispermaceae	Rhizome	zyc145	83.8	4.5	67.1
26	<i>Melastoma dodecandrum</i> Roxb.	Melastomataceae	Whole plant	zyc175	39.6	23.4	96.1
27	<i>Cistanche deserticola</i> Y.C.Ma	Orobanchaceae	Rhizome	hch102	-0.5	5.6	12.6
28	<i>Zea mays</i> L.	Poaceae	Stigmas and style	hch108	23.8	4.1	82.7
29	<i>Lophatherum gracile</i> Brongn.	Poaceae	Leaves	hch110	1.1	4.1	81.3
30	<i>Reynoutria multiflora</i> (Thunb.) Moldenke	Polygonaceae	Tuber	hch101	-2.9	5.5	23.5
31	<i>Persicaria bistorta</i> (L.) Samp.	Polygonaceae	Rhizome	zyc085	88.9	44.6	99.4

32	<i>Portulaca oleracea</i> L.	Portulacaceae	Whole plant	zyc225	10.8	5.7	95.7
33	<i>Coptis chinensis</i> Franch.	Ranunculaceae	Rhizome	hch106	20.4	1.7	-3.7
34	<i>Semiaquilegia adoxoides</i> (DC.) Makino	Ranunculaceae	Root	zyc126	39.1	-0.3	77.9
35	<i>Potentilla kleiniana</i> Wight & Arn.	Rosaceae	Whole plant	zyc243	6.2	11.2	49.9
36	<i>Gardenia jasminoides</i> Ellis	Rubiaceae	Fruit	zyc315	8.1	6.1	56.2
37	<i>Paederia scandens</i> (Lour.) Merr.	Rubiaceae	Whole plant	zyc309	60.1	4.2	95.0
38	<i>Dimocarpus longan</i> Lour.	Sapindaceae	Seed	zyc295	41.3	9.8	27.4
39	<i>Scrophularia ningpoensis</i> Hemsl.	Scrophulariaceae	Tuber	hch105	0.6	6.6	65.9
40	<i>Boehmeria nivea</i> (L.) Gaudich	Urticaceae	Root	zyc105	84.8	8.6	97.2

^a Values expressed as mean of three independent experiments.

Table S2. Identification metabolites with HRMS and NMR data.

Peak	Detected precursor ion (m/z , positive)	Molecular formula	Compound	NMR Data ^a
1	155.0347 [M+H] ⁺ (calcd for C ₇ H ₇ O ₄ ⁺ : 155.0339) ΔM -5.0 ppm	C ₇ H ₆ O ₄	 3,4-Dihydroxybenzoic acid	¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.43 (1H, d, <i>J</i> = 1.6 Hz, H-2'), 7.41 (1H, dd, <i>J</i> = 8.1, 1.6 Hz, H-6'), 6.79 (1H, d, <i>J</i> = 8.1 Hz, H-5')
2	291.0864 [M+H] ⁺ (calcd for C ₁₅ H ₁₅ O ₆ ⁺ : 291.0863) ΔM -0.3 ppm	C ₁₅ H ₁₄ O ₆	 Catechin	¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 6.84 (1H, d, <i>J</i> = 1.8 Hz, H-2'), 6.76 (1H, d, <i>J</i> = 8.2 Hz, H-5'), 6.72 (1H, dd, <i>J</i> = 8.2, 1.8 Hz, H-6'), 5.93 (1H, s, H-8), 5.86 (1H, s, H-6), 4.56 (1H, d, <i>J</i> = 7.4 Hz, H-2), 3.98 (1H, m, H-3), 2.85 (1H, dd, <i>J</i> = 16.0, 2.4 Hz, H-4b), 2.50 (1H, dd, <i>J</i> = 16.0, 8.6 Hz, H-4a).
3	241.0706 [M+H] ⁺ (calcd for C ₁₁ H ₁₃ O ₆ ⁺ : 241.0707) ΔM 0.3 ppm	C ₁₁ H ₁₂ O ₆	 Eucomic acid	¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.07 (1H, d, <i>J</i> = 8.3 Hz, H-2' and H-6'), 6.66 (1H, d, <i>J</i> = 8.3 Hz, H-3' and H-5'), 2.96 (1H, d, <i>J</i> = 13.5 Hz, H-4a), 2.84 (1H, d, <i>J</i> = 16.2 Hz, H-2b), 2.82 (1H, d, <i>J</i> = 13.5 Hz, H-4b), 2.58 (1H, d, <i>J</i> = 16.2 Hz, H-2a)
4	291.0872 [M+H] ⁺ (calcd for C ₁₅ H ₁₅ O ₆ ⁺ : 291.0863) ΔM -3.1 ppm	C ₁₅ H ₁₄ O ₆	 Epicatechin	¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 6.97 (1H, d, <i>J</i> = 1.9 Hz, H-2'), 6.80 (1H, dd, <i>J</i> = 8.2, 1.9 Hz, H-6'), 6.76 (1H, d, <i>J</i> = 8.2 Hz, H-5'), 5.94 (1H, d, <i>J</i> = 2.2 Hz, H-8), 5.91 (1H, d, <i>J</i> = 2.2 Hz, H-6), 4.69 (1H, m, H-3), 2.87 (1H, dd, <i>J</i> = 16.8, 4.7 Hz, H-4b), 2.73 (1H, dd, <i>J</i> = 16.8, 2.8 Hz, H-4a).

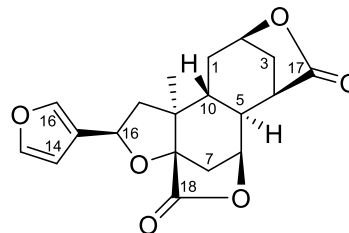
5	245.0795 [M+H] ⁺ (calcd for C ₁₄ H ₁₃ O ₄ ⁺ : 245.0808) ΔM 5.6 ppm	C ₁₄ H ₁₂ O ₄		¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.88 (1H, s, H-8), 6.59 (1H, s, H-5), 6.23 (1H, d, <i>J</i> = 2.4 Hz, H-3), 6.19 (1H, d, <i>J</i> = 2.4 Hz, H-1), 2.59 (2H, m, H-10), 2.57 (2H, m, H-9)
6	247.0955 [M+H] ⁺ (calcd for C ₁₄ H ₁₅ O ₄ ⁺ : 247.0965) ΔM 4.0 ppm	C ₁₄ H ₁₄ O ₄		¹ H NMR (methanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 6.65 (1H, d, <i>J</i> = 8.0 Hz, H-5), 6.61 (1H, d, <i>J</i> = 2.0 Hz, H-2), 6.47 (1H, dd, <i>J</i> = 8.0, 2.0 Hz, H-6), 6.13 (2H, d, <i>J</i> = 1.8 Hz, H-2', H-6'), 6.08 (1H, d, <i>J</i> = 1.8 Hz, H-4'), 2.68 (4H, m, H-7, 8)
7	331.0811 [M+H] ⁺ (calcd for C ₁₇ H ₁₅ O ₇ ⁺ : 331.0812) ΔM 0.3 ppm	C ₁₇ H ₁₄ O ₇		¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.48 (1H, d, <i>J</i> = 2.0 Hz, H-2'), 7.36 (1H, dd, <i>J</i> = 8.2, 2.0 Hz, H-6'), 6.87 (1H, d, <i>J</i> = 8.2 Hz, H-5'), 6.43 (1H, d, <i>J</i> = 1.8 Hz, H-8), 6.35 (1H, d, <i>J</i> = 1.8 Hz, H-6), 3.79 (3H, s, 5-OMe), 3.69 (3H, s, 3-OMe)
8	243.0649 [M+H] ⁺ (calcd for C ₁₄ H ₁₁ O ₄ ⁺ : 243.0652) ΔM 1.0 ppm	C ₁₄ H ₁₀ O ₄		See as Table 2 in manuscript

9	257.0449 [M+H] ⁺ (calcd for C ₁₄ H ₉ O ₅ ⁺ : 257.0444) ΔM -1.9 ppm	C ₁₄ H ₈ O ₅		See as Table 2 in manuscript
2,6,7-Trihydroxy-1,4-phenanthrenedione				
10	483.1064 [M+H] ⁺ (calcd for C ₂₈ H ₁₉ O ₈ ⁺ : 483.1074) ΔM 2.2 ppm	C ₂₈ H ₁₈ O ₈		See as Table 2 in manuscript
[1,1'-Biphenanthren]-2,2',3,3',6,6',7,7'-octaol				
11	485.1231 [M+H] ⁺ (calcd for C ₂₈ H ₂₁ O ₈ ⁺ : 485.1231) ΔM 5.0 ppm	C ₂₈ H ₂₀ O ₈		<p>¹H NMR (methanol-<i>d</i>₄, 600 MHz, δ in ppm, <i>J</i> in Hz) δ:</p> <p>9.20 (1H, s, H-5), 7.31 (1H, d, <i>J</i> = 8.9 Hz, H-9), 7.08 (1H, s, H-8), 7.00 (1H, d, <i>J</i> = 8.9 Hz, H-10), 6.79 (1H, d, <i>J</i> = 2.0 Hz, H-2'), 6.75 (1H, d, <i>J</i> = 8.1 Hz, H-5'), 6.70 (1H, s, H-3), 6.68 (1H, dd, <i>J</i> = 8.1, 2.0 Hz, H-6'), 6.14 (1H, t, <i>J</i> = 2.2 Hz, H-4''), 6.11 (2H, d, <i>J</i> = 2.2, H-2'', 6''), 5.34 (1H, d, <i>J</i> = 5.8 Hz, H-7'), 4.59 (1H, d, <i>J</i> = 5.8 Hz, H-8').</p> <p>¹³C NMR (methanol-<i>d</i>₄, 150 MHz, δ in ppm) δ: 114.2 (C-1), 158.6 (C-2), 96.5 (C-3), 115.4 (C-4a), 131.3 (C-4b), 113.7 (C-5), 146.0 (C-6), 144.7 (C-7), 112.6 (C-8), 127.3 (C-8a), 128.3 (C-9), 121.2 (C-10), 127.3 (C-10a), 135.0 (C-1'), 113.4 (C-2'), 146.0 (C-3'), 146.0 (C-4'), 116.0 (C-5'), 118.2 (C-6'), 95.0 (C-7'), 58.7 (C-8'), 147.7 (C-1''), 107.0 (C-2''), 107.0 (C-6''), 159.7 (C-3''), 159.7 (C-5''), 101.9 (C-4'').</p>
Cassigarol D				

12

345.1318 [M+H]⁺ (calcd for C₁₉H₂₁O₆⁺:
345.1333) ΔM 4.2 ppm

C₁₉H₂₀O₆



Diosbulbin B

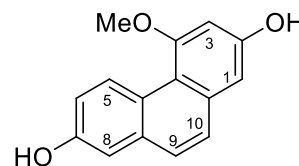
¹H NMR (acetone-*d*₆, 600 MHz, δ in ppm, *J* in Hz) δ: 7.62 (1H, s, H-16), 7.47 (1H, d, *J* = 1.5 Hz, H-15), 6.94 (1H, d, *J* = 1.5 Hz, H-14), 5.30 (1H, dd, *J* = 10.9, 5.5 Hz, H-12), 4.84 (1H, m, H-2), 4.75 (1H, d, *J* = 5.9 Hz, H-6), 2.71 (1H, m, H-4), 2.50 (1H, m, H-3β), 1.99 (1H, m, H-3α), 2.46 (1H, m, H-7β), 2.26 (1H, m, H-7α), 2.25 (1H, m, H-5), 2.06 (1H, m, H-11β), 1.98 (1H, m, H-11α), 2.01 (1H, m, H-1β), 1.77 (1H, m, H-1α), 1.90 (1H, m, H-10), 1.29 (3H, s, H-19).

¹³C NMR (acetone-*d*₆, 150 MHz, δ in ppm) δ: 29.6 (C-1), 77.2 (C-2), 39.4 (C-3), 42.7 (C-4), 42.7 (C-5), 77.9 (C-6), 37.8 (C-7), 90.2 (C-8), 45.9 (C-9), 39.4 (C-10), 42.8 (C-11), 75.6 (C-12), 126.9 (C-13), 111.0 (C-14), 143.3 (C-15), 141.3 (C-16), 175.7 (C-17), 177.3 (C-18), 16.6 (C-19).

13

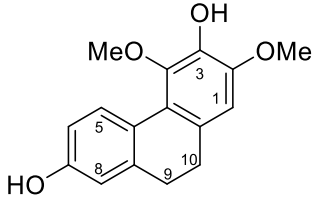
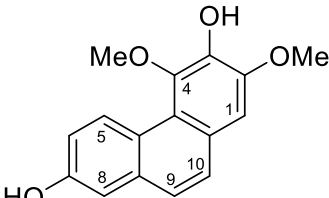
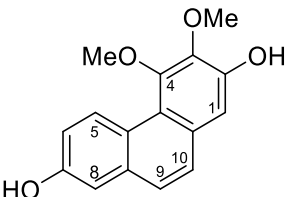
241.0860 [M+H]⁺ (calcd for C₁₅H₁₃O₃⁺:
241.0859) ΔM -0.4 ppm

C₁₅H₁₂O₃

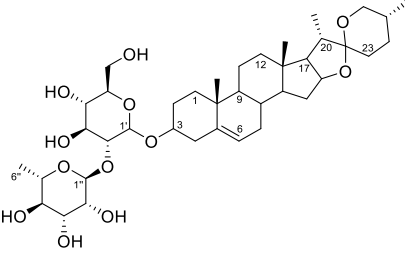
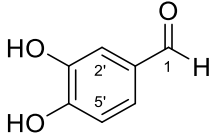
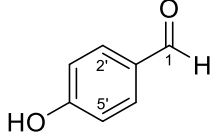
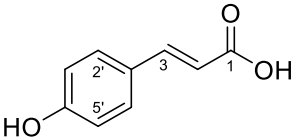


Flavanthrinin

¹H NMR (acetone-*d*₆, 600 MHz, δ in ppm, *J* in Hz) δ: 9.37 (1H, d, *J* = 9.2 Hz, H-5), 7.55 (1H, d, *J* = 8.8 Hz, H-9), 7.51 (1H, d, *J* = 8.8 Hz, H-10), 7.23 (1H, d, *J* = 2.8 Hz, H-6), 7.14 (1H, dd, *J* = 9.2, 2.8 Hz, H-6), 6.90 (1H, d, *J* = 2.4 Hz, H-1), 6.82 (1H, d, *J* = 2.4 Hz, H-3), 4.09 (3H, s, 4-OMe).

14	273.1112 [M+H] ⁺ (calcd for C ₁₆ H ₁₇ O ₄ ⁺ : 273.1121) ΔM 3.6 ppm	C ₁₆ H ₁₆ O ₄	 <p style="text-align: center;">Flavanthridin</p>	¹ H NMR (acetone- <i>d</i> ₆ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 8.09 (1H, d, <i>J</i> = 8.5 Hz, H-5), 6.73-6.65 (2H, m, H-6, 8), 6.56 (1H, s, H-1), 3.83 (3H, s, 3-OMe), 3.64 (3H, s, 4-OMe), 2.64 (4H, s, H-9, 10). ¹³ C NMR (acetone- <i>d</i> ₆ , 150 MHz, δ in ppm) δ: 107.6 (C-1), 128.4 (C-5), 114.6 (C-6), 113.4 (C-8), 30.0 (C-9), 30.3 (C-10), 59.4 (2-OMe), 56.0 (4-OMe).
15	271.0962 [M+H] ⁺ (calcd for C ₁₆ H ₁₅ O ₄ ⁺ : 271.0965) ΔM 1.1 ppm	C ₁₆ H ₁₄ O ₄	 <p style="text-align: center;">2,4-Dimethoxy-3,7-phenanthrenediol</p>	¹ H NMR (acetone- <i>d</i> ₆ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 9.34 (1H, d, <i>J</i> = 9.2, H-5), 7.60 (1H, d, <i>J</i> = 8.8, H-10), 7.46 (1H, d, <i>J</i> = 8.8, H-9), 7.24 (1H, s, H-8), 7.23 (1H, s, H-1), and 7.19 (1H, d, <i>J</i> = 9.2, H-6), 3.99 (3H, s, 2-OMe) and 3.93 (3H, s, 4-OMe) ¹³ C NMR (acetone- <i>d</i> ₆ , 150 MHz, δ in ppm) δ: 106.0 (C-1), 129.0 (C-5), 117.4 (C-6), 112.2 (C-8), 125.3 (C-9), 128.1 (C-10), 59.6 (4-OMe), 56.3 (2-OMe). ^b
16	271.0967 [M+H] ⁺ (calcd for C ₁₆ H ₁₅ O ₄ ⁺ : 271.0965) ΔM -1.0 ppm	C ₁₆ H ₁₄ O ₄	 <p style="text-align: center;">Nudol</p>	¹ H NMR (acetone- <i>d</i> ₆ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 9.32 (1H, d, <i>J</i> = 9.2, H-5), 7.50 (1H, d, <i>J</i> = 8.8, H-10), 7.49 (1H, d, <i>J</i> = 8.8, H-9), 7.24 (1H, m, H-6), 7.23 (1H, s, H-8), 7.19 (1H, d, <i>J</i> = 9.2, H-6), 4.00 (3H, s, 3-OMe), 3.97 (3H, s, 4-OMe) ¹³ C NMR (acetone- <i>d</i> ₆ , 150 MHz, δ in ppm) δ: 108.2 (C-1), 128.3 (C-5), 116.5 (C-6), 111.8 (C-8), 126.3 (C-9), 127.1 (C-10), 61.2 (4-OMe), 59.7 (2-OMe). ^b

17	275.1267 [M+H] ⁺ (calcd for C ₁₆ H ₁₉ O ₄ ⁺ : 275.1278) ΔM 4.1 ppm	C ₁₆ H ₁₈ O ₄		¹ H NMR (chloroform- <i>d</i> , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.10 (1H, d, <i>J</i> = 7.5, H-6), 7.08 (1H, m, H-4), 6.87 (1H, m, H-5), 6.75 (1H, d, <i>J</i> = 7.5, H-3), 6.49 (1H, d, <i>J</i> = 1.8, H-2'), 6.25 (1H, d, <i>J</i> = 1.8, H-6'), 5.70 (1H, brs, 3'-OH), 4.61 (1H, brs, 2-OH), 3.87 (3H, s, 4'-OMe) and 3.80 (3H, s, 5'-OMe), 2.89 (2H, m, H-7), 2.83 (2H, m, H-8).
18	289.1443 [M+H] ⁺ (calcd for C ₁₇ H ₂₁ O ₄ ⁺ : 289.1434) ΔM -3.0 ppm	C ₁₇ H ₂₀ O ₄		¹ H NMR (chloroform- <i>d</i> , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.10 (1H, d, <i>J</i> = 7.5, H-6), 7.08 (1H, m, H-4), 6.88 (1H, m, H-5), 6.75 (1H, d, <i>J</i> = 7.5, H-3), 6.38 (1H, s, H-2', H-6'), 4.55 (1H, brs, 2-OH), 3.83 (3H, s, 4'-OMe) and 3.81 (6H, s, 3', 5'-OMe), 2.90 (2H, m, H-7), 2.86 (2H, m, H-8).
19	445.2111 [M+H] ⁺ (calcd for C ₂₇ H ₂₉ N ₂ O ₄ ⁺ : 445.2122) ΔM 2.3 ppm	C ₂₇ H ₂₈ N ₂ O ₄		¹ H NMR (chloroform- <i>d</i> , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.71 (2H, m, ring A, H-2, H-6), 7.51 (1H, m, ring C, H-4), 7.42 (2H, m, ring C, H-3, H-5), 7.30 (2H, m, ring C, H-2, H-6), 7.24 (4H, m, ring B), 7.17 (3H, m, ring A), 7.10 (1H, t, <i>J</i> = 6.7, ring B), 4.30 (1H, m, H-2), 3.90 (1H, dd, <i>J</i> = 11.4, 4.5, H-1a), 3.13 (1H, m, H-1b), 3.09 (1H, dd, <i>J</i> = 13.8, 6.5 Hz, H-3β), 2.98 (1H, dd, <i>J</i> = 13.8, 8.5 Hz, H-β), 2.82 (1H, dd, <i>J</i> = 13.8, 8.5 Hz, H-3a), 2.75 (1H, m, H-3b), 1.98 (3H, s, Me).

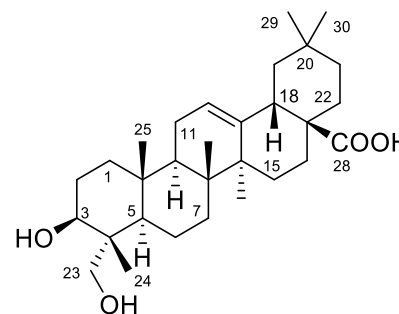
20	723.4301 [M+H] ⁺ (calcd for C ₃₉ H ₆₃ O ₁₂ ⁺ : 723.4314) ΔM 1.8 ppm	C ₃₉ H ₆₂ O ₁₂		¹ H NMR (pyridine- <i>d</i> ₅ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 6.35 (s, 1H, H-1''), 5.27 (br d, 1H, <i>J</i> = 3.8 Hz, H-6), 5.00 (1H, m, H-1'), 4.76 (1H, br s, H-2''), 4.59 (1H, m, H-3''), 4.53-4.45 (2H, m, H-16, H-5''), 4.28-4.24 (3H, m, H-2', H-4', H-4''), 4.13 (1H, m, H-3'), 3.91-3.84 (2H, m, H-3, H-50), 3.56 (1H, m, H-26), 3.46 (1H, t, <i>J</i> = 9.6 Hz, H-26), 1.73 (3H, d, <i>J</i> = 6.5 Hz, 6''-Me), 1.10 (3H, d, <i>J</i> = 6.7 Hz, 21-Me), 1.01 (3H, s, 18-Me), 0.78 (3H, s, 18-Me), 0.65 (3H, br s, 27-Me)
21	139.0391 [M+H] ⁺ (calcd for C ₇ H ₇ O ₃ ⁺ : 139.0390) ΔM -1.1 ppm	C ₇ H ₆ O ₃		¹ H NMR (metanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 9.66 (1H, s, H-1), 7.30 (1H, dd, <i>J</i> = 8.0, 1.6 Hz, H-6'), 7.29 (1H, d, <i>J</i> = 1.6 Hz, H-2'), 6.89 (1H, d, <i>J</i> = 8.0 Hz, H-5')
22	123.0438 [M+H] ⁺ (calcd for C ₇ H ₇ O ₂ ⁺ : 123.0441) ΔM 2.1 ppm	C ₇ H ₆ O ₂		¹ H NMR (metanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 9.76 (1H, s, H-1), 7.78 (1H, d, <i>J</i> = 8.7 Hz, H-2', 6'), 6.92 (1H, d, <i>J</i> = 8.7 Hz, H-3', 5')
23	165.0540 [M+H] ⁺ (calcd for C ₉ H ₉ O ₃ ⁺ : 165.0546) ΔM 3.6 ppm	C ₉ H ₈ O ₃		¹ H NMR (metanol- <i>d</i> ₄ , 600 MHz, δ in ppm, <i>J</i> in Hz) δ: 7.49 (1H, d, <i>J</i> = 15.9 Hz, H-3), 7.41 (1H, d, <i>J</i> = 8.6 Hz, H-2', 6'), 6.79 (1H, d, <i>J</i> = 8.6 Hz, H-3', 5'), 6.30 (1H, d, <i>J</i> = 15.9 Hz, H-2).

24

473.3638 [M+H]⁺ (calcd for C₃₀H₄₉O₄⁺;

473.3625) ΔM -1.3 ppm

C₃₀H₄₈O₄



Hederagenin

¹H NMR (DMSO-*d*₆, 600 MHz, δ in ppm, *J* in Hz) δ: 5.15 (1H, s, H-12), 3.43 (1H, overlapped, H-3), 3.32 (1H, overlapped, H-23a), 3.07 (1H, d, *J* = 10.6 Hz, H-23b), 2.74 (1H, dd, *J* = 3.2, 13.7 Hz, H-18), 1.89 (1H, t, *J* = 12.6 Hz, H-16β), 1.80 (2H, m, H-11α, H-11β), 1.66 (1H, m, H-15β), 1.61 (1H, m, H-22β), 1.60 (1H, m, H-19β), 1.50 (1H, overlapped, H-9), 1.48 (1H, m, H-16α), 1.47 (1H, m, H-1β), 1.46 (2H, m, H-2), 1.43 (1H, m, H-7β), 1.42 (1H, m, H-22α), 1.40 (1H, m, H-6α), 1.31 (1H, m, H-21α), 1.23 (1H, m, H-6β), 1.16 (1H, m, H-7α), 1.12 (1H, m, H-21α), 1.04 (1H, m, H-19α), 0.97 (1H, m, H-15α), 1.10 (1H, m, H-5α), 1.09 (3H, s, H-27), 0.87 (9H, s, H-25, H-29, H-30), 0.84 (1H, m, H-1α), 0.71 (3H, s, H-26), 0.53 (3H, s, H-24).

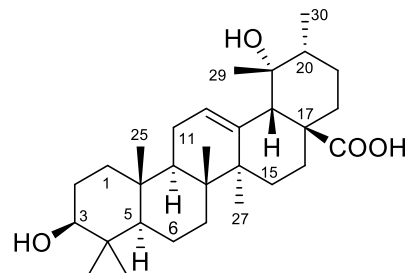
¹³C NMR (DMSO-*d*₆, 150 MHz, δ in ppm) δ: 37.7 (C-1), 26.3 (C-2), 70.0 (C-3), 41.6 (C-4), 46.1 (C-5), 17.3 (C-6), 31.8 (C-7), 38.6 (C-8), 46.9 (C-9), 35.9 (C-10), 22.8 (C-11), 121.3 (C-12), 143.6 (C-13), 40.8 (C-14), 27.0 (C-15), 22.5 (C-16), 45.1 (C-17), 40.6 (C-18), 45.6 (C-19), 30.2 (C-20), 33.2 (C-21), 31.9 (C-22), 64.4 (C-23), 12.4 (C-24), 15.2 (C-25), 16.7 (C-26), 25.5 (C-27), 32.6 (C-29), 23.2 (C-30).

25

473.3613 [M+H]⁺ (calcd for C₃₀H₄₉O₄⁺;

473.3625) ΔM 2.6 ppm

C₃₀H₄₈O₄



Pomolic acid

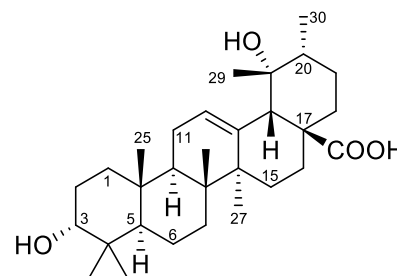
¹H NMR (DMSO-*d*₆, 600 MHz, δ in ppm, *J* in Hz) δ: 5.15

(1H, s, H-12), 3.00 (1H, m, H-3), 2.48 (1H, m, H-16β),
2.39 (1H, m, H-18), 1.89 (1H, m, H-11β), 1.83 (1H, m, H-
11α), 1.69 (1H, m, H-15α), 1.68 (1H, m, H-2β), 1.61 (1H,
m, H-21α), 1.59 (1H, m, H-22β), 1.58 (1H, m, H-9), 1.52
(1H, m, H-1β), 1.50 (1H, m, H-22α), 1.49 (1H, m, H-1α),
1.46 (1H, m, H-6β), 1.45 (1H, m, H-7β), 1.38 (1H, m, H-
16α), 1.31 (1H, m, H-6α), 1.28 (3H, s, H-27), 1.26 (1H,
m, H-20), 1.22 (1H, m, H-7α), 1.12 (1H, m, H-21β), 1.07
(3H, s, H-29), 0.89 (3H, s, H-23), 0.85 (3H, s, H-25), 0.87
(1H, m, H-15β), 0.84 (3H, s, H-30), 0.70 (3H, s, H-
26), 0.68 (3H, s, H-24), 0.67 (1H, m, H-5).

¹³C NMR (DMSO-*d*₆, 150 MHz, δ in ppm) δ: 37.9 (C-1),
27.9 (C-2), 76.7 (C-3), 38.2 (C-4), 54.6 (C-5), 17.9 (C-6),
32.6 (C-7), 39.1 (C-8), 46.5 (C-9), 38.1 (C-10), 23.0 (C-
11), 126.8 (C-12), 138.6 (C-13), 40.9 (C-14), 27.9 (C-15),
25.0 (C-16), 46.8 (C-17), 53.1 (C-18), 73.4 (C-19), 41.3
(C-20), 25.7 (C-21), 37.1 (C-22), 28.1 (C-23), 15.8 (C-24),
15.4 (C-25), 16.4 (C-26), 23.8 (C-27), 178.8 (C-28), 26.1
(C-29), 16.1 (C-30).

26

473.3607 [M+H]⁺ (calcd for C₃₀H₄₉O₄⁺:
473.3625) ΔM 3.9 ppm

C₃₀H₄₈O₄3-*epi*-pomolic acid

¹H NMR (DMSO-*d*₆, 600 MHz, δ in ppm, *J* in Hz) δ: 5.17 (1H, s, H-12), 3.00 (1H, m, H-3), 2.48 (1H, m, H-16β), 2.39 (1H, m, H-18), 1.90 (1H, m, H-11β), 1.82 (1H, m, H-11α), 1.69 (1H, m, H-15β), 1.69 (1H, m, H-9), 1.61 (1H, m, H-21α), 1.60 (1H, m, H-22β), 1.45 (1H, m, H-1β), 1.45 (1H, m, H-7β), 1.51 (1H, m, H-22α), 1.38 (1H, m, H-16α), 1.34 (1H, m, H-6β), 1.30 (1H, m, H-1α), 1.29 (3H, s, H-27), 1.29 (1H, m, H-6α), 1.26 (1H, m, H-20), 1.23 (1H, m, H-5), 1.22 (1H, m, H-7α), 1.12 (1H, m, H-21β), 1.08 (3H, s, H-29), 0.87 (1H, m, H-15α), 0.86 (3H, s, H-25), 0.84 (3H, s, H-30), 0.83 (3H, s, H-23), 0.76 (3H, s, H-24), 0.70 (3H, s, H-26).

¹³C NMR (DMSO-*d*₆, 150 MHz, δ in ppm) δ: 32.4 (C-1), 76.0 (C-3), 36.6 (C-4), 47.9 (C-5), 17.6 (C-6), 32.4 (C-7), 40.2 (C-8), 46.4 (C-9), 36.1 (C-10), 23.2 (C-11), 126.7 (C-12), 138.3 (C-13), 40.2 (C-14), 27.6 (C-15), 25.0 (C-16), 46.7 (C-17), 53.0 (C-18), 73.5 (C-19), 41.0 (C-20), 25.5 (C-21), 37.0 (C-22), 28.4 (C-23), 22.0 (C-24), 14.7 (C-25), 16.4 (C-26), 23.8 (C-27), 26.2 (C-29), 16.0 (C-30).

27

279.2306 [M+H]⁺ (calcd for C₁₈H₃₁O₂⁺:
279.2319) ΔM 4.4 ppm

C₁₈H₃₀O₂

α-Linolenic acid

¹H NMR (chloroform-*d*, 600 MHz, δ in ppm, *J* in Hz) δ: 5.39-5.35 (6H, m, H-9, H-10, H-12, H-13, H-15, H-16), 2.81 (4H, br t, *J* = 5.4 Hz, H-11 and H-14), 2.34 (2H, t, *J* = 7.4 Hz, H-2), 2.10-2.04 (4H, m, H-8 and H-17), 1.64-1.61 (2H, m, H-3), 1.35-1.25 (8H, m, H-4, H-5, H-6, H-7), 0.96 (3H, t, *J* = 7.4 Hz, H-18).

28

281.2464 [M+H]⁺ (calcd for C₁₈H₃₃O₂⁺:

281.2475) ΔM 3.9 ppm

C₁₈H₃₂O₂

Linolenic acid

¹H NMR (chloroform-*d*, 600 MHz, δ in ppm, *J* in Hz) δ:
5.37-5.32 (4H, m, H-9, H-10, H-12, H-13), 2.76 (2H, t, *J* =
6.8 Hz, H-11), 2.33 (2H, t, *J* = 7.6 Hz, H-2), 2.03-2.06
(4H, m, H-8 and H-14), 1.62 (2H, p, *J* = 7.3 Hz, H-3),
1.36-1.25 (14H, m, H-4, H-5, H-6, H-7, H-15, H-16 and
H-17), 0.88 (3H, t, *J* = 7.2 Hz, H-18).

^a ¹³C NMR data were obtained from HSQC and/or HMBC spectra. ^b Quaternary carbon was not determined.

Figure S1. α -Glucosidase (A) and PTP1B (B) inhibition curves of crude ethyl acetate extracts of *Persicaria bistorta* (L.) Samp., *Dioscorea bulbifera* L., *Boehmeria nivea* (L.) Gaudich, and *Tinospora sagittata* Gagnep.

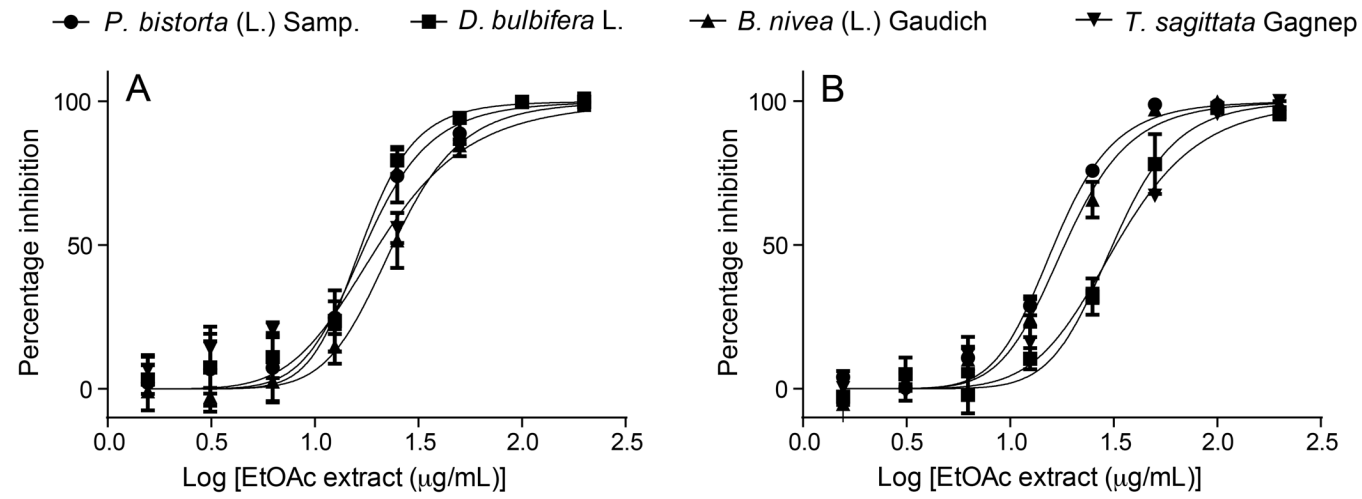


Figure S2. UV and HRMS spectra of compound **8** obtained in the HPLC-HRMS mode.

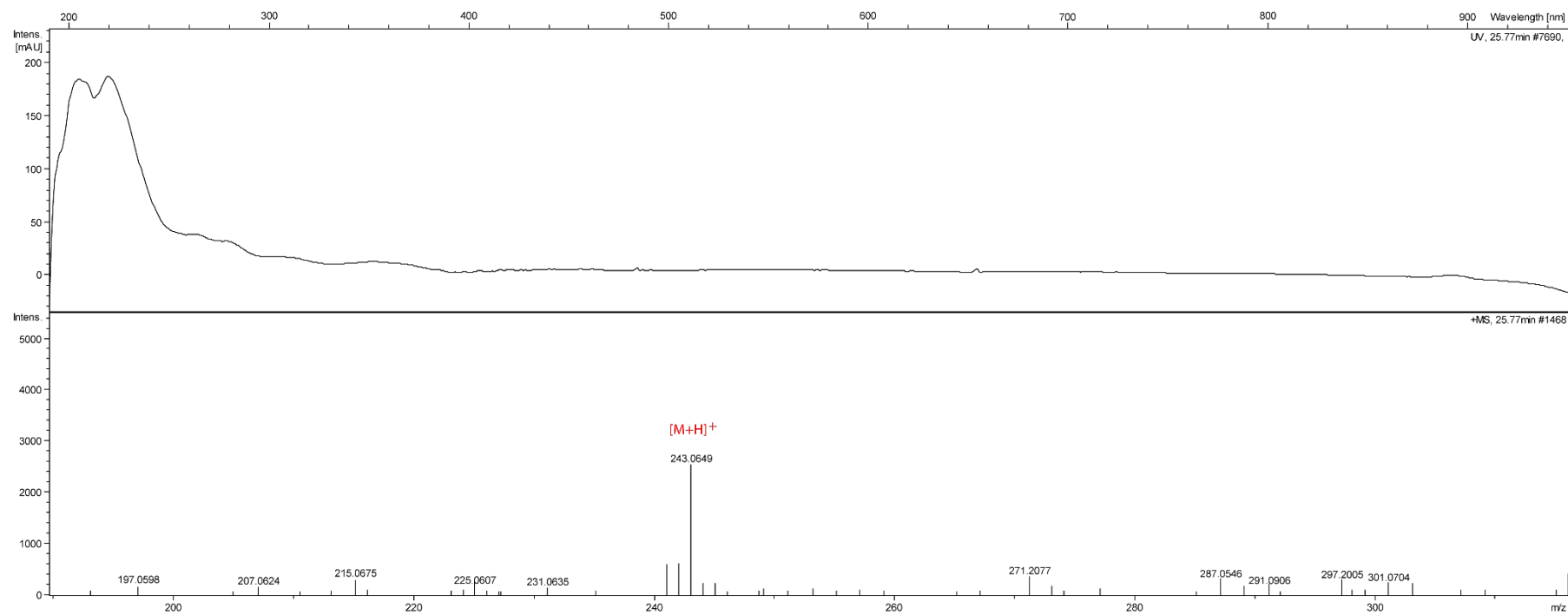


Figure S3. ¹H-NMR spectrum of the mixture of compound **7** and **8** (600 MHz, Methanol-*d*₄).

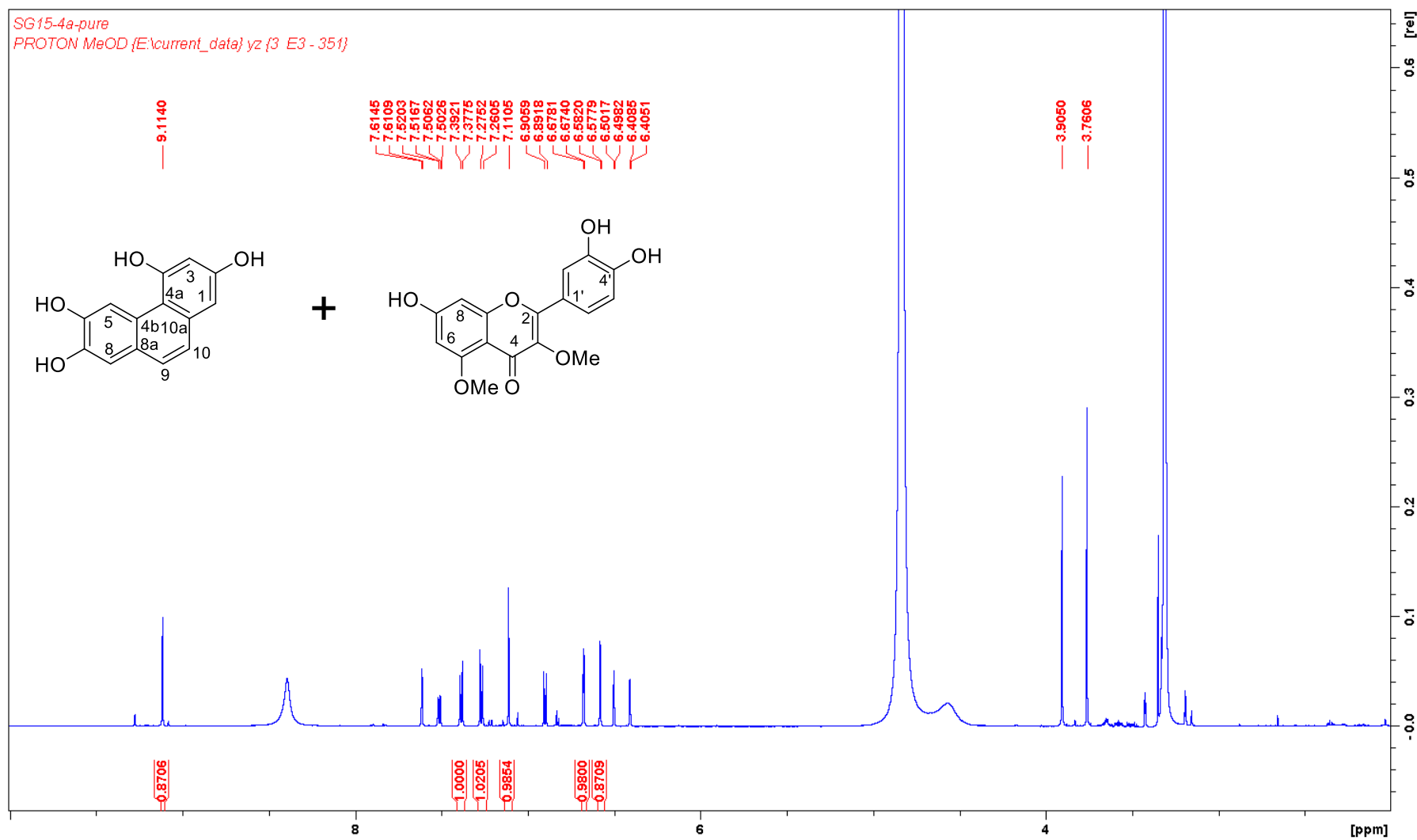


Figure S4. $^1\text{H-NMR}$ spectrum of compound **7** and the mixture of compound **7** and **8** (600 MHz, Methanol- d_4).

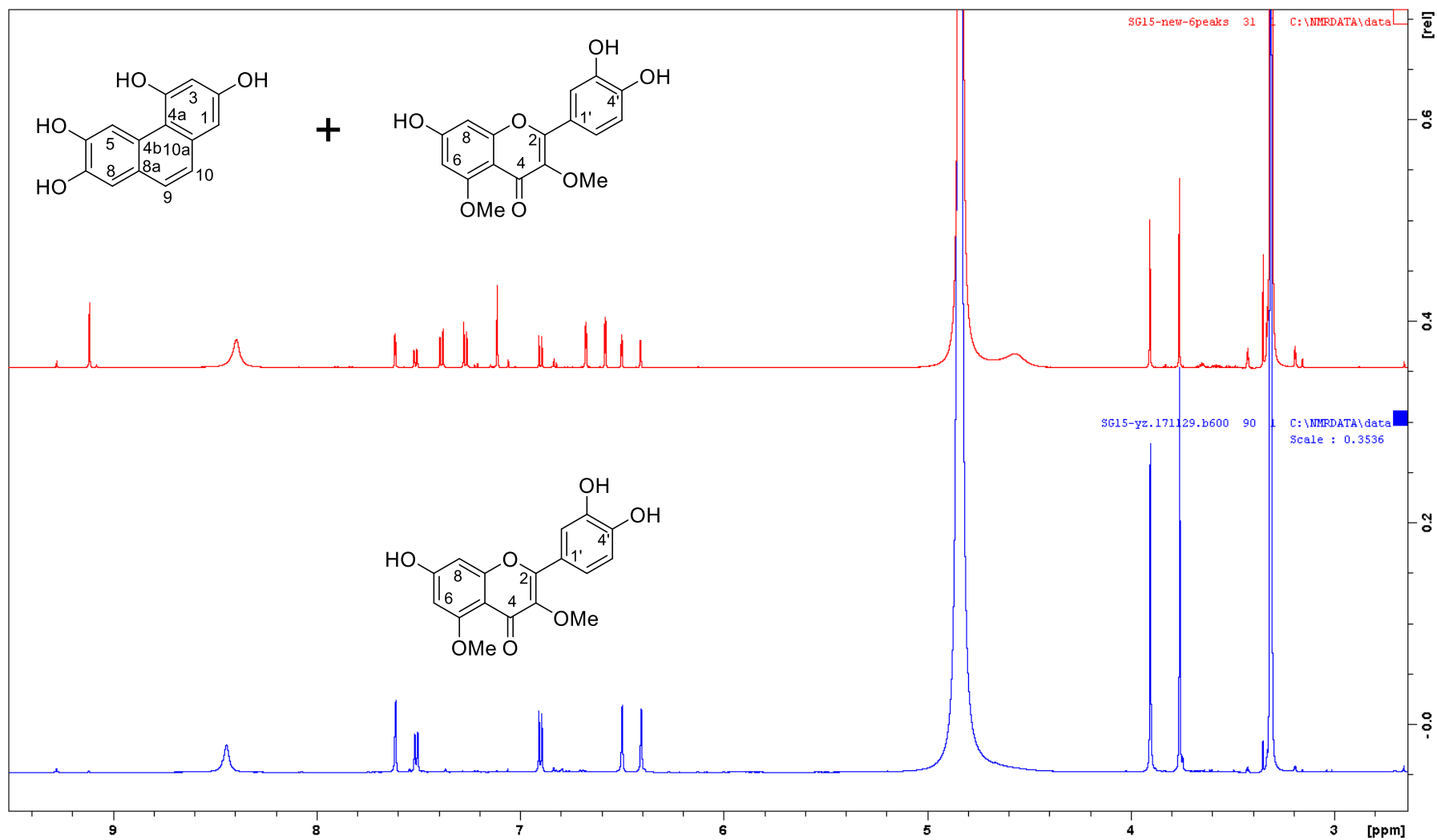


Figure S5. HSQC spectrum of the mixture of compound 7 and 8 (600 MHz, Methanol- d_4).

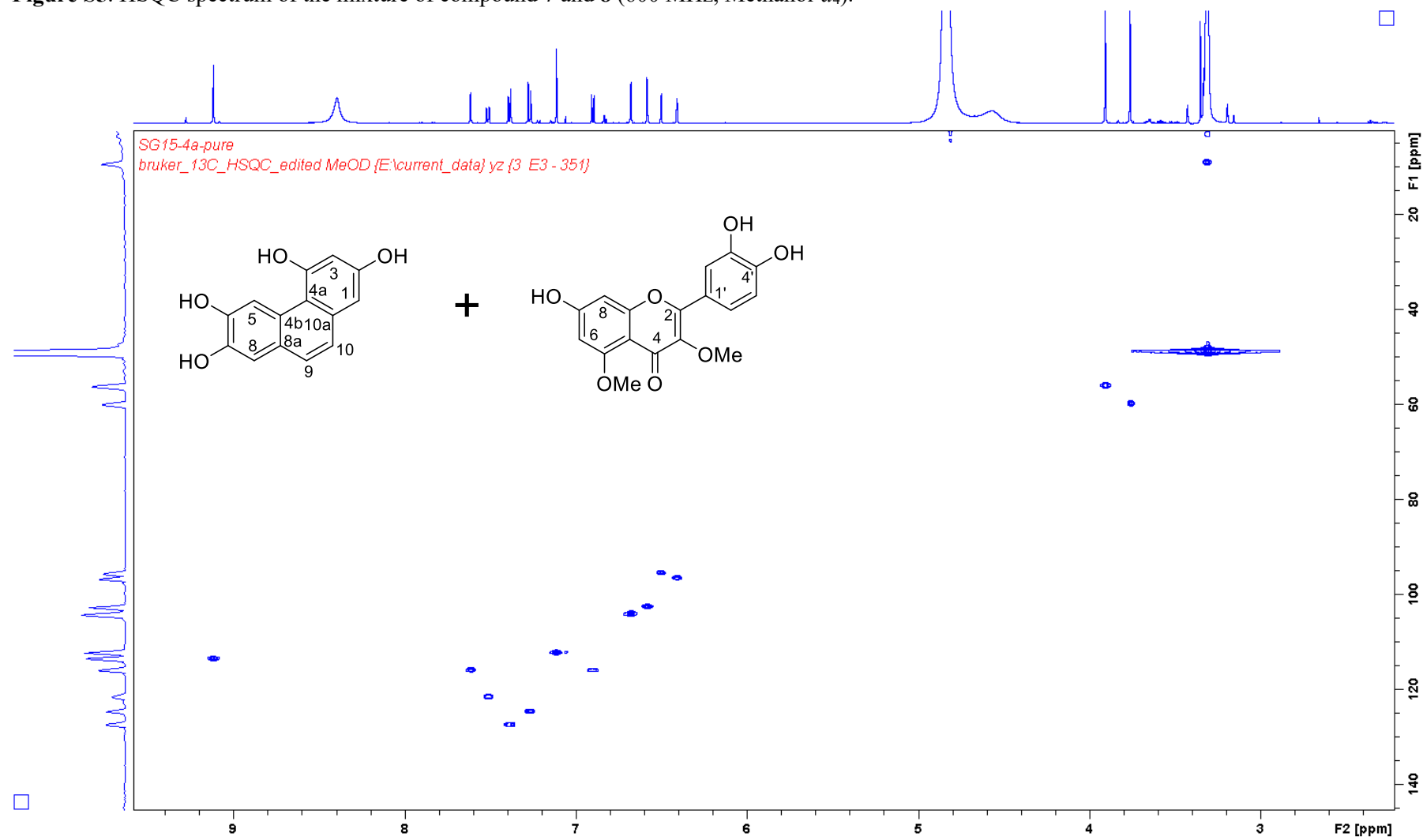


Figure S6. HMBC spectrum of the mixture of compound **7** and **8** (600 MHz, Methanol-*d*₄).

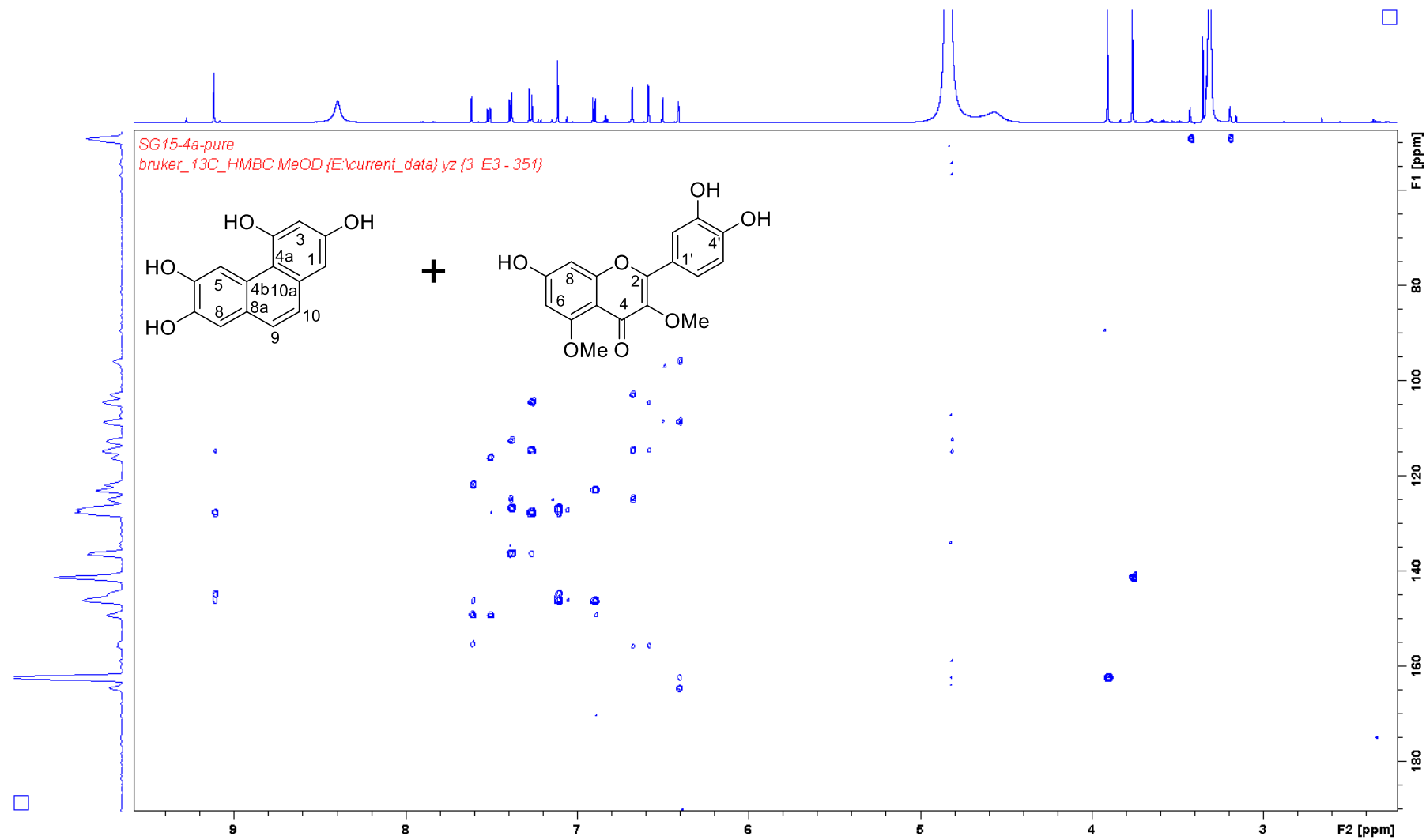


Figure S7. COSY spectrum of the mixture of compound **7** and **8** (600 MHz, Methanol-*d*₄).

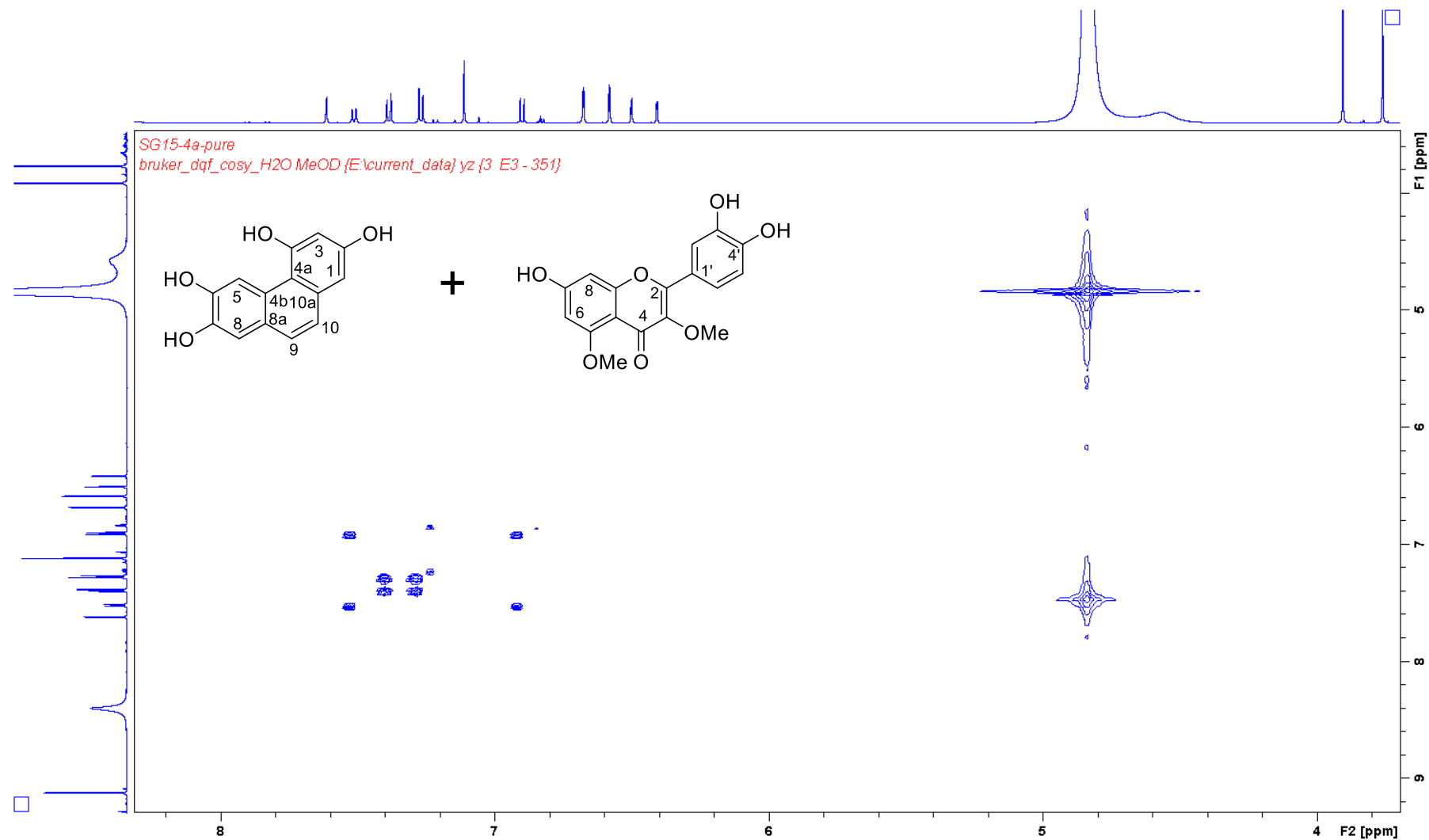


Figure S8. UV and HRMS spectra of compound **9** obtained in the HPLC-HRMS mode.

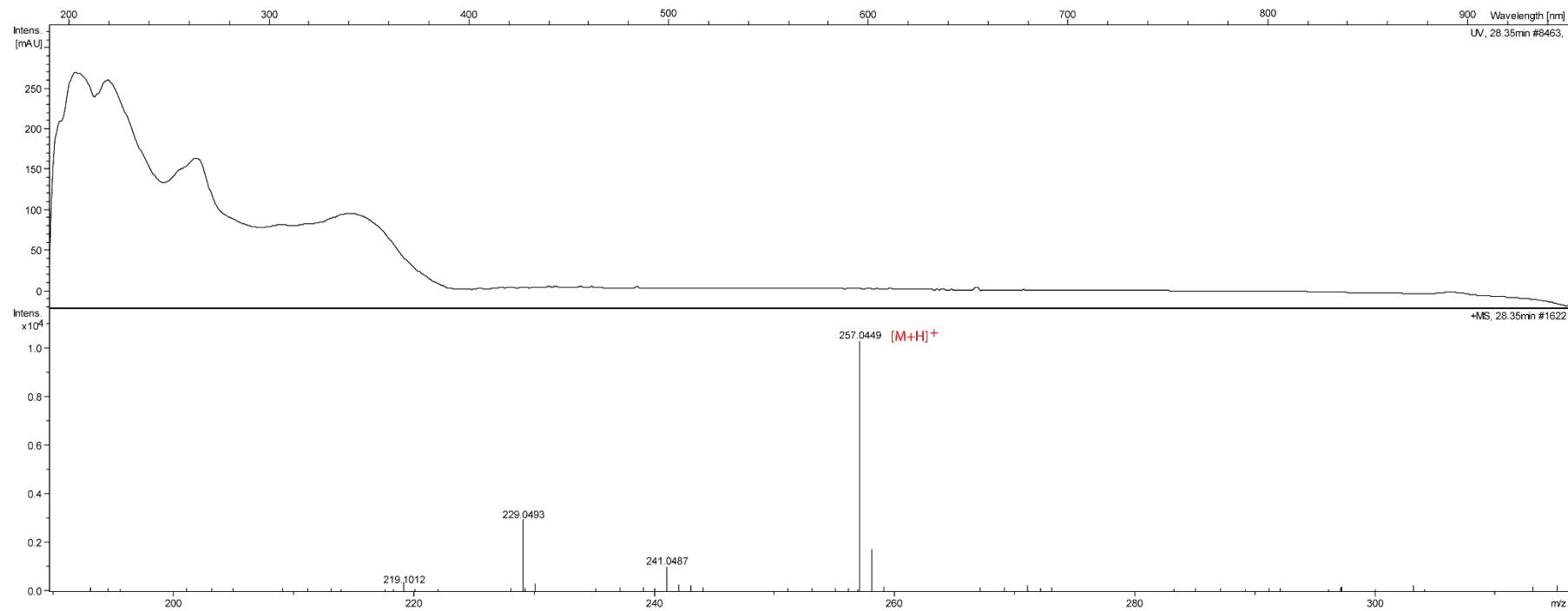


Figure S9. $^1\text{H-NMR}$ spectrum of compound **9** (600 MHz, $\text{DMSO-}d_6$).

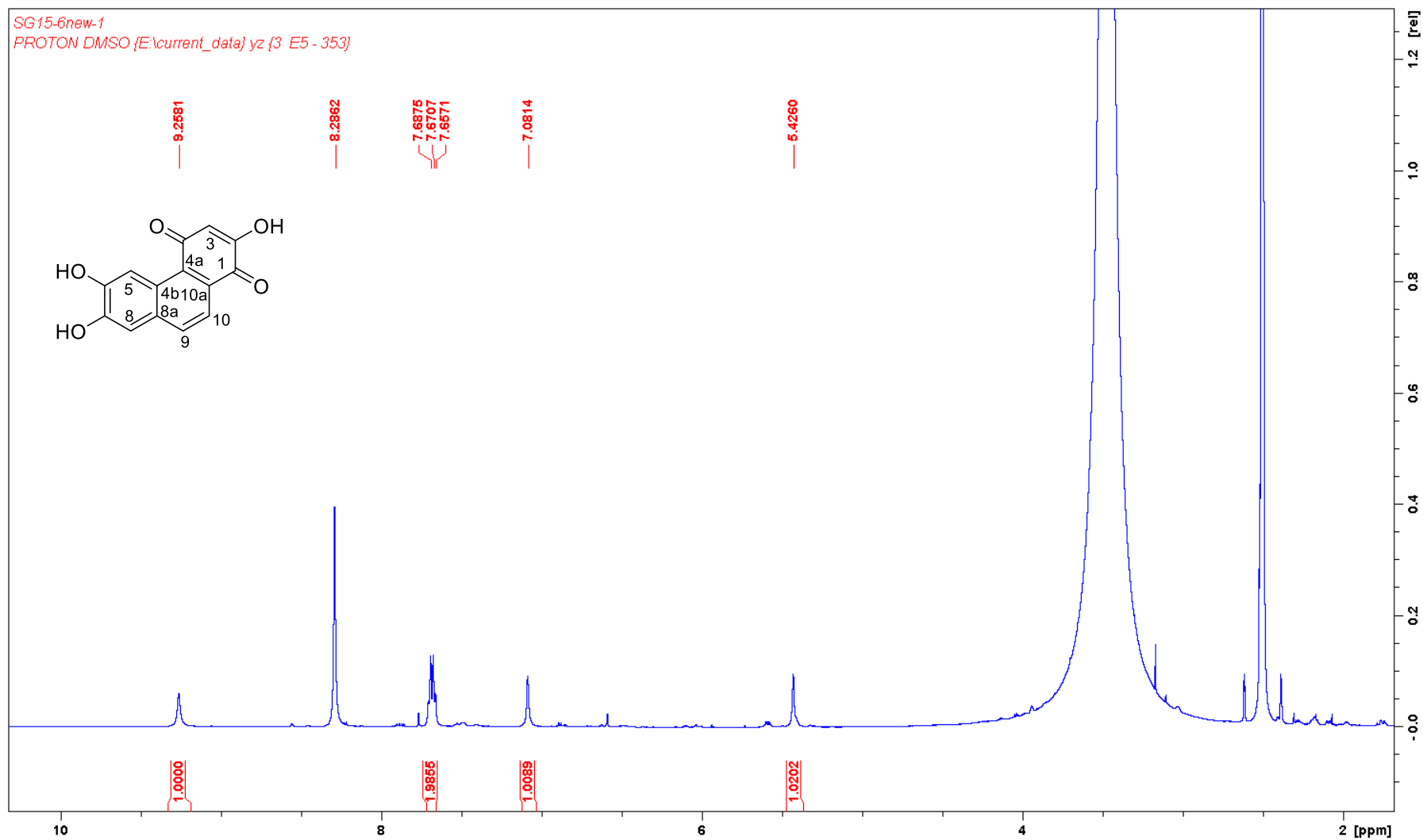


Figure S10. HSQC spectrum of compound **9** (600 MHz, DMSO-*d*₆).

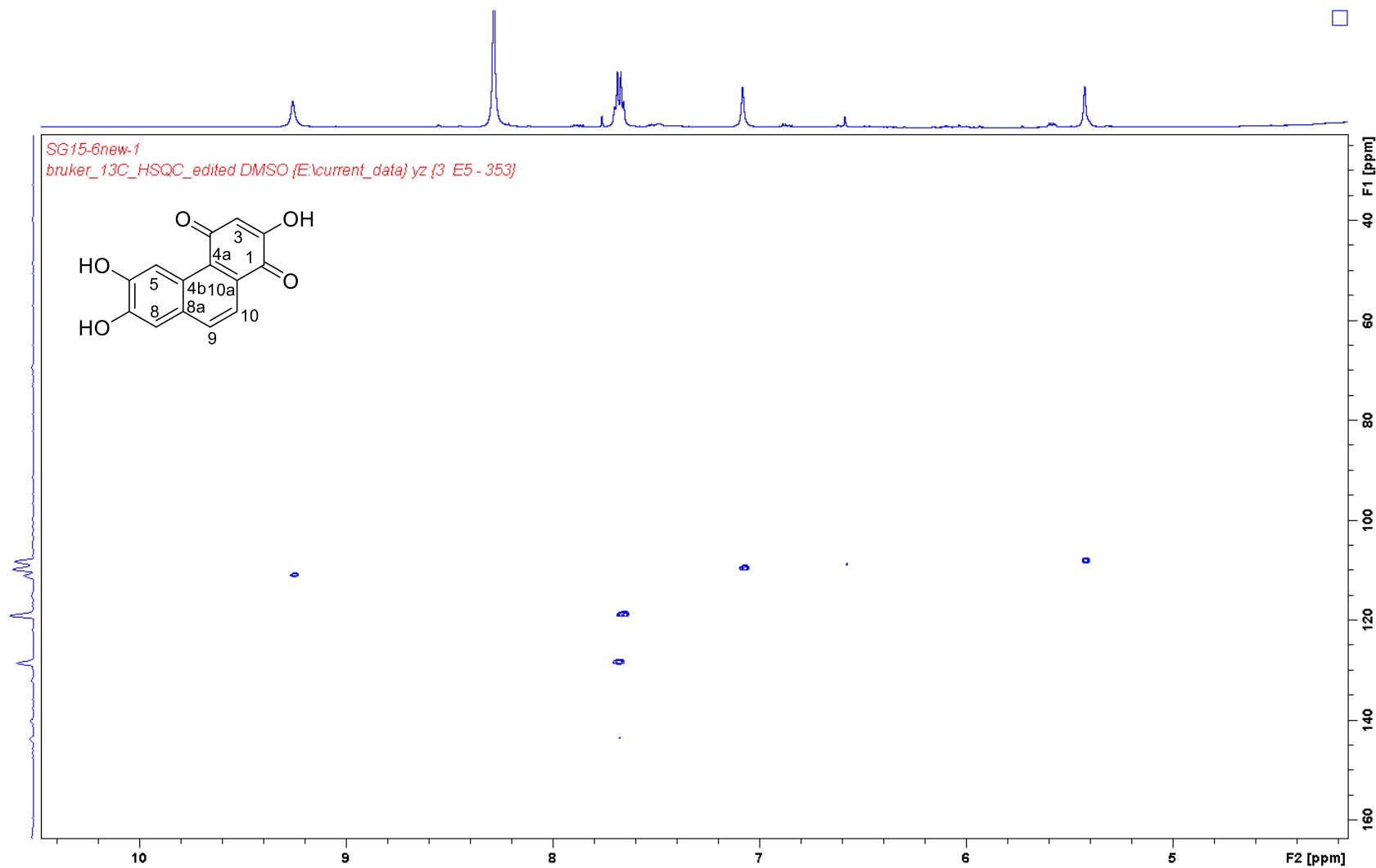


Figure S11. HMBC spectrum of compound **9** (600 MHz, DMSO-*d*₆).

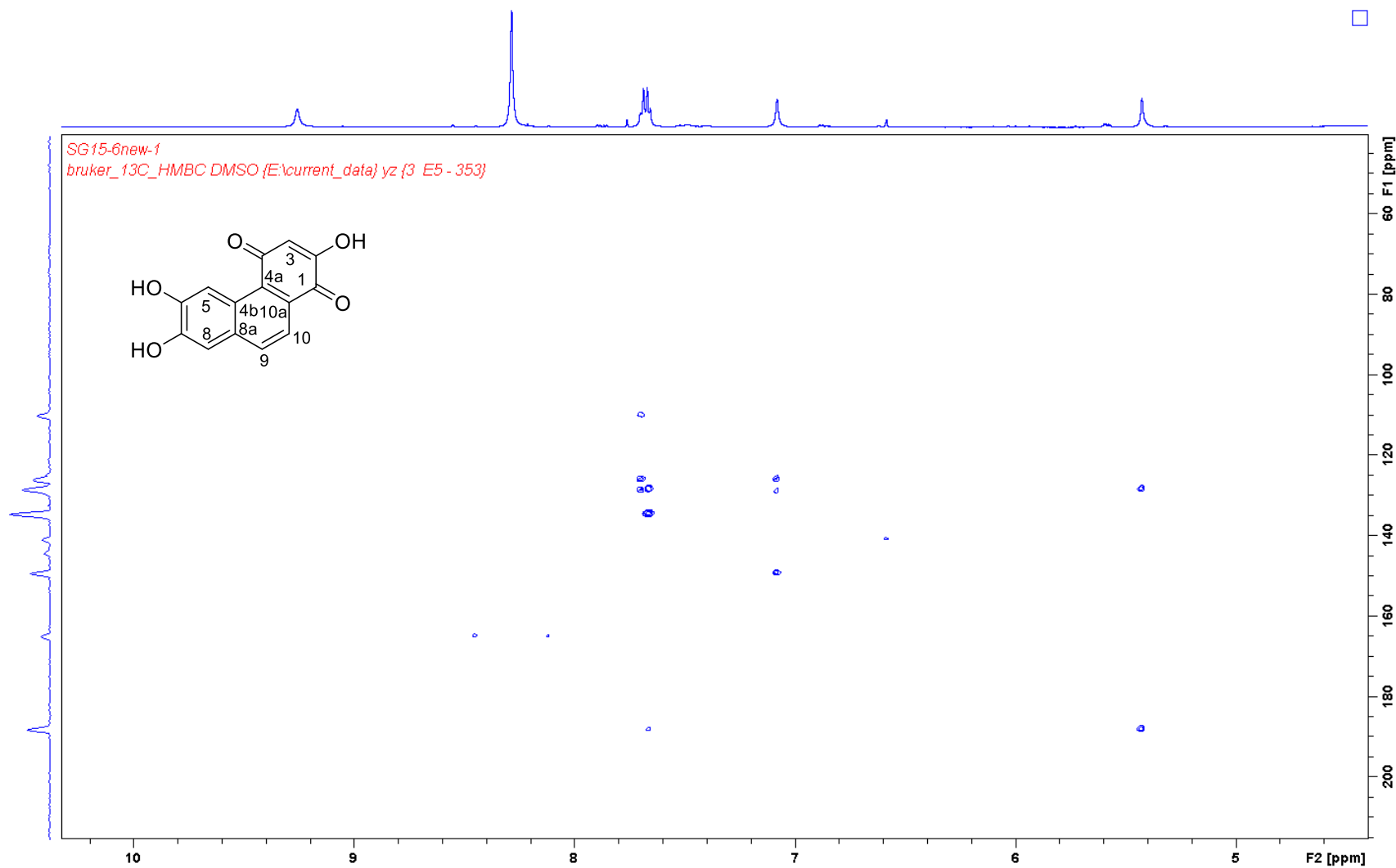


Figure S12. COSY spectrum of compound **9** (600 MHz, DMSO-*d*₆).

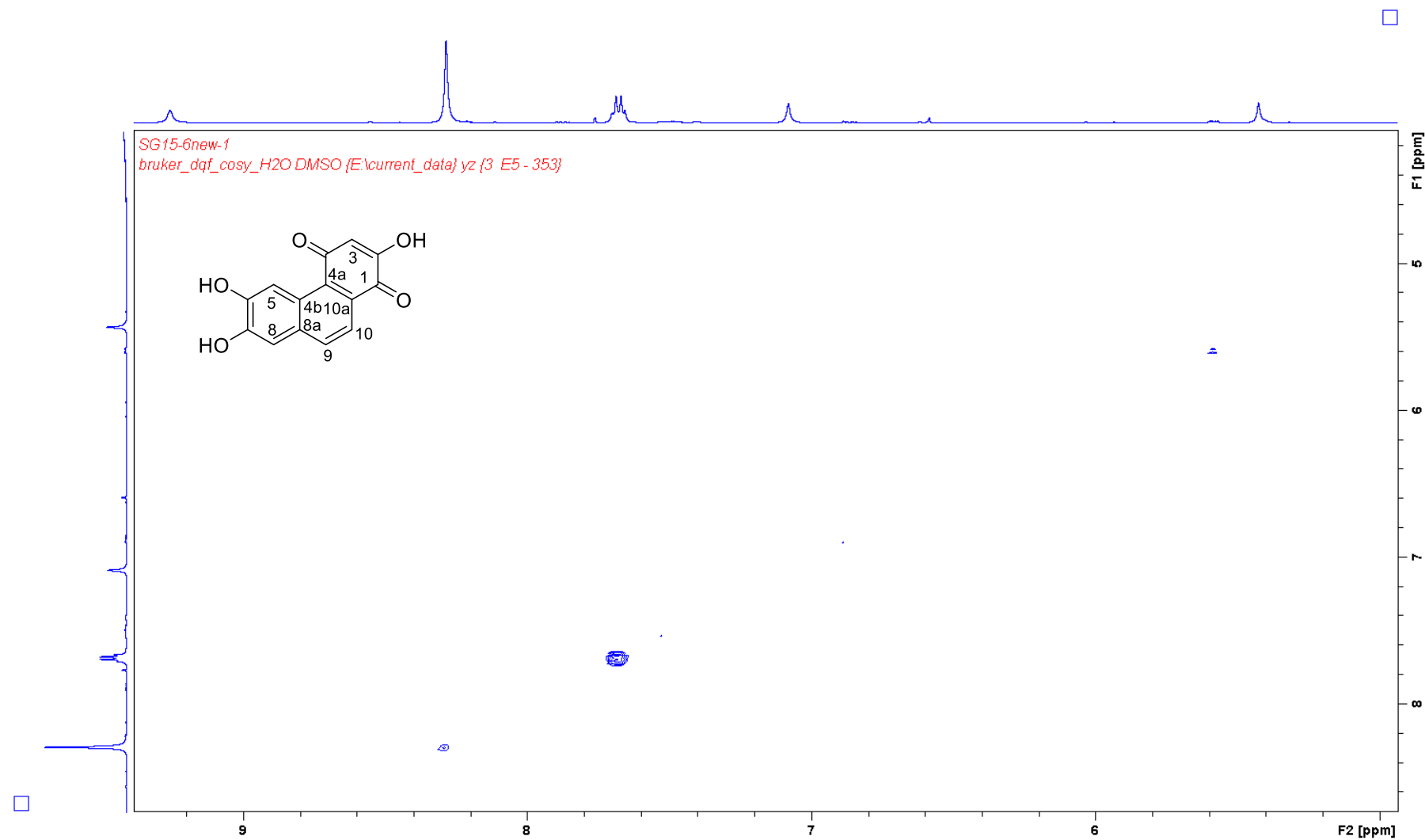


Figure S13. UV and HRMS spectra of compound **10** obtained in the HPLC-HRMS mode.

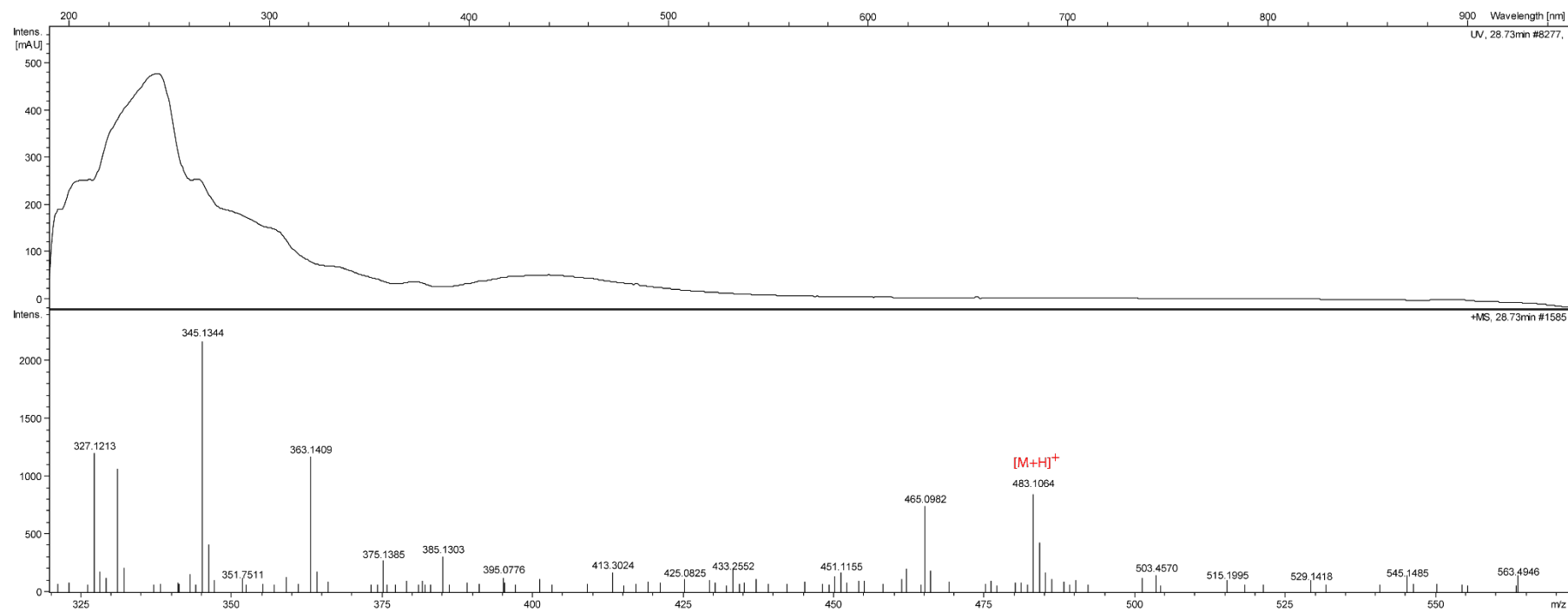


Figure S14. $^1\text{H-NMR}$ spectrum of compound **10** (600 MHz, Methanol- d_4).

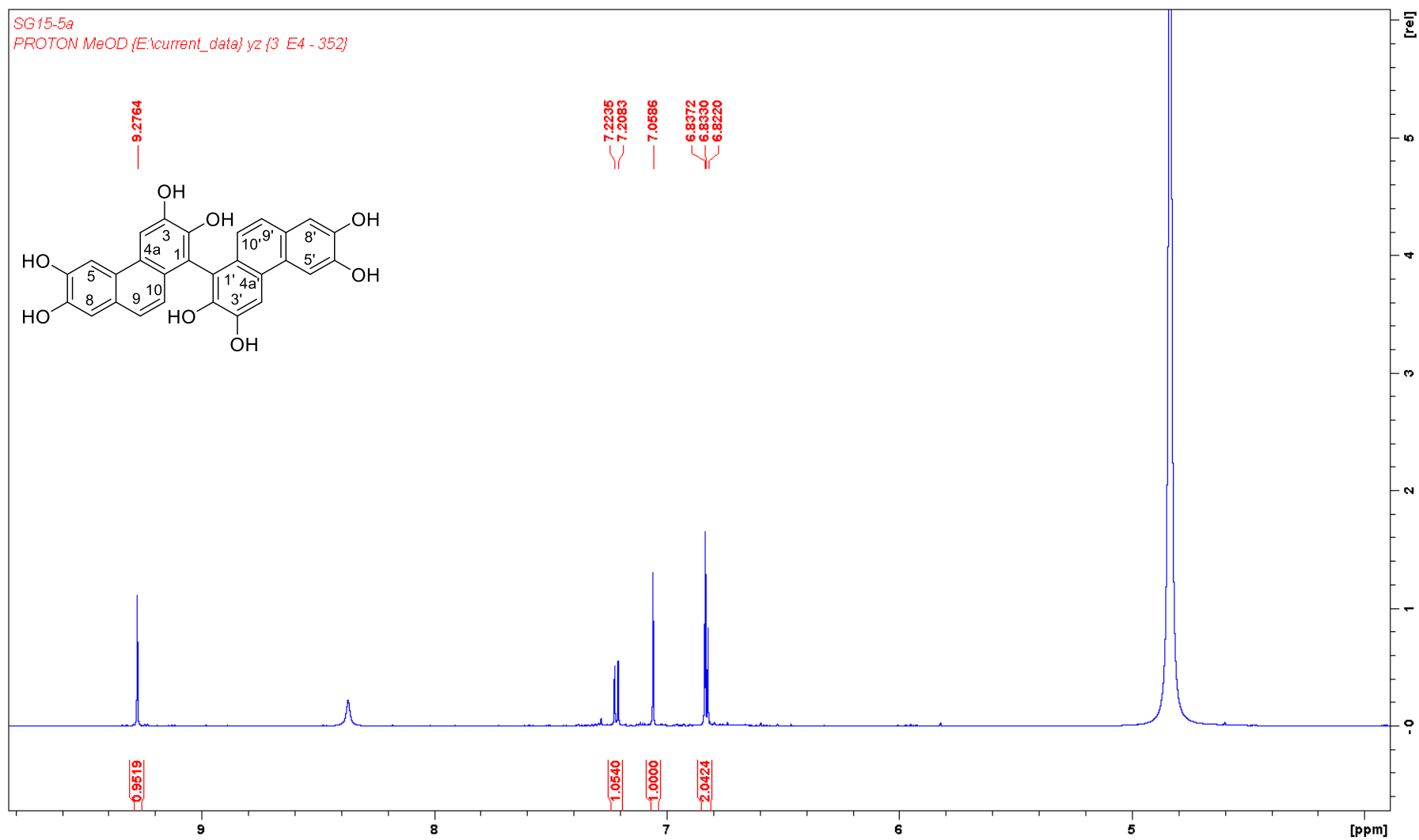


Figure S15. HSQC spectrum of compound **10** (600 MHz, Methanol-*d*₄).

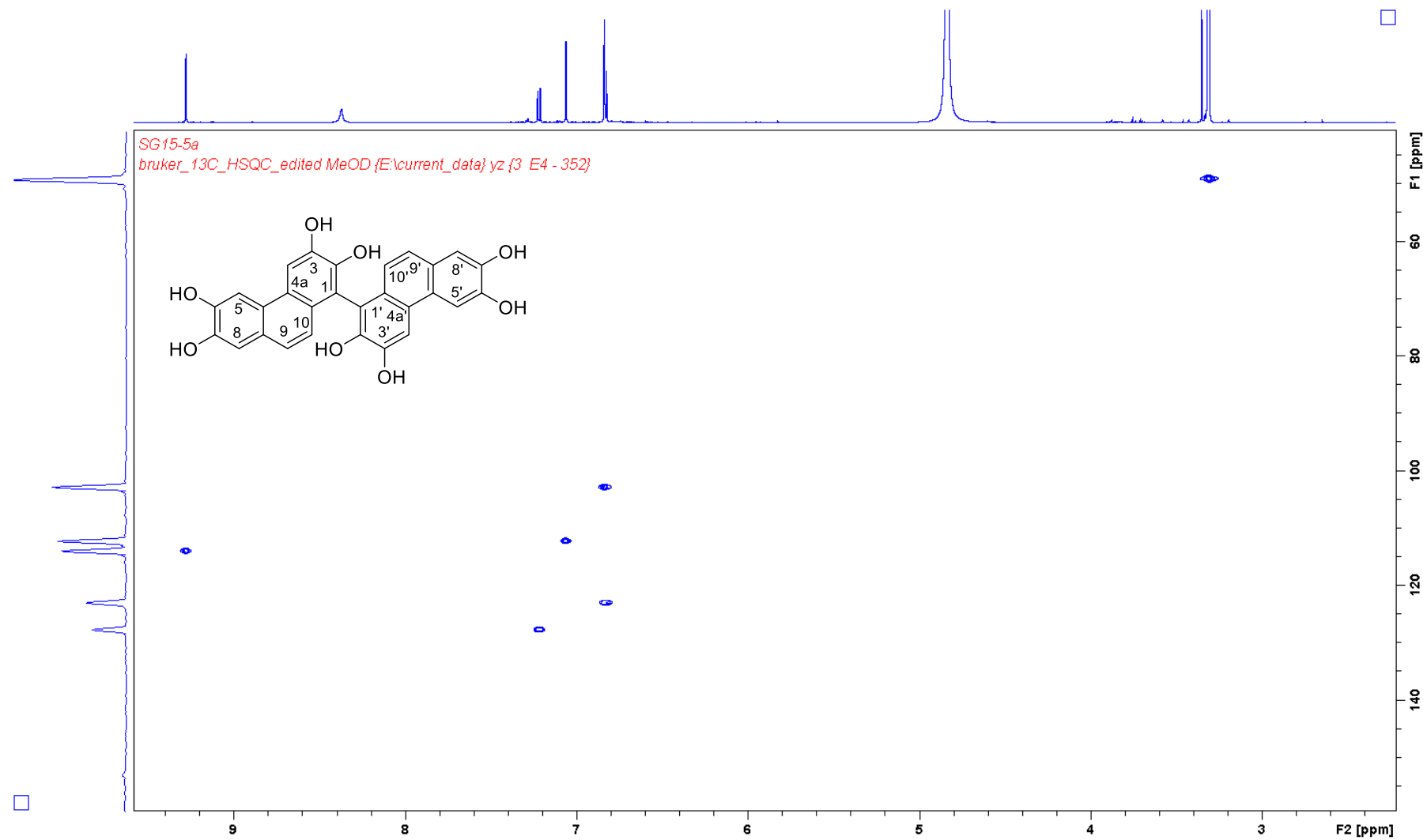


Figure S16. HMBC spectrum of compound **10** (600 MHz, Methanol- d_4).

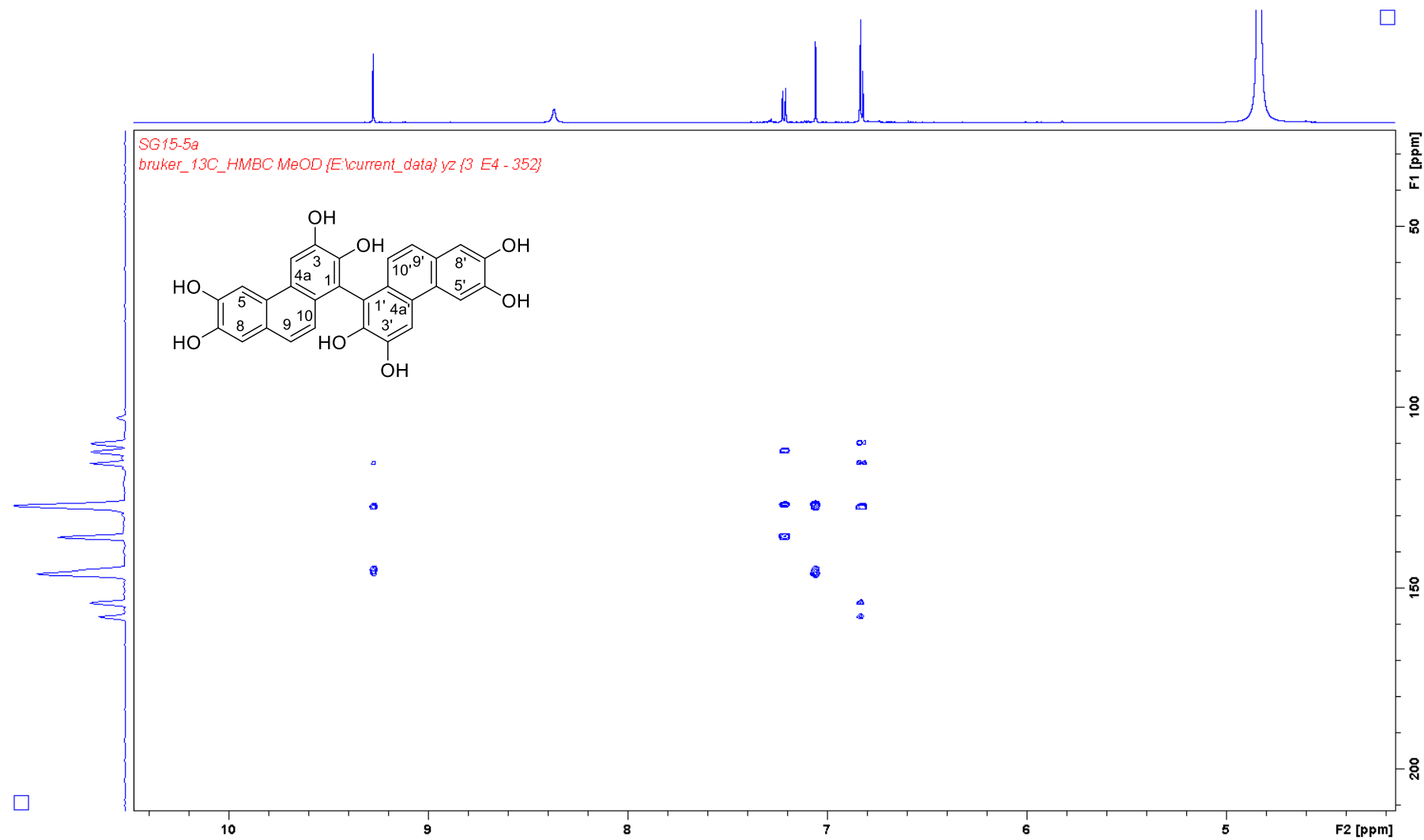


Figure S17. COSY spectrum of compound **10** (600 MHz, Methanol-*d*₄).

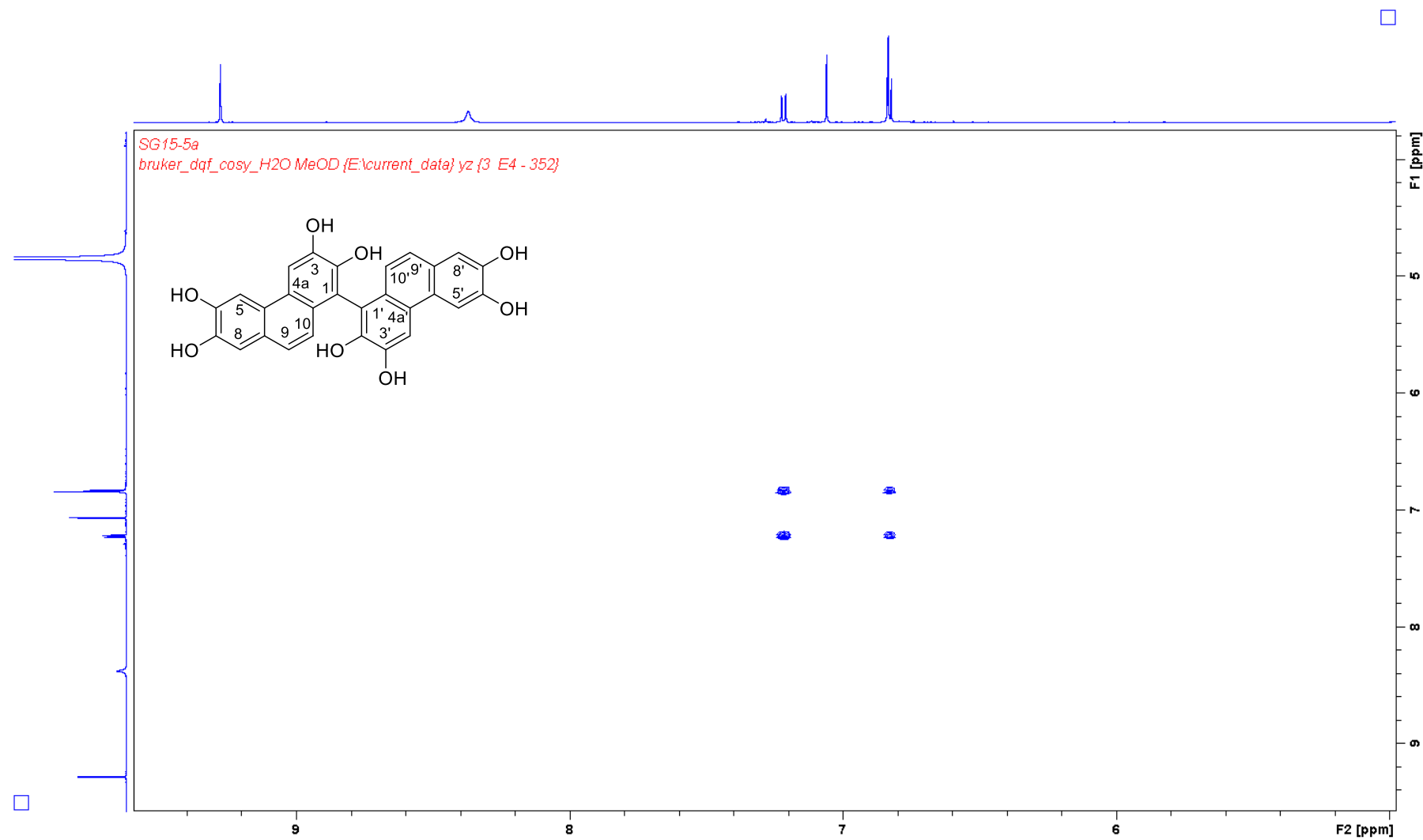


Figure S18. HPLC chromatogram of crude *P. bistorta* ethyl acetate extract monitored at 254 nm (black) as well as high-resolution α -glucosidase inhibition profile (red), high-resolution PTP1B inhibition profile (green) and high-resolution α -amylase inhibition profile (blue) [40 mg/mL, 10 μ L injection].

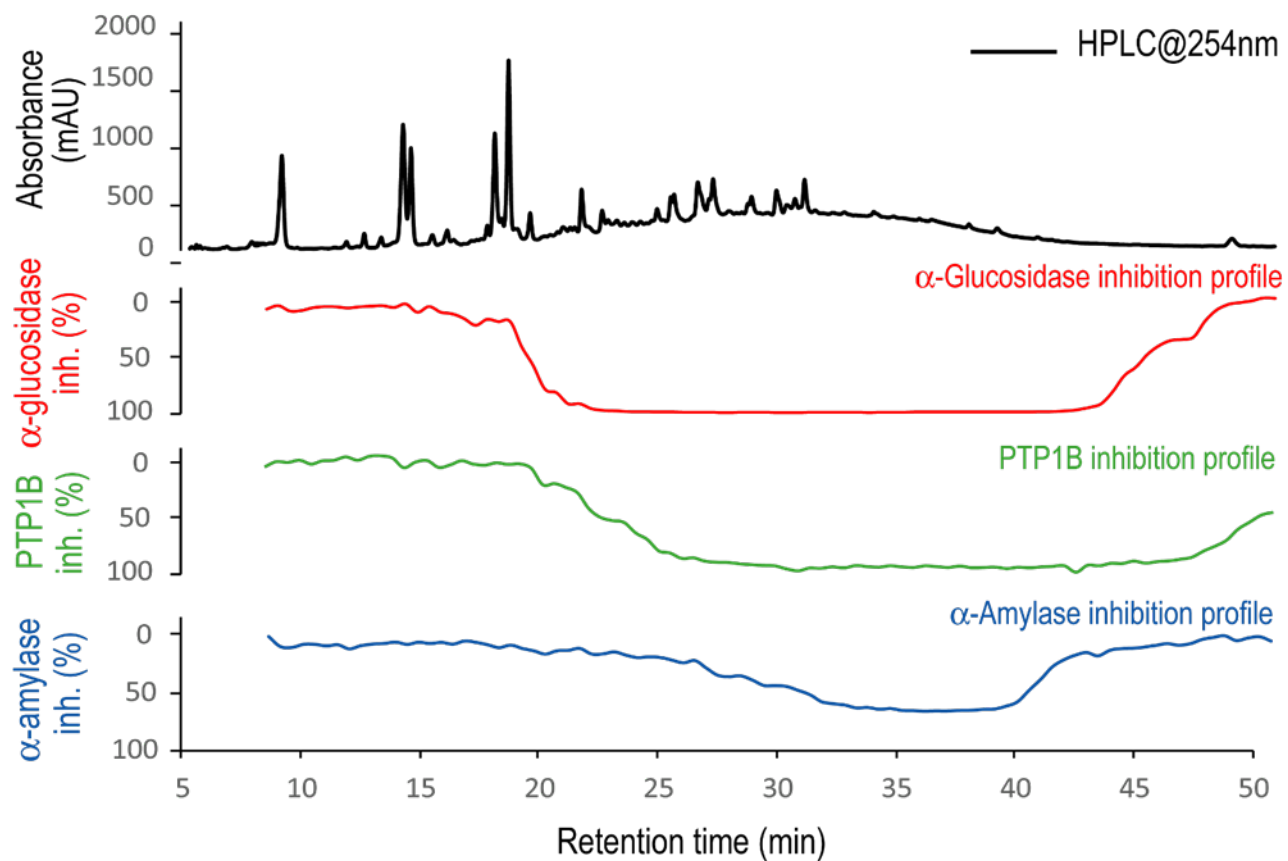


Figure S19. IC₅₀ curves of isolated compounds and Acarbose (reference compound) in the α-glucosidase inhibition assays.

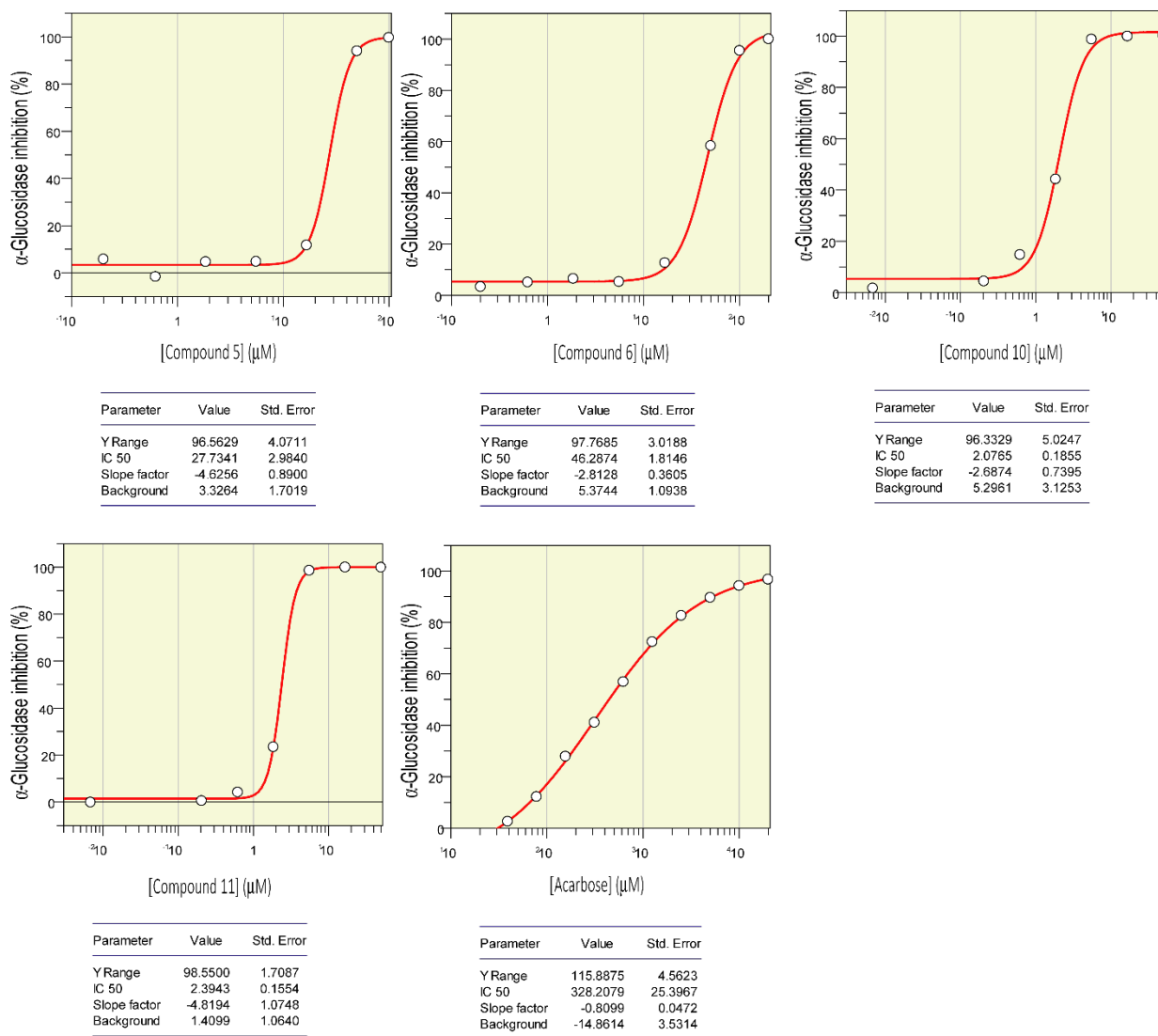


Figure S20. IC₅₀ curves of isolated compounds and RK682 (reference compound) in the PTP1B inhibition assays.

