

Synthesis and properties of Kojic acid dimer and its potential for the treatment of Alzheimer's disease

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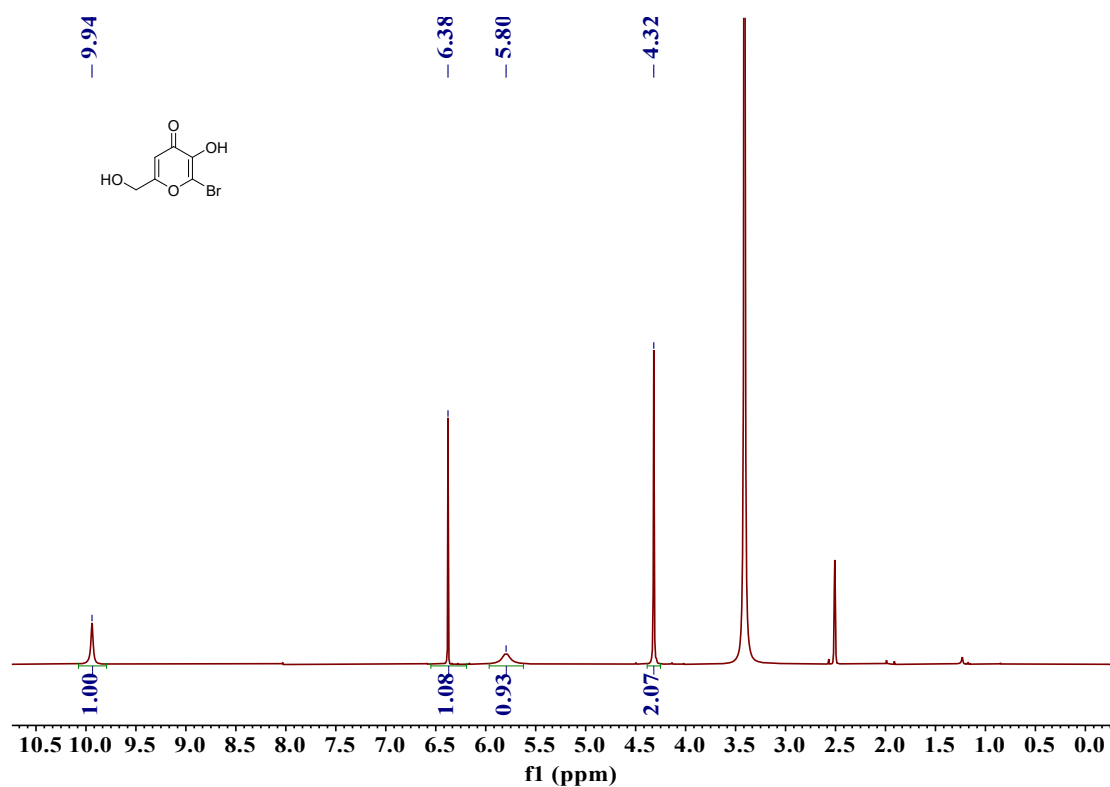


Figure S1. $^1\text{H-NMR}$ spectrum of **2a** (solvent $\text{DMSO-}d_6$, 400 MHz)

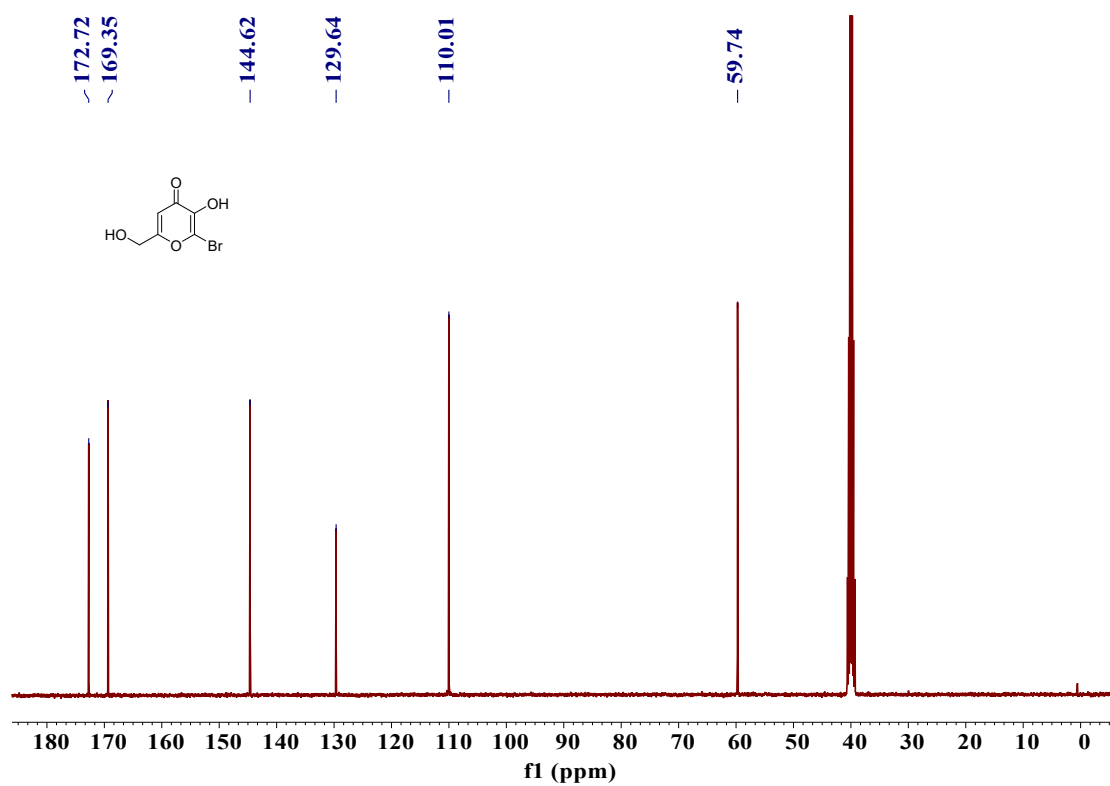
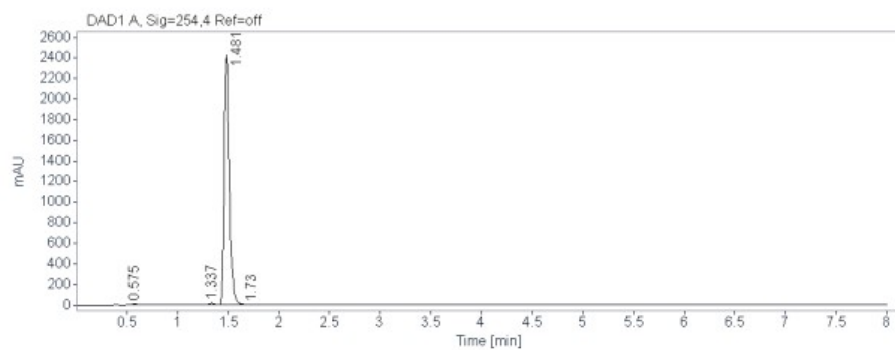


Figure S2. $^{13}\text{C-NMR}$ spectrum of **2a** (solvent $\text{DMSO-}d_6$, 100 MHz)



Signal: DAD1 A, Sig=254,4 Ref=off

Name	RT [min]	Height	Area	Area%
	0.575	12.64760	39.15671	0.444
	1.337	16.87968	59.13989	0.671
	1.481	2436.12012	8710.54102	98.819
	1.730	2.13698	5.80268	0.066
	Sum		8814.64029	

Signal: MSD1 TIC, MS File

Name	RT [min]	Height	Area	Area%
	1.557	1668619.25000	10434263.0000	100.000
	Sum		10434263.0000	

Figure S3. HPLC of compound **2a** ($t_R = 1.481$ min, 98.8% purity)

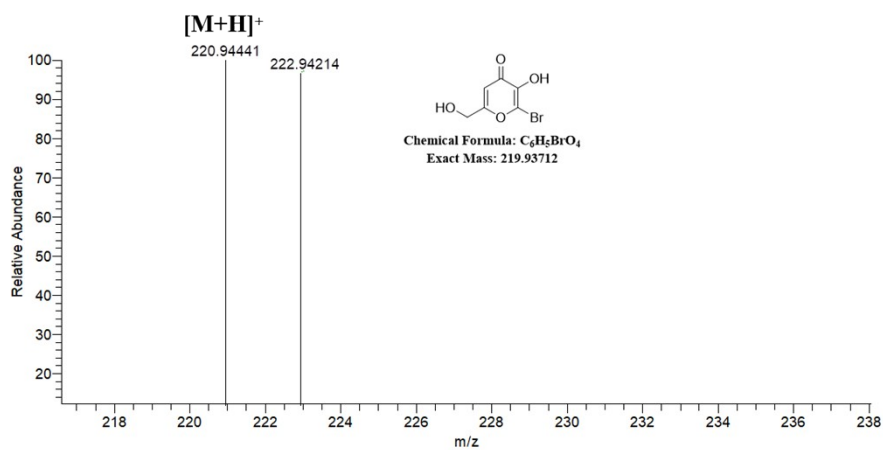


Figure S4. HRMS of compound **2a**

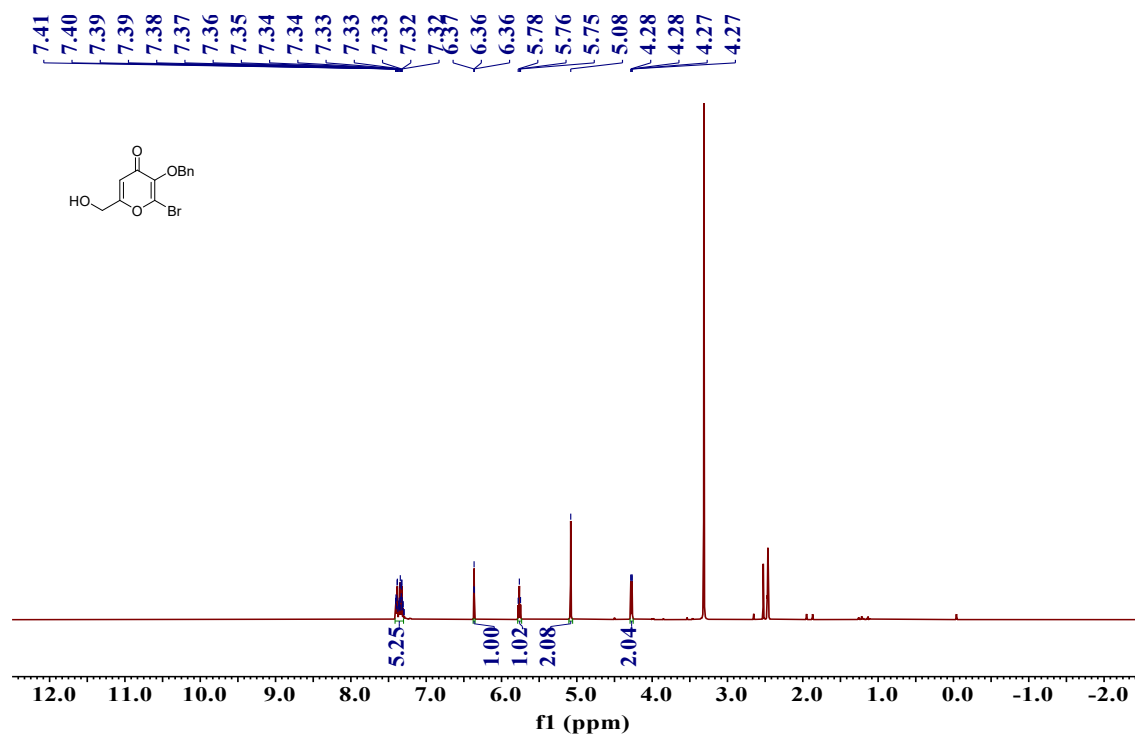


Figure S5. ¹H-NMR spectrum of **2b** (solvent DMSO-*d*₆, 400 MHz)

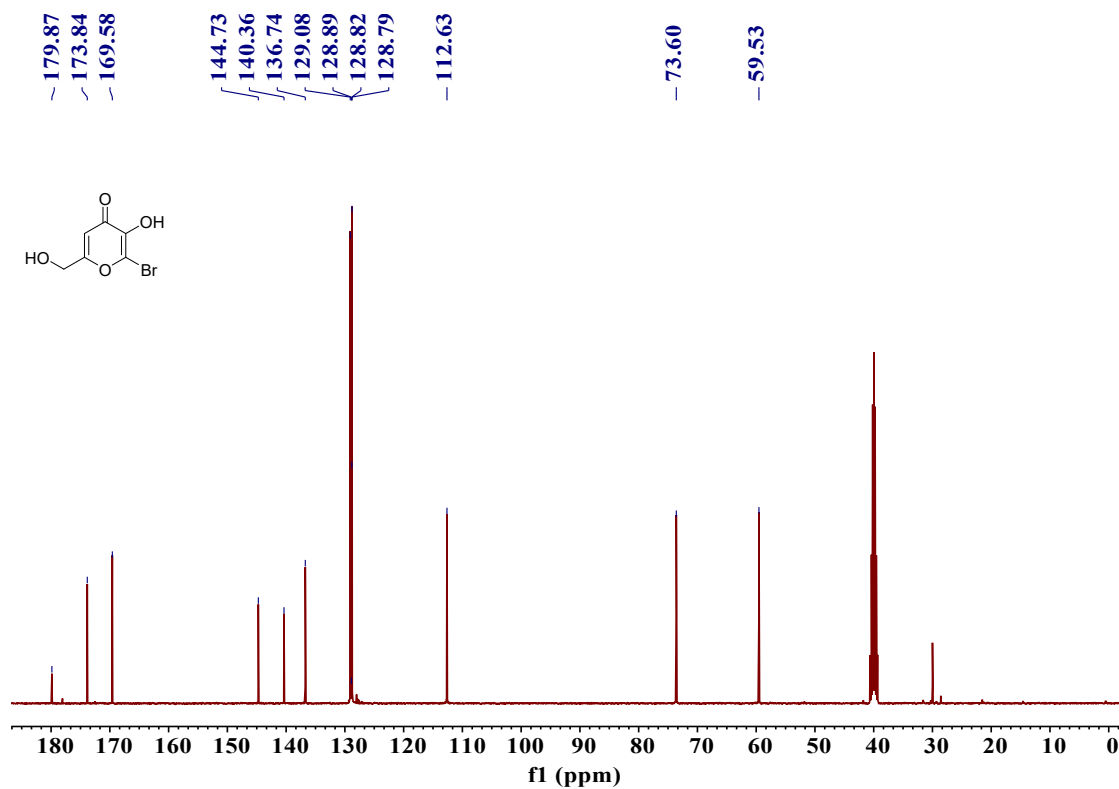


Figure S6. ¹³C-NMR spectrum of **2b** (solvent DMSO-*d*₆, 100 MHz)

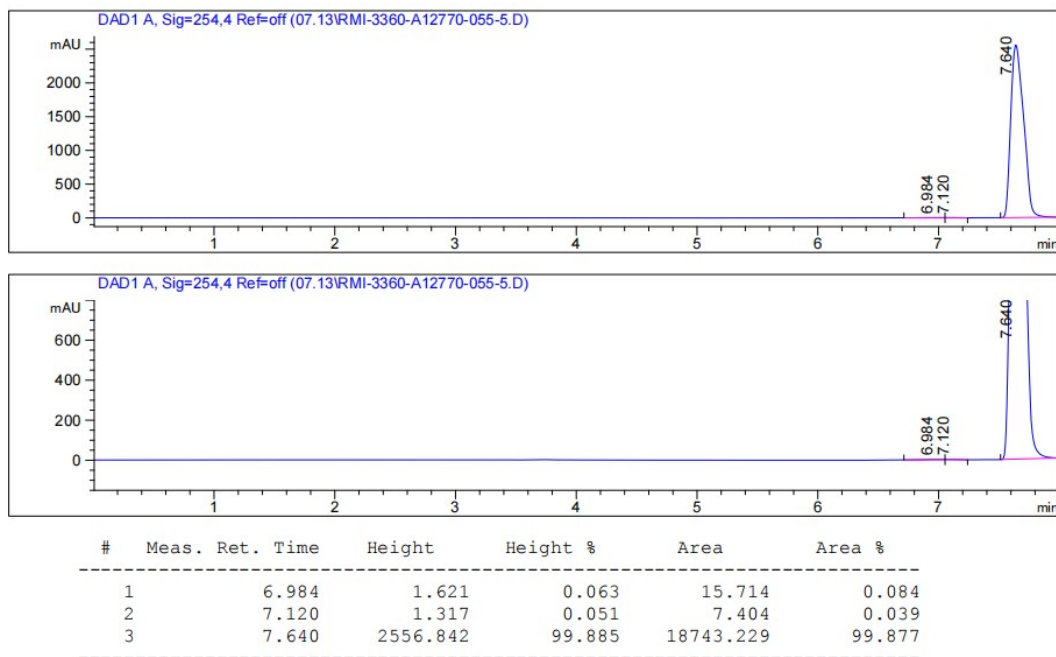


Figure S7. HPLC of compound **2b** ($t_R = 7.640$ min, 99.9% purity)

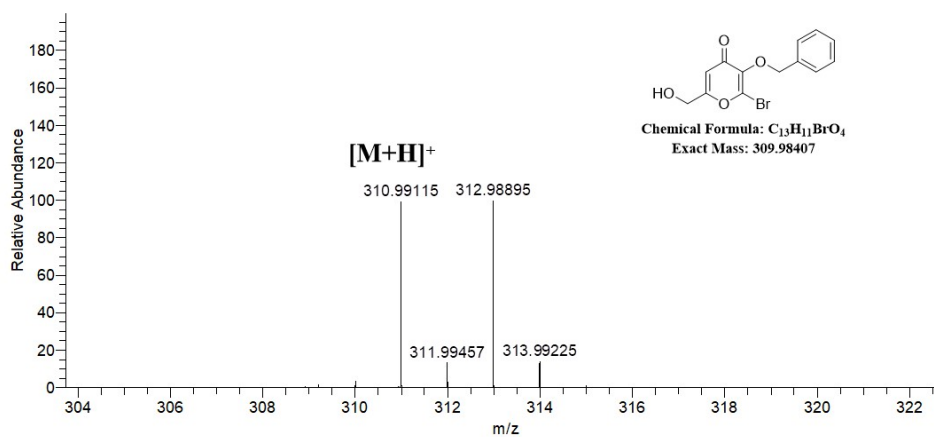


Figure S8. HRMS of compound **2b**

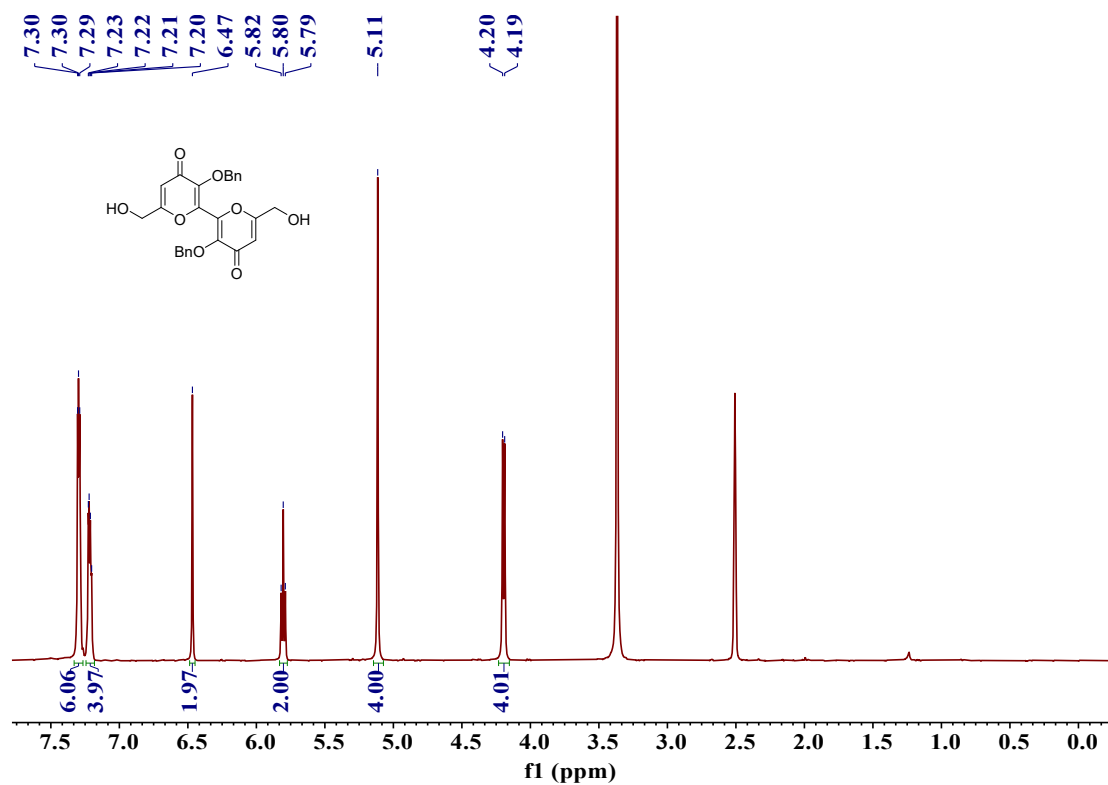


Figure S9. ¹H-NMR spectrum of **2c** (solvent DMSO-*d*₆, 400 MHz)

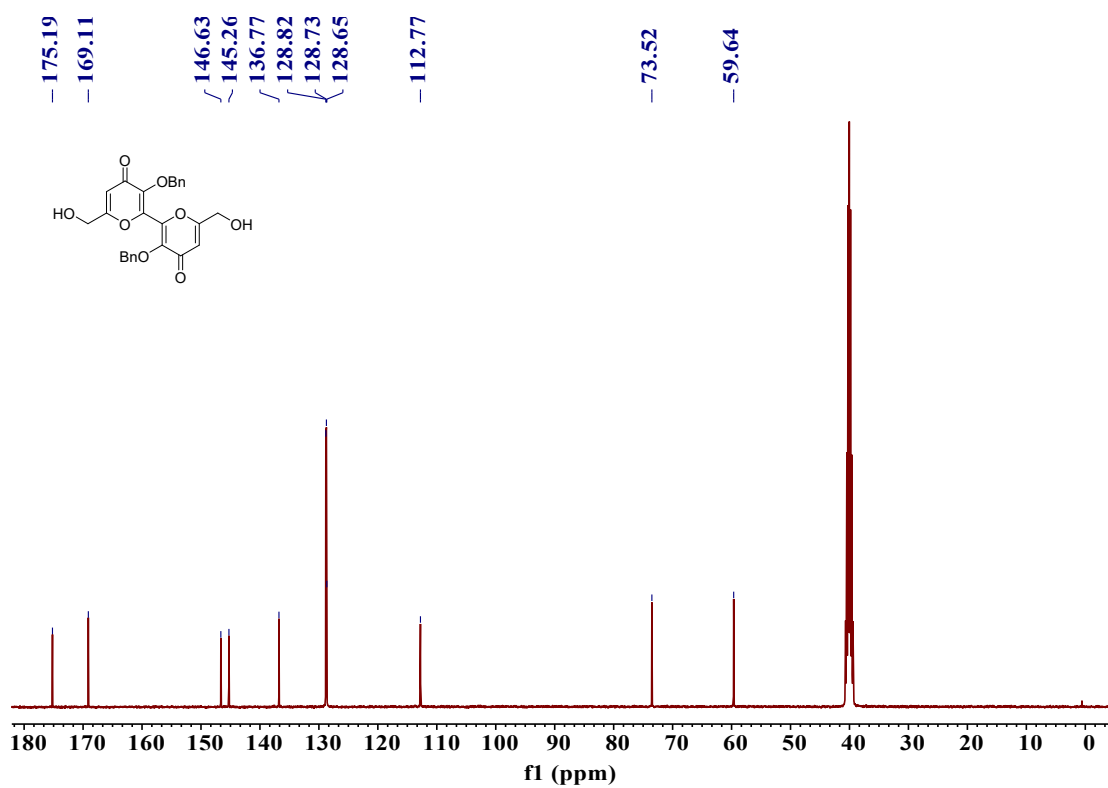
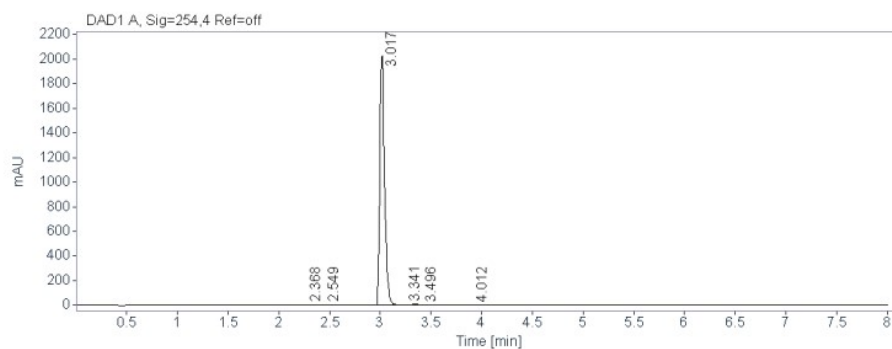


Figure S10. ¹³C-NMR spectrum of **2c** (solvent DMSO-*d*₆, 100 MHz)



Signal: DAD1 A, Sig=254,4 Ref=off

Name	RT [min]	Height	Area	Area%
	2.368	4.79719	14.25930	0.214
	2.549	2.21418	5.57477	0.084
	3.017	2046.45203	6605.24219	98.990
	3.341	9.05428	28.72350	0.430
	3.496	3.78189	12.18742	0.183
	4.012	1.98748	6.65501	0.100
Sum			6672.64219	

Signal: MSD1 TIC, MS File

Name	RT [min]	Height	Area	Area%
	3.099	3810789.50000	27842504.0000	100.000
Sum			27842504.0000	

Figure S11. HPLC of compound **2c** ($t_R = 3.017$ min, 99.0% purity)

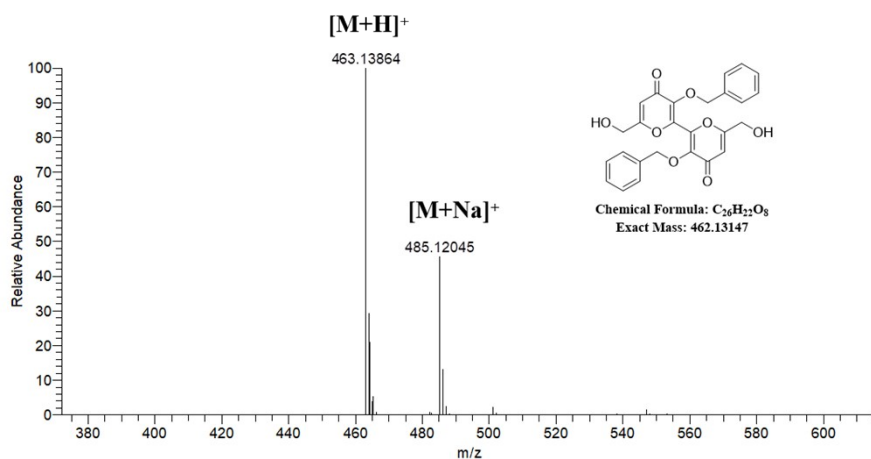


Figure S12. HRMS of compound **2c**

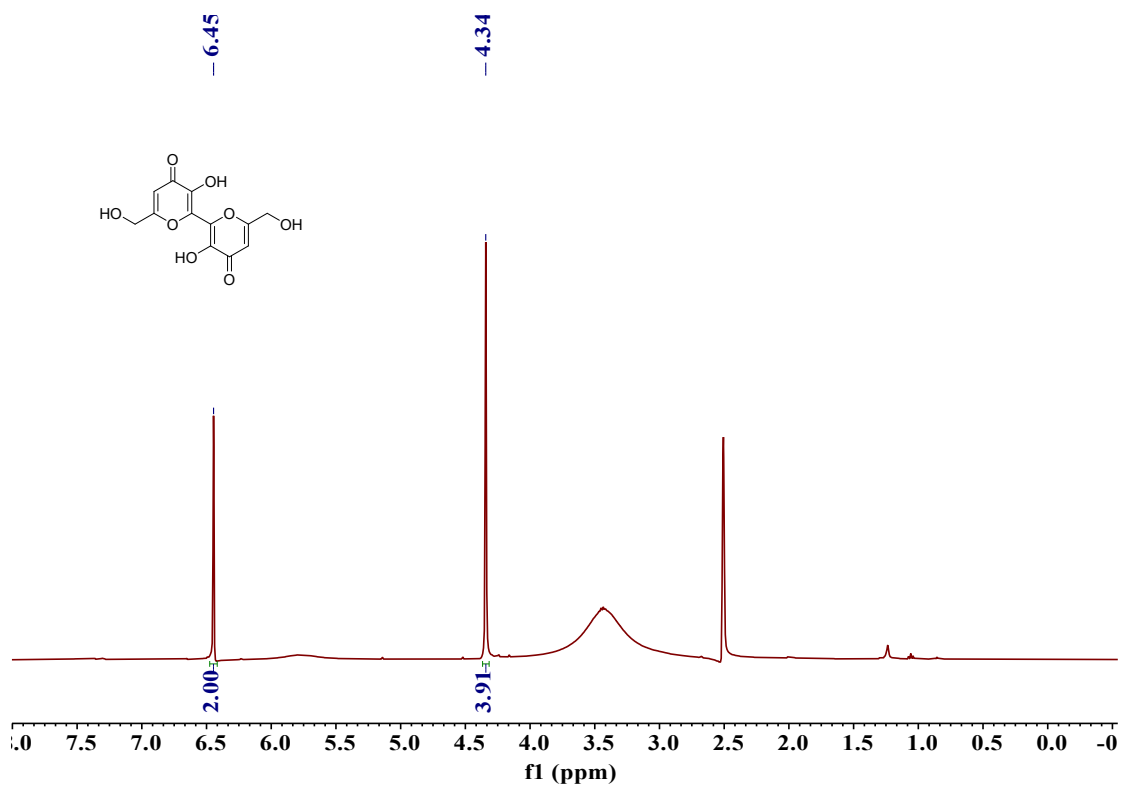


Figure S13. $^1\text{H-NMR}$ spectrum of **KAD** (solvent $\text{DMSO-}d_6$, 400 MHz)

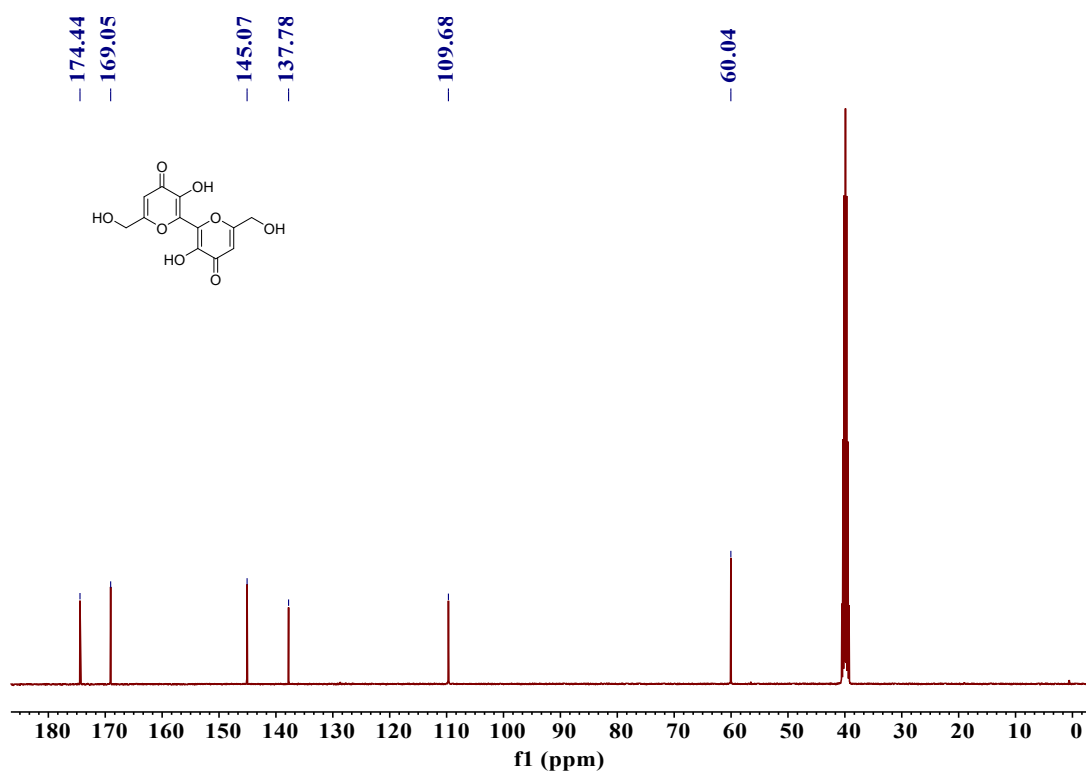
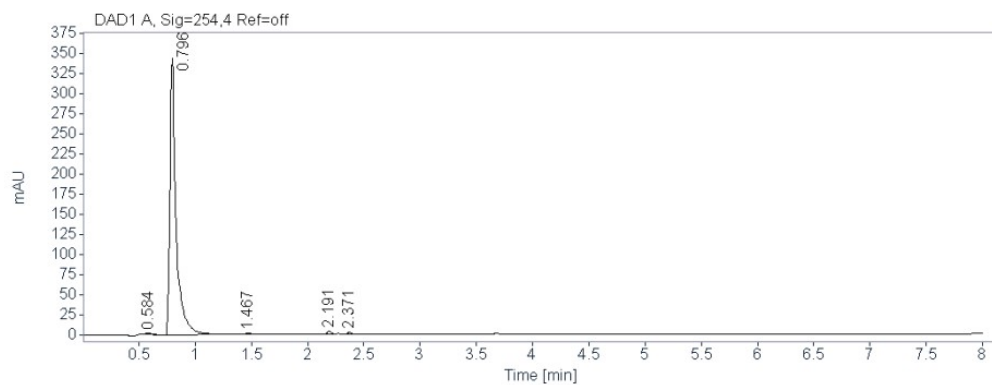


Figure S14. $^{13}\text{C-NMR}$ spectrum of **KAD** (solvent $\text{DMSO-}d_6$, 100 MHz)



Signal: DAD1 A, Sig=254,4 Ref=off

Name	RT [min]	Height	Area	Area%
	0.584	2.10841	7.02529	0.512
	0.796	342.85400	1343.36609	97.842
	1.467	1.55136	7.09679	0.517
	2.191	3.22409	8.54370	0.622
	2.371	2.24691	6.96297	0.507
	Sum		1372.99484	

Signal: MSD1 TIC, MS File

Name	RT [min]	Height	Area	Area%
	0.873	932102.00000	6942991.00000	100.000
	Sum		6942991.00000	

Figure S15. HPLC of compound **KAD** ($t_R = 0.796$ min, 97.8% purity)

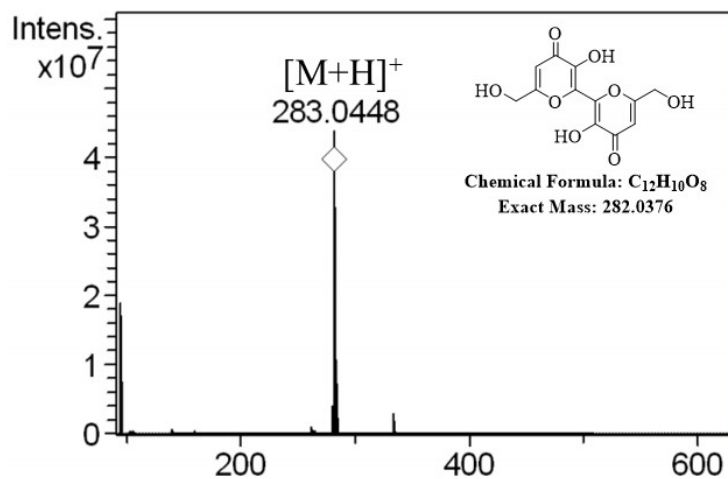


Figure S16. HRMS of compound **KAD**

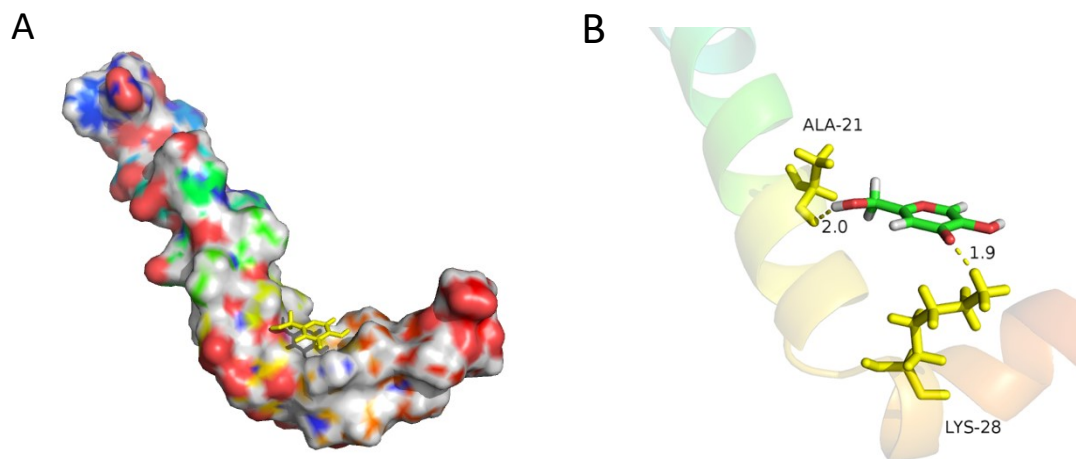


Figure S17. Docking study of KA with A β ₁₋₄₂ (PDB:1IYT). (A) Full view of KA (colored yellow) binding to A β ₁₋₄₂. (B) The possible hydrogen bonds between KA and residues Ala 21 and Lys 28 are indicated by yellow dotted lines.

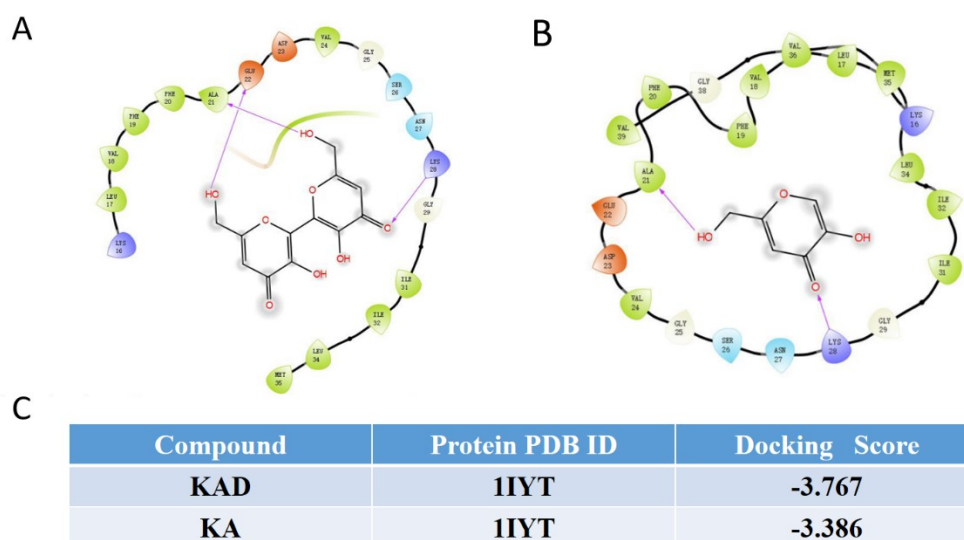


Figure S18. 2D schematic diagram and predicted docking scoring of KAD and KA with A β ₁₋₄₂. (A) 2D schematic diagram of KAD and A β ₁₋₄₂ docking models; (B) 2D schematic diagram of KA and A β ₁₋₄₂ docking models; (C) Docking Score of KAD and KA with A β ₁₋₄₂.

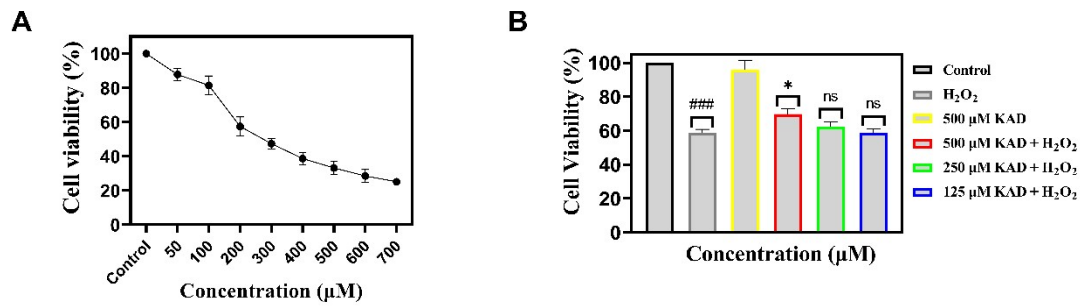


Figure S19. KAD inhibits H₂O₂-induced injury in H9c2 cells. (A) H₂O₂ reduced H9c2 cell viability in a concentration-dependent manner (2 h); (B) Effect of KAD on the viability of H9c2 cells treated by H₂O₂. Cell viability was determined by MTT assay. The values are presented as mean ± SEM (n = 3). ###P < 0.001 vs. the control group; P* < 0.05 vs. the H₂O₂-treated group.

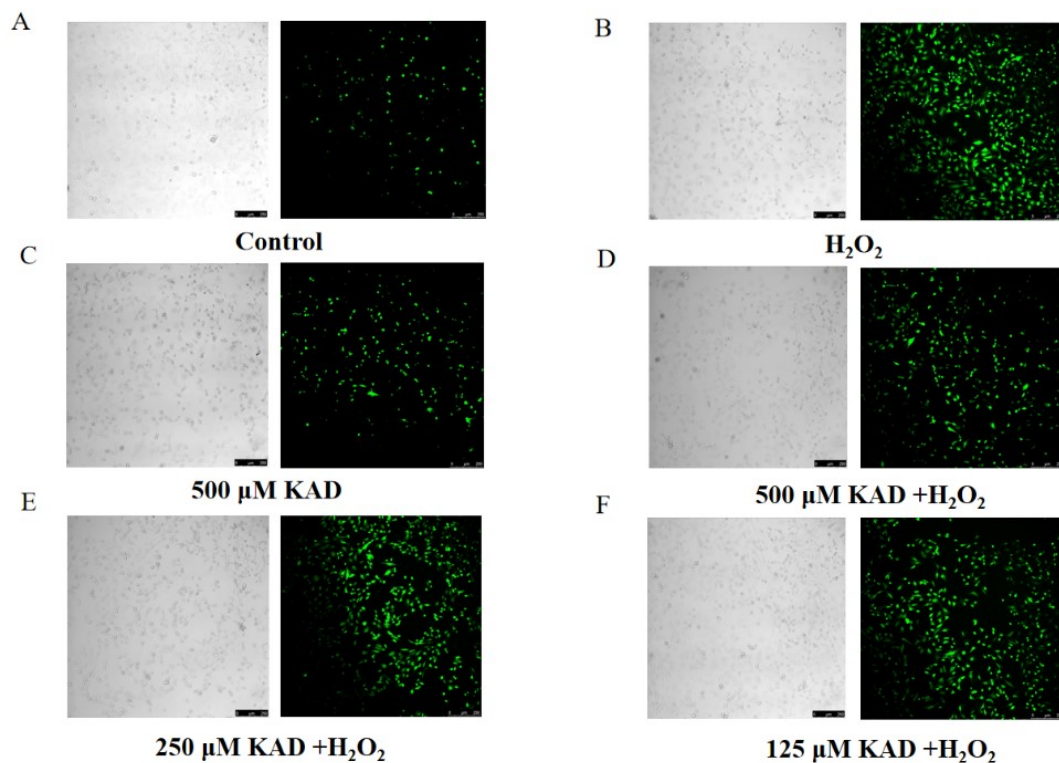


Figure S20. Inhibitory effect of KAD on intracellular ROS accumulation in H9c2 cells exposed to H₂O₂ (10×). Images of cells stained with 2',7'-dichlorofluorescein (DCF) showing the ROS contents in H9c2 cells: (A) Control; (B) H₂O₂; (C) 500 μM KAD; (D) 500 μM KAD + H₂O₂; (E) 250 μM KAD + H₂O₂; and (F) 125 μM KAD + H₂O₂.

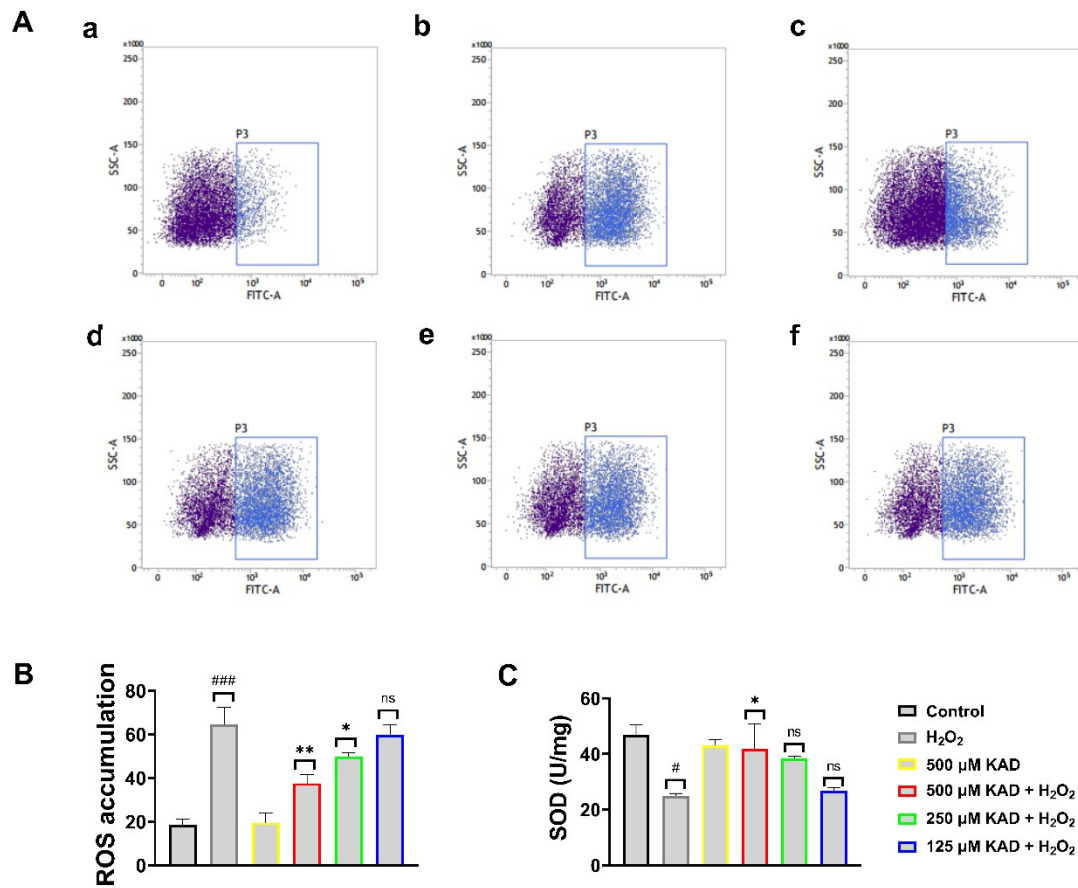


Figure S21. KAD alleviated intracellular ROS accumulation and increased the SOD activity induced by H₂O₂ in H9c2 cells. (A) Flow cytometry images for each group: (a) Control; (b) H₂O₂; (c) 500 μM KAD; (d) 500 μM KAD + H₂O₂; (e) 250 μM KAD + H₂O₂; and (f) 125 μM KAD + H₂O₂; (B) ROS quantification by DCFH-DA using flow cytometry (20000 cells); (C) Effect of KAD on SOD activity. All data are presented as mean ± SEM of three independent experiments. #P < 0.05 and ###P < 0.001 vs. the control group; P* < 0.05 and **P < 0.01 vs. the H₂O₂-treated group.

Crystallographic data was collected on a Mercury single crystal diffractometer at room temperature. The structures were solved with direct methods by using OlexSys or SHELXS-97 and refined with the full-matrix least-squares technique based on F2 by using the OlexSys or SHELXL-97

Table S1. Summary of crystallographic data for KAD

Formula	C ₁₂ H ₁₀ O ₈	$\beta / ^\circ$	99.527(8)
Formula weight	282.21	$\gamma / ^\circ$	90.000
T/K	293(2)	$V/ \text{\AA}^3$	557.23(9)
Crystallization solvent	methanol	Z	2
Color	white	$D_x / \text{g cm}^{-3}$	1.682
Crystal system	monoclinic	μ / mm^{-1}	0.803
Space group	$P2_1/n$	$F(000)$	292
$a / \text{\AA}$	3.8200(4)	θ range / $^\circ$	4.395 to 60.445
$b / \text{\AA}$	17.5109(17)	GOF on F ²	1.055
$c / \text{\AA}$	8.4469(7)	$R_1 [I > 2\sigma(I)]$	0.0371
$\alpha / ^\circ$	90.000	wR_2 (all data)	0.1030