

Supplementary material

Tyrosinase inhibition and kinetic details of puerol A having but-2-enolide structure from *Amorpha fruticosa*

► Characterization Data

- Figure S1–S7: NMR and HREIMS data of compound **1**
- Figure S8–14: NMR and HREIMS data of compound **2**
- Figure S15: The HPLC peak of compound **1** and **2**
- Figure S16: The fluorescence quenching spectra of compound **2**
- Figure S17: The B16F10 cell experiment data of compound **1**
- Table 1: ^1H and ^{13}C NMR data of compound **1** in $\text{MeOH-}d_4$.
- Table 2: ^1H and ^{13}C NMR data of compound **2** in $\text{Acetone-}d_6$

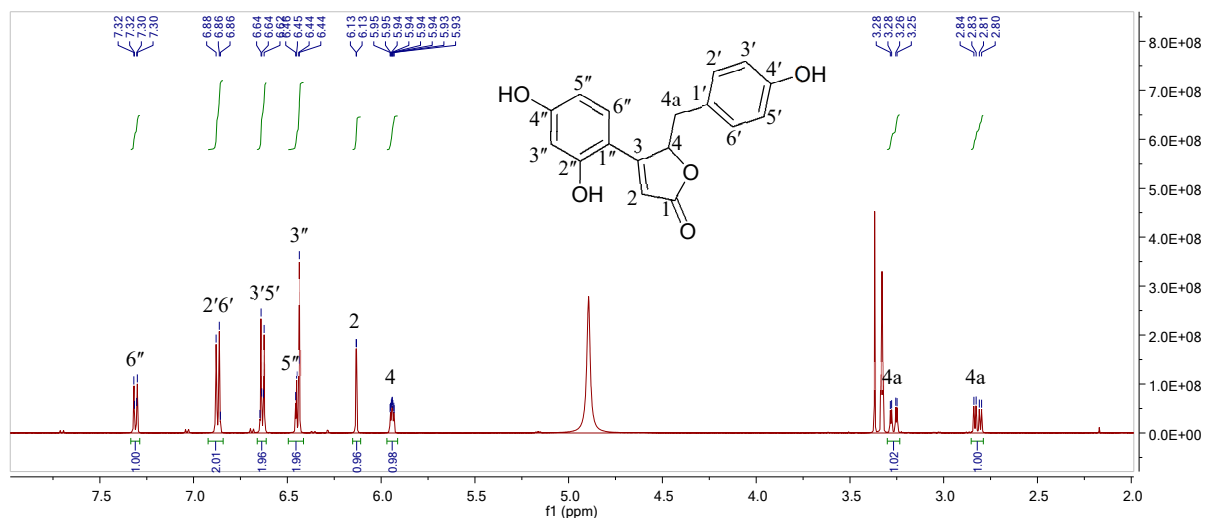


Figure S1. ^1H -NMR spectrum of compound 1 (500MHz, $\text{MeOH-}d_4$)

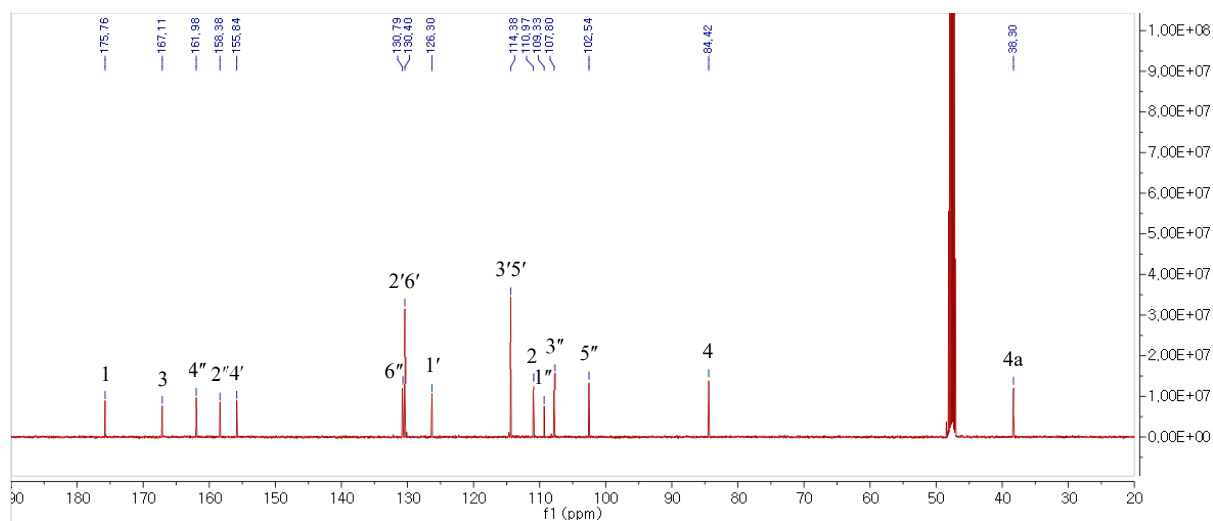


Figure S2. ^{13}C -NMR spectrum of compound 1 (500MHz, $\text{MeOH-}d_4$)

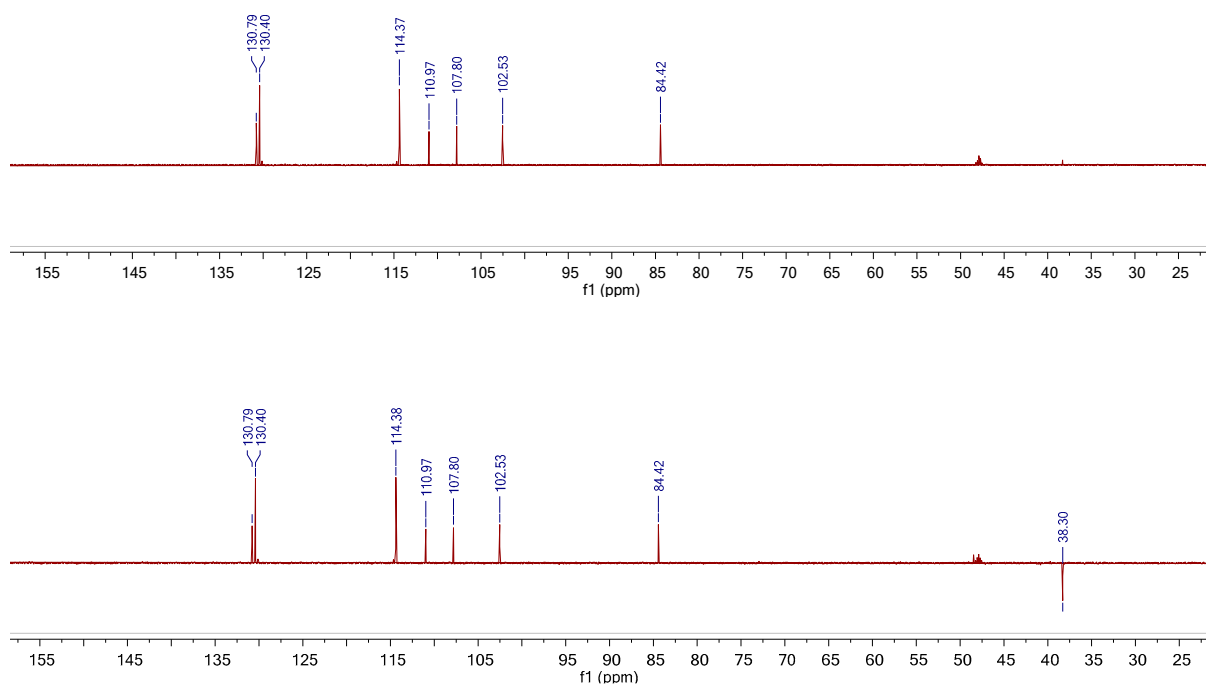


Figure S3. DEPT-90 and -135 spectrum of compound 1.

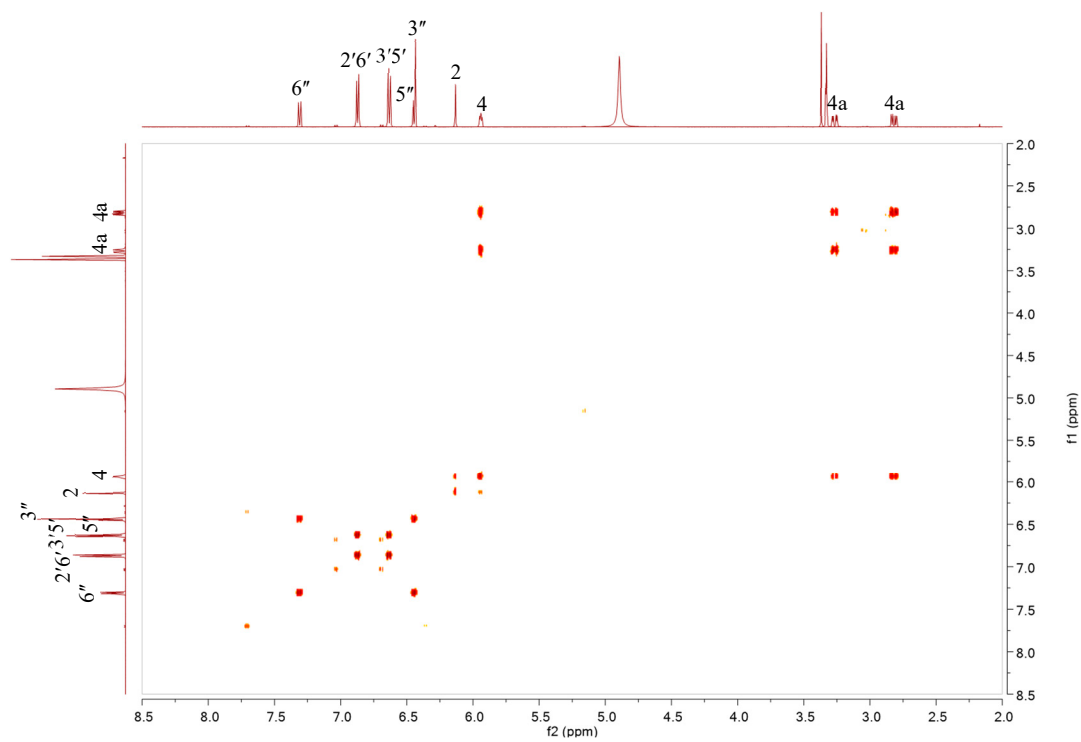


Figure S4. COSY spectrum of compound 1.

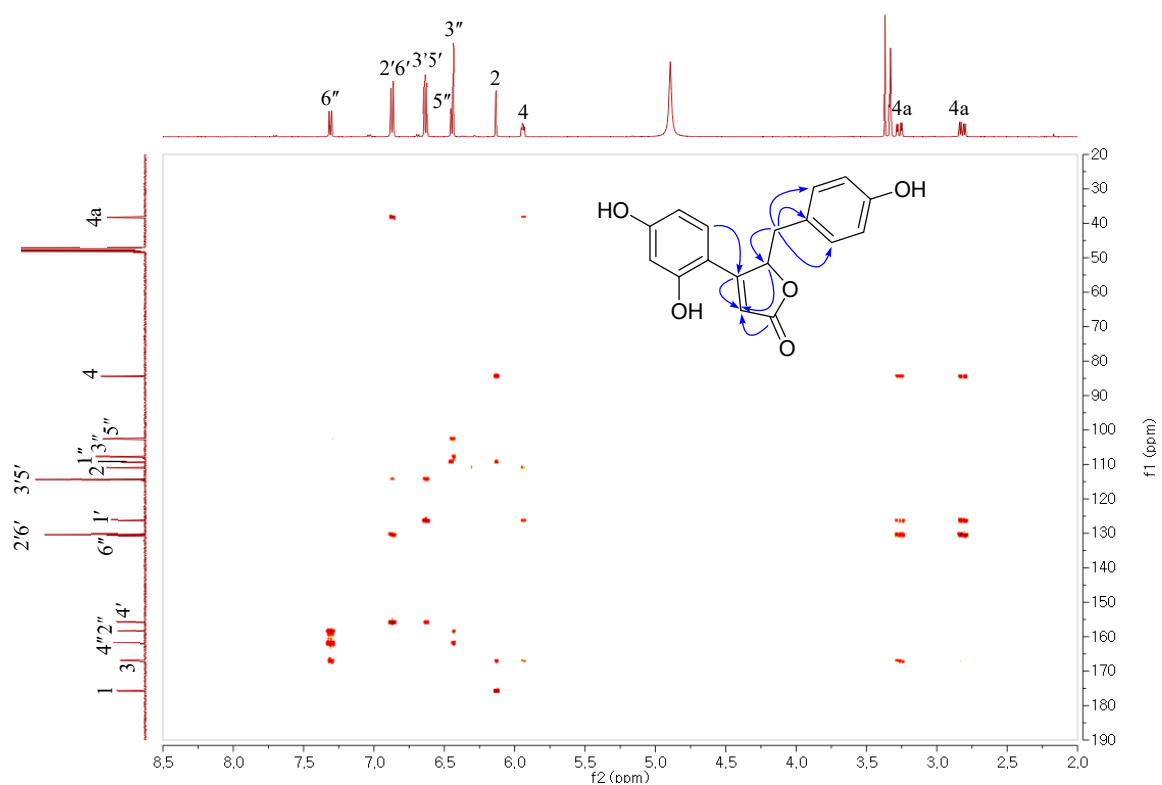


Figure S5. HMBC spectrum of compound 1.

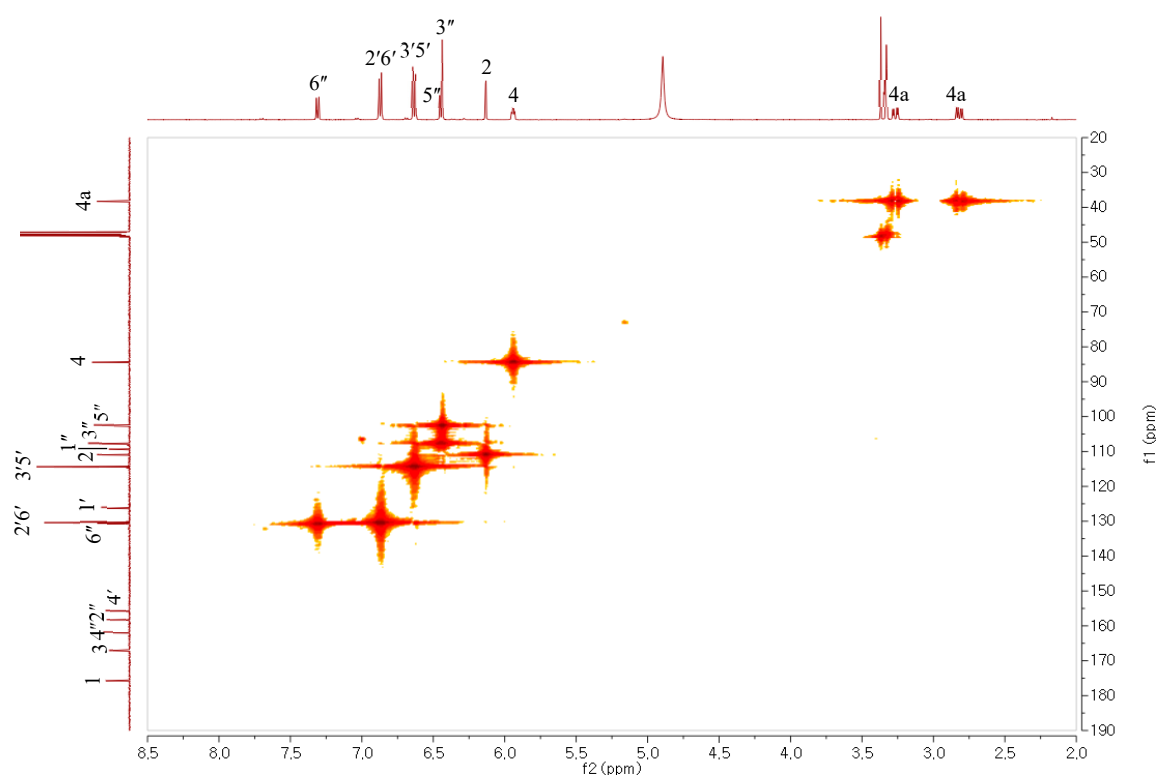
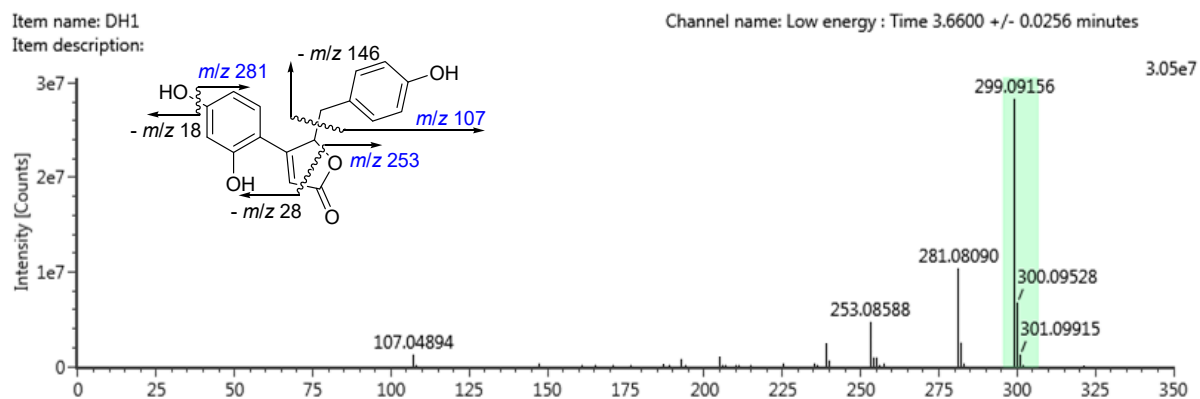


Figure S6. HMQC spectrum of compound 1.



Component name	Identification status	Formula	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Detector counts	Adducts
Compound 1	Identified	C ₁₇ H ₁₄ O ₅	298.08412	298.0843	299.0916	0.2	0.5	3.66	4762262	+H

Figure S7. HRESIMS data of compound **1**.

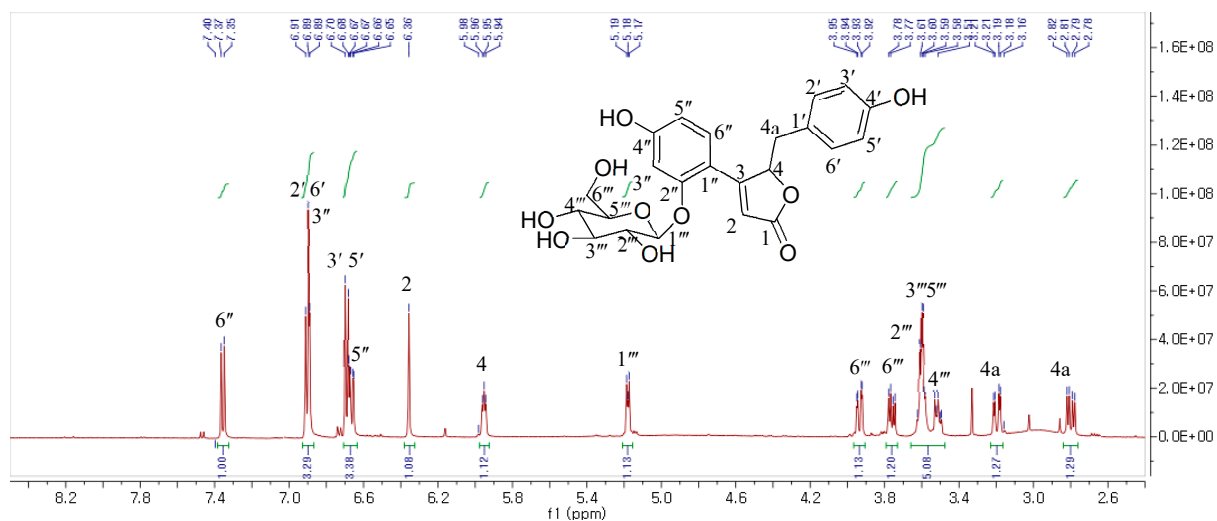


Figure S8. ^1H -NMR spectrum of compound 2 (500MHz, Acetone- d_6)

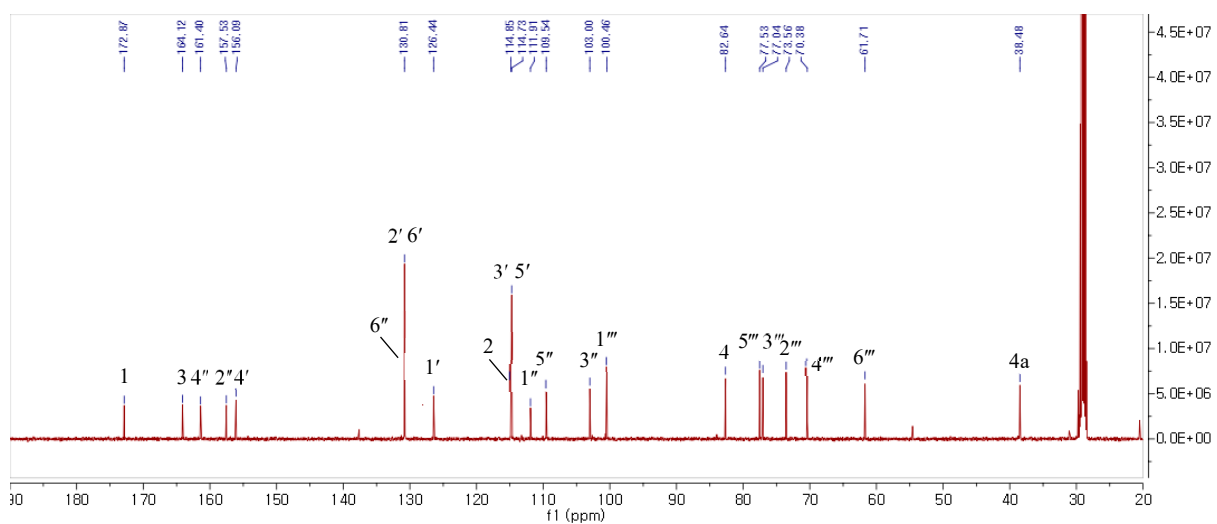


Figure S9. ^{13}C -NMR spectrum of compound 2 (500MHz, Acetone- d_6)

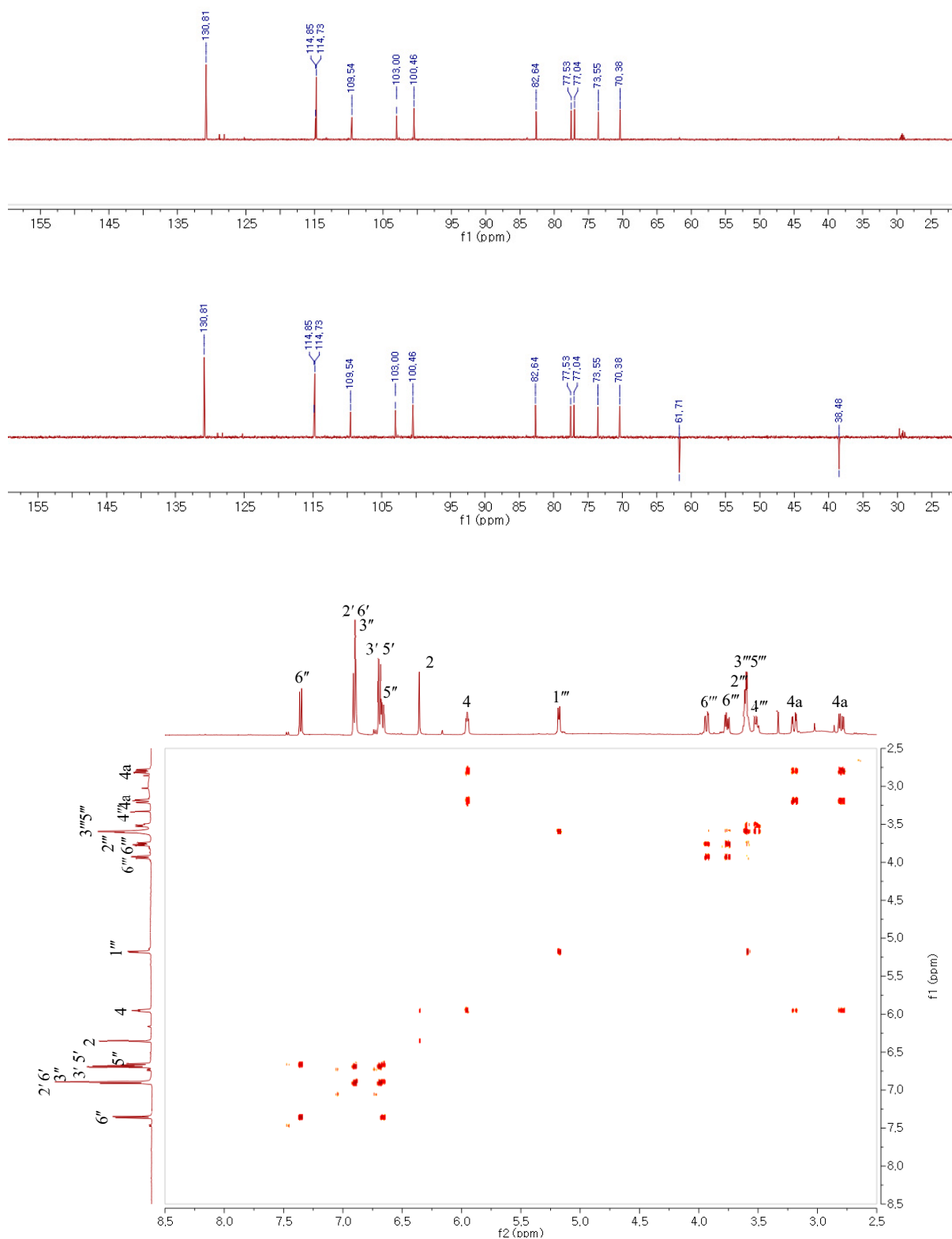


Figure S10. COSY spectrum of compound 2.

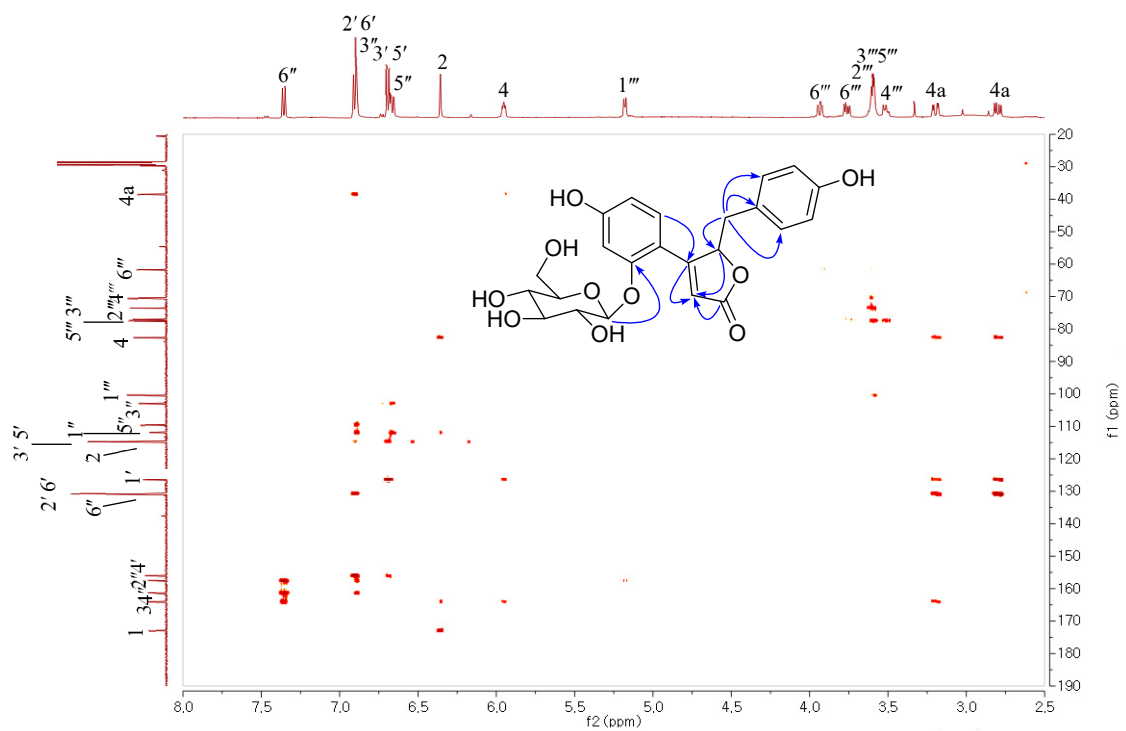


Figure S11. HMBC spectrum of compound 2

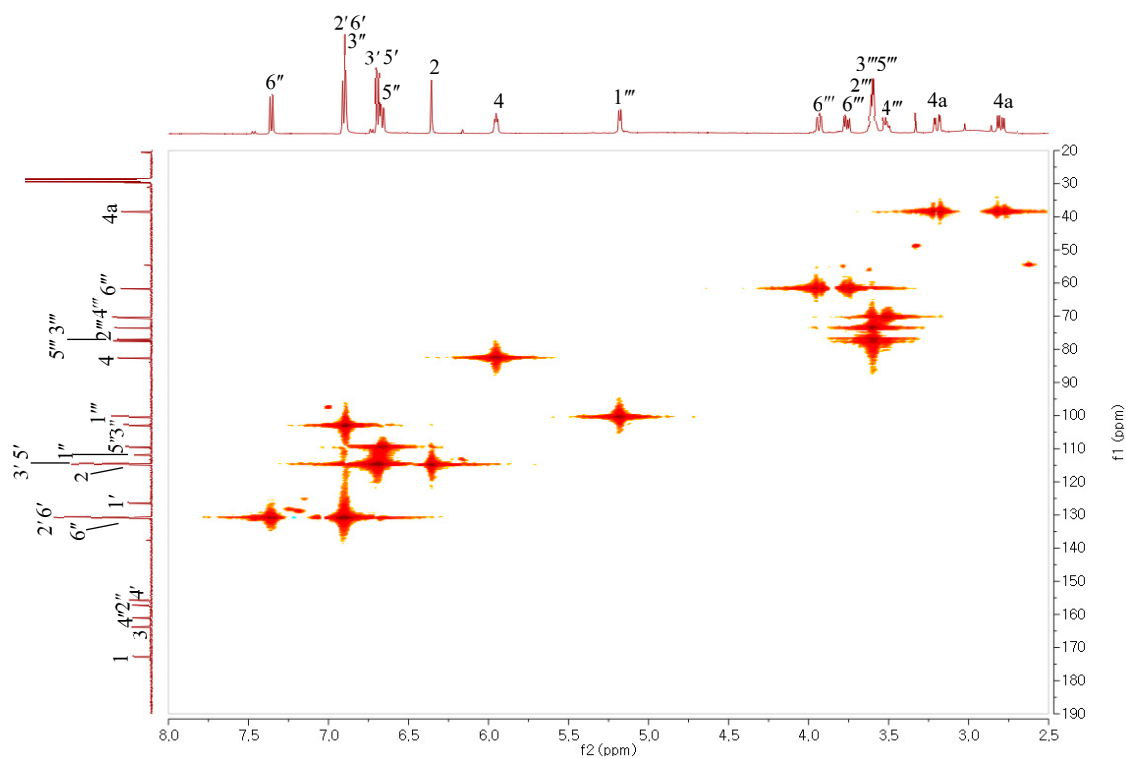
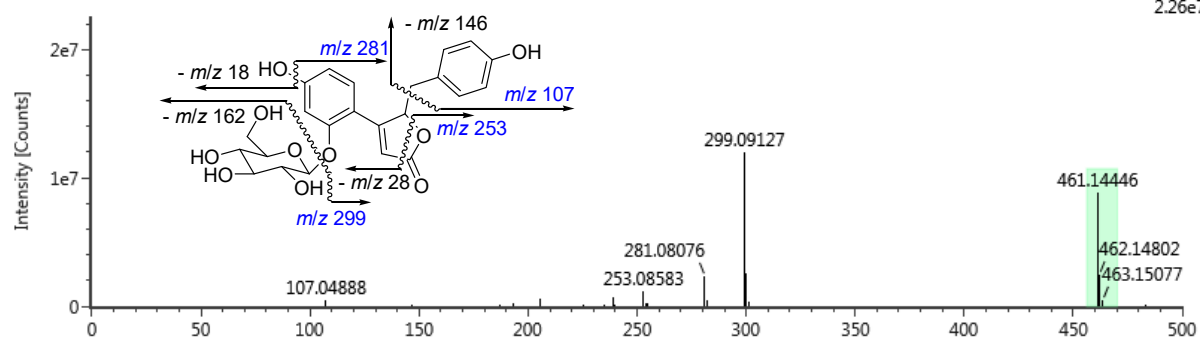


Figure S12. HMQC spectrum of compound 2.

Item name: DH2
Item description:

Channel name: Low energy : Time 3.1134 +/- 0.0241 minutes

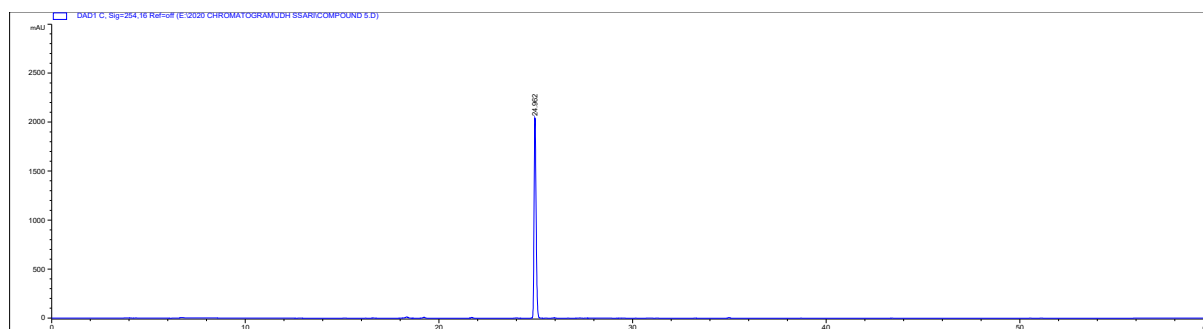
2.26e7



Component name	Identification status	Formula	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Detector counts	Adducts
Compound 2	Identified	C ₂₃ H ₂₄ O ₁₀	460.13695	460.1372	461.1445	0.2	0.5	3.11	1940588	+H

Figure S13. HRESIMS data of compound 2.

(A)



(B)

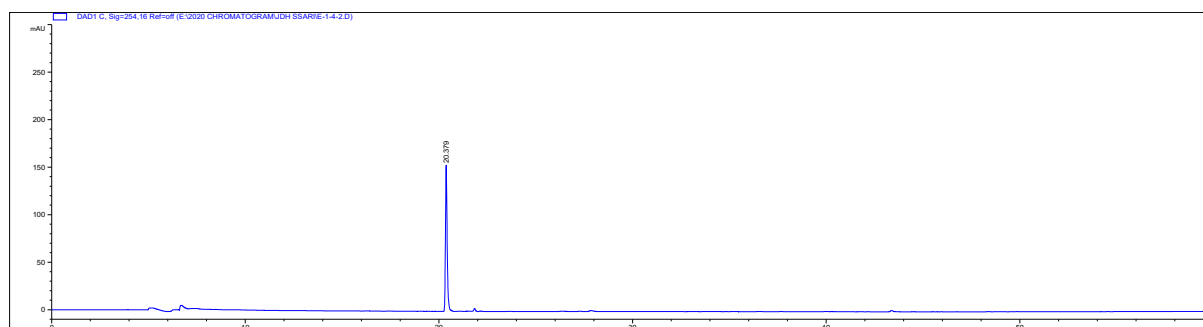


Figure S15. HPLC peak of (A) puerol A and (B) kuzubutenolide A

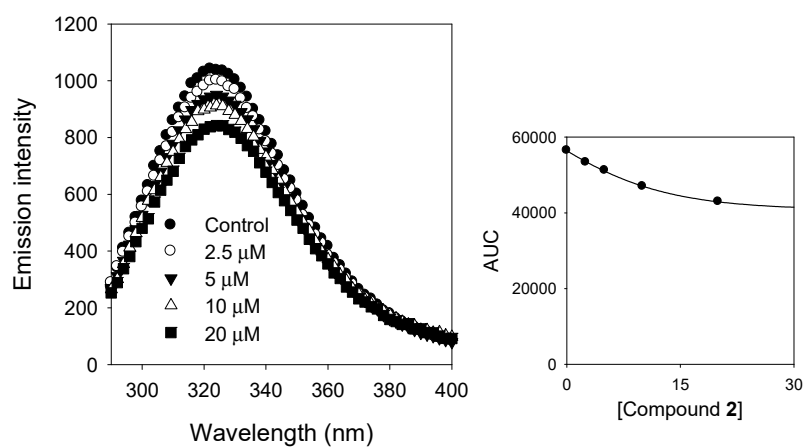


Figure S15. The fluorescence emission spectra of tyrosinase at different concentrations of compound 2 (Inset) Normalized intensities of fluorescence for tyrosinase are shown for compounds 2.

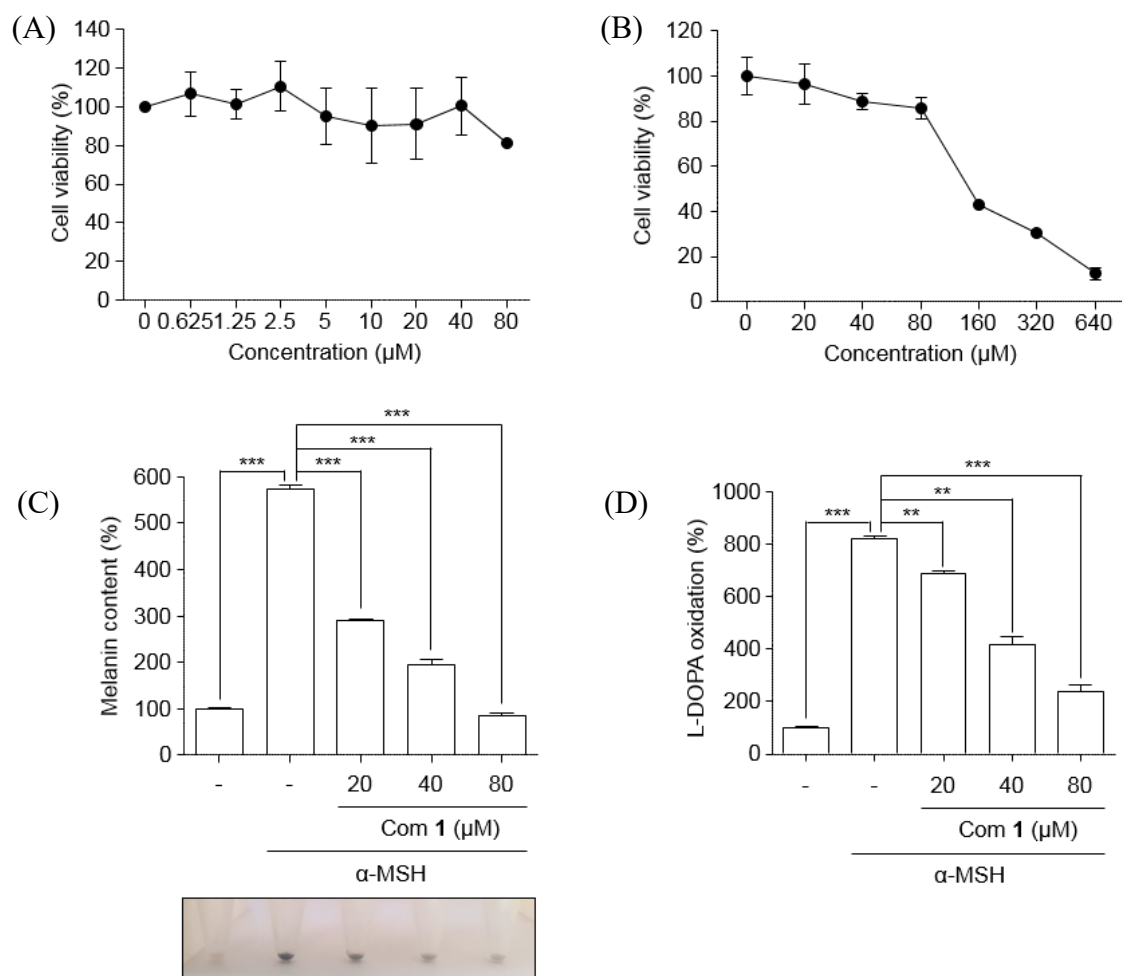


Figure S17. (A and B) Effects of compound 1 on B16F10 melanoma cell viability incubated at each concentration for 48 h. (C) Effect of compound 1 lowering melanin synthesis in B16F10 cells. (D) Effect of compound 1 reducing L-DOPA oxidation in B16F10 cells.

Table S1. ^1H NMR and ^{13}C NMR data of compound **1** (500 MHz, MeOD)

Position	δ_{H} (multi, J in Hz)	δ_{C} (ppm)
1	-	175.7
2	6.13 (1H, d, J = 1.1 Hz)	110.9
3	-	167.1
4	5.95 (1H, ddd, J = 1.1, 3.5, 5.8 Hz)	84.4
4a	2.80 (1H, dd, J = 6.0, 14.5 Hz)	38.3
	3.25 (1H, dd, J = 3.5, 14.5 Hz)	
1'	-	126.2
2'	6.86 (2H, d, J = 8.5 Hz)	130.4
3'	6.62 (2H, d, J = 8.5 Hz)	114.3
4'	-	155.8
5'	6.62 (2H, d, J = 8.5 Hz)	114.3
6'	6.86 (2H, d, J = 8.5 Hz)	130.4
1''	-	109.3
2''	-	158.3
3''	6.44 (1H, d, J = 1.5 Hz)	107.7
4''	-	161.9
5''	6.45 (1H, d, J = 2.3 Hz)	102.5
6''	7.30 (1H, dd, J = 1.6, 7.4 Hz)	130.7

Table S2. ¹H NMR and ¹³C NMR data of compound **2** (500 MHz, Aceton-d₆)

Position	δ _H (multi, <i>J</i> in Hz)	δ _C (ppm)
1	-	172.8
2	6.36 (1H, d, <i>J</i> = 1.1 Hz)	114.8
3	-	164.1
4	5.95 (1H, ddd, <i>J</i> = 1.4, 3.4, 6.4 Hz)	82.6
4a	2.79 (1H, dd, <i>J</i> = 6.2, 14.7 Hz)	38.4
	3.19 (1H, dd, <i>J</i> = 3.7, 14.6 Hz)	
1'	-	126.4
2'	6.91 (2H, d, <i>J</i> = 8.6 Hz)	130.8
3'	6.70 (2H, d, <i>J</i> = 8.6 Hz)	114.7
4'	-	156.0
5'	6.70 (2H, d, <i>J</i> = 8.6 Hz)	114.7
6'	6.91 (2H, d, <i>J</i> = 8.6 Hz)	130.8
1''	-	111.9
2''	-	157.5
3''	6.89 (1H, d, <i>J</i> = 1.6 Hz)	103.0
4''	-	161.4
5''	6.67 (1H, dd, <i>J</i> = 2.2, 8.6 Hz)	109.5
6''	7.37 (1H, d, <i>J</i> = 8.5 Hz)	130.8
1'''	5.18 (1H, t, <i>J</i> = 7.1 Hz)	100.4
2'''	3.60 (1H, m)	73.5
3'''	3.58 (1H, m)	77.0
4'''	3.52 (1H, m)	70.3
5'''	3.59 (1H, m)	77.5
6'''	3.93 (1H, m)	61.7