Supplementary Information

Furoxan-Piplartine Hybrids as Effective NO Donors and ROS Inducers in PC3 Cancer Cells: Design, Synthesis, and Biological Evaluations

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A. Characterization data of compounds

A.1. ^{1}H- and ^{13}C-NMR spectra of compounds 4 – 9

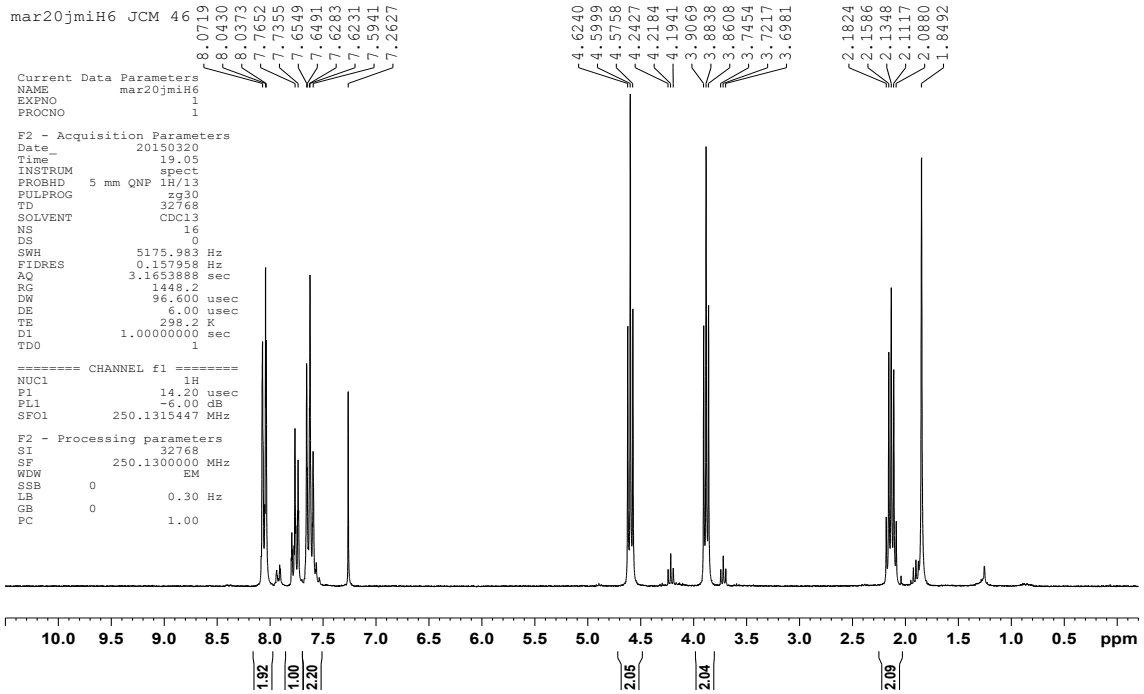


Figure S1. ^{1}H NMR spectrum of compound **4** (250 MHz, $CDCl_3$, 298 K).

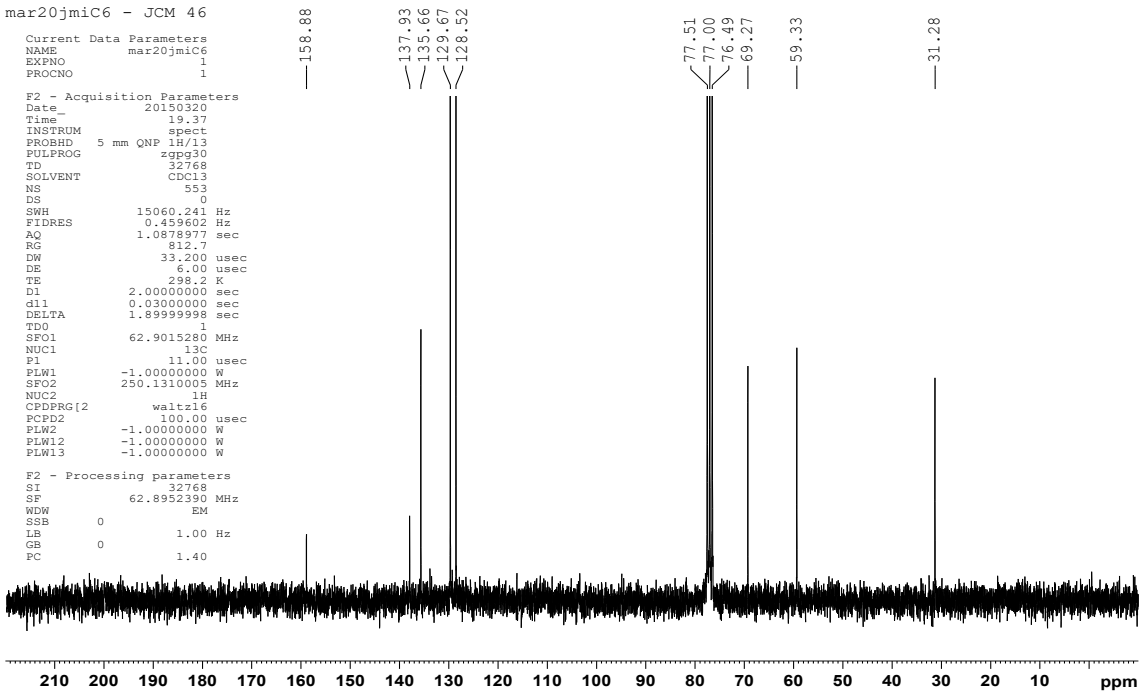


Figure S2. ^{13}C NMR spectrum of compound **4** (62.5 MHz, $CDCl_3$, 298 K).

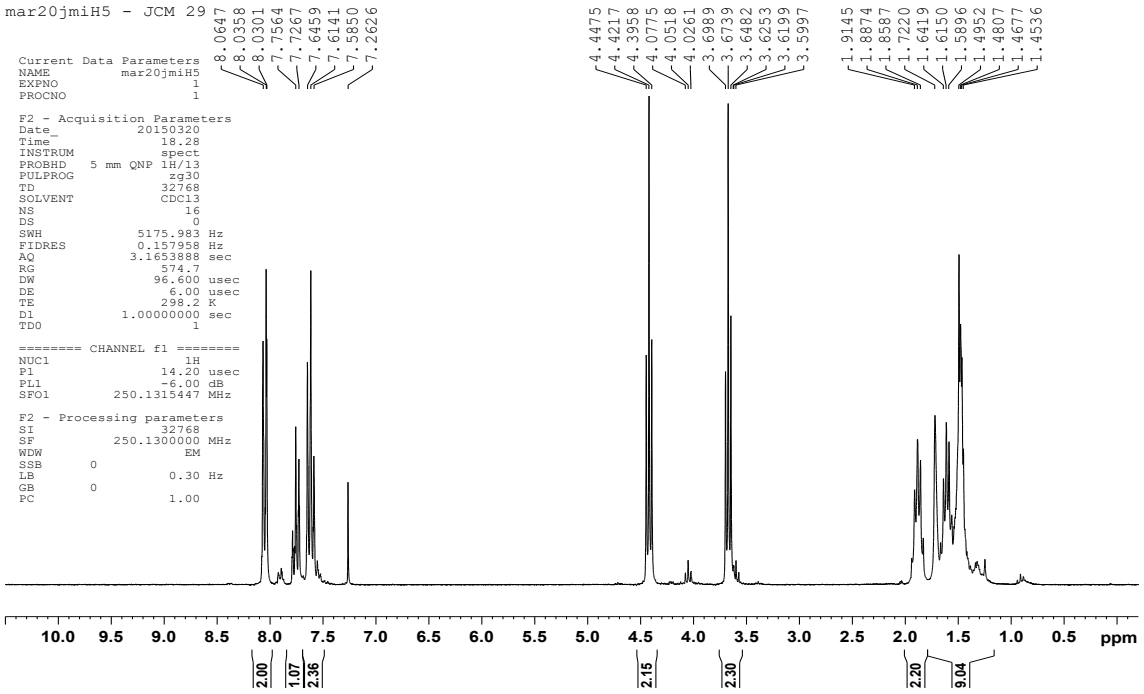


Figure S3. ^{1}H NMR spectrum of compound **5** (250 MHz, $CDCl_3$, 298 K).

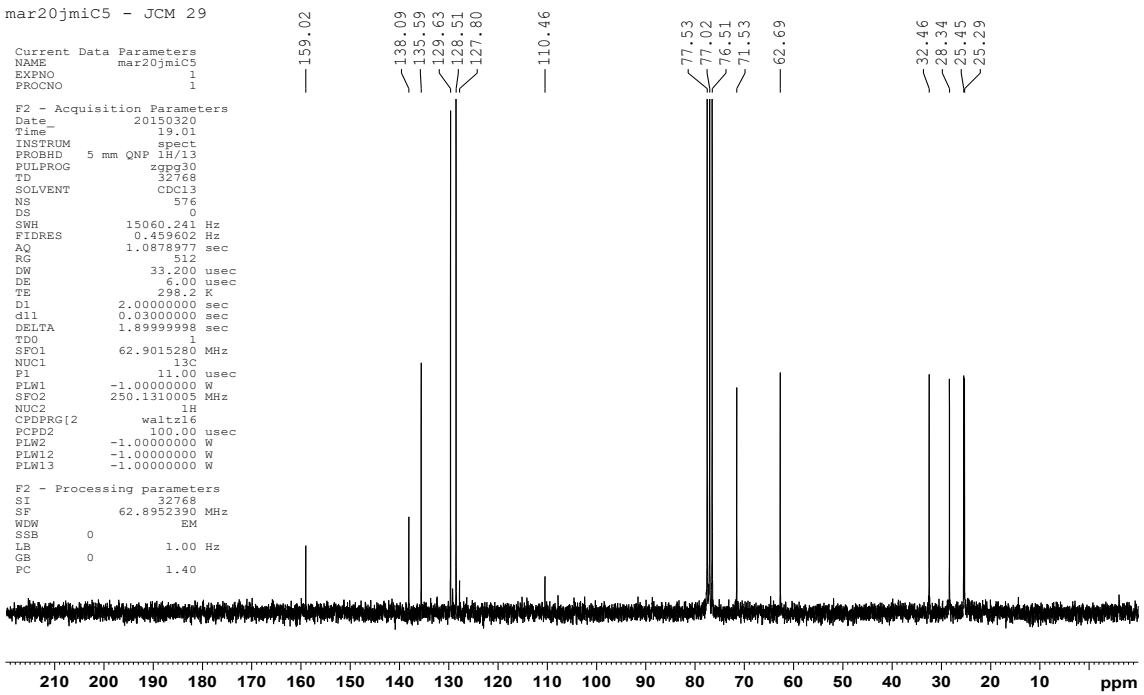


Figure S4. ^{13}C NMR spectrum of compound **5** (62.5 MHz, $CDCl_3$, 298 K).

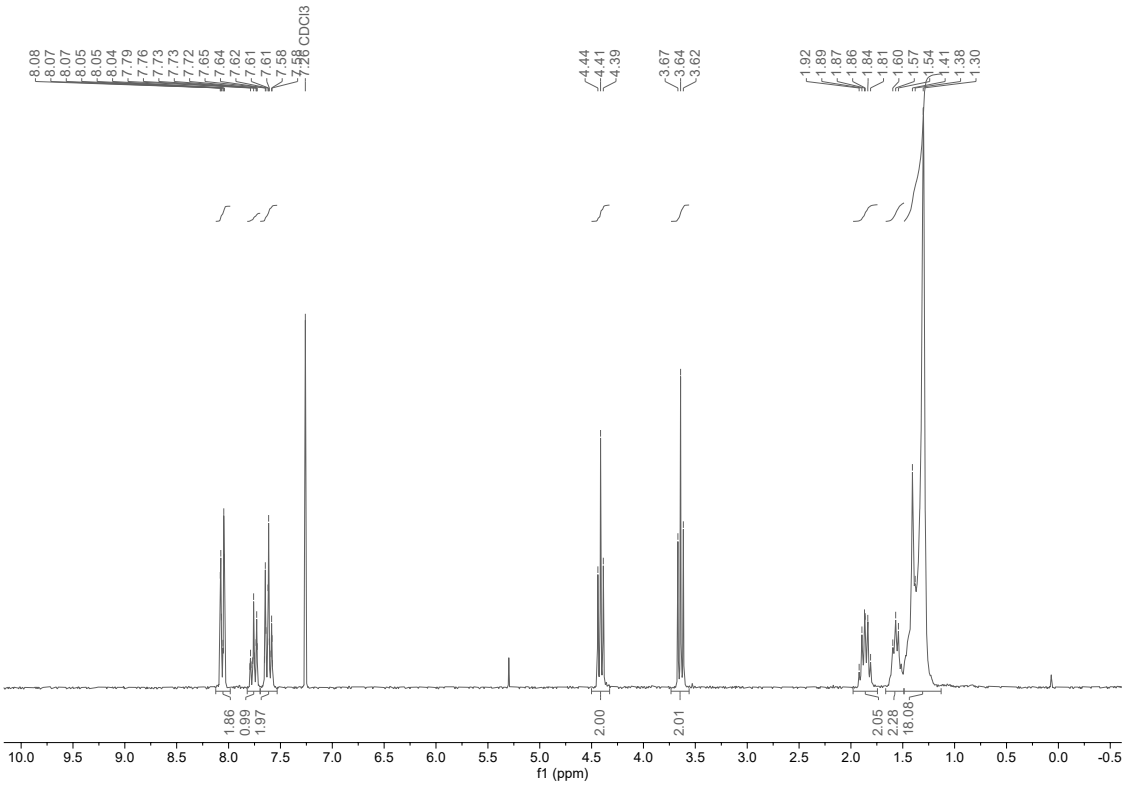


Figure S5. ^{1}H NMR spectrum of compound **6** (250 MHz, $CDCl_3$, 298 K).

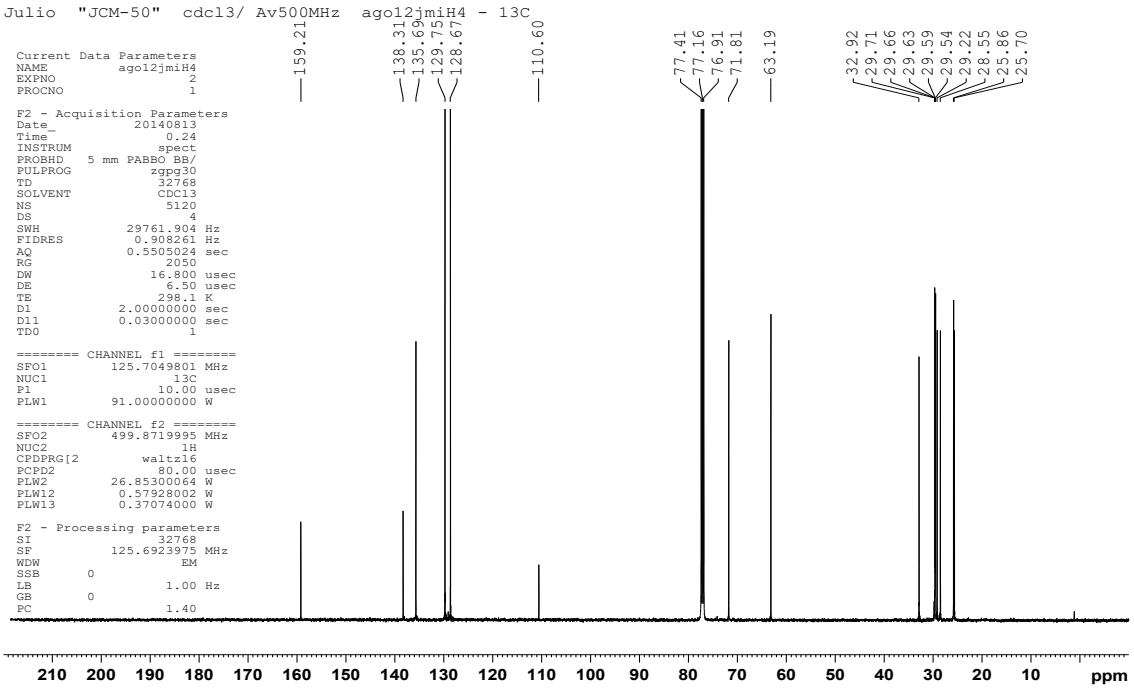


Figure S6. ^{13}C NMR spectrum of compound **6** (125 MHz, $CDCl_3$, 298 K).

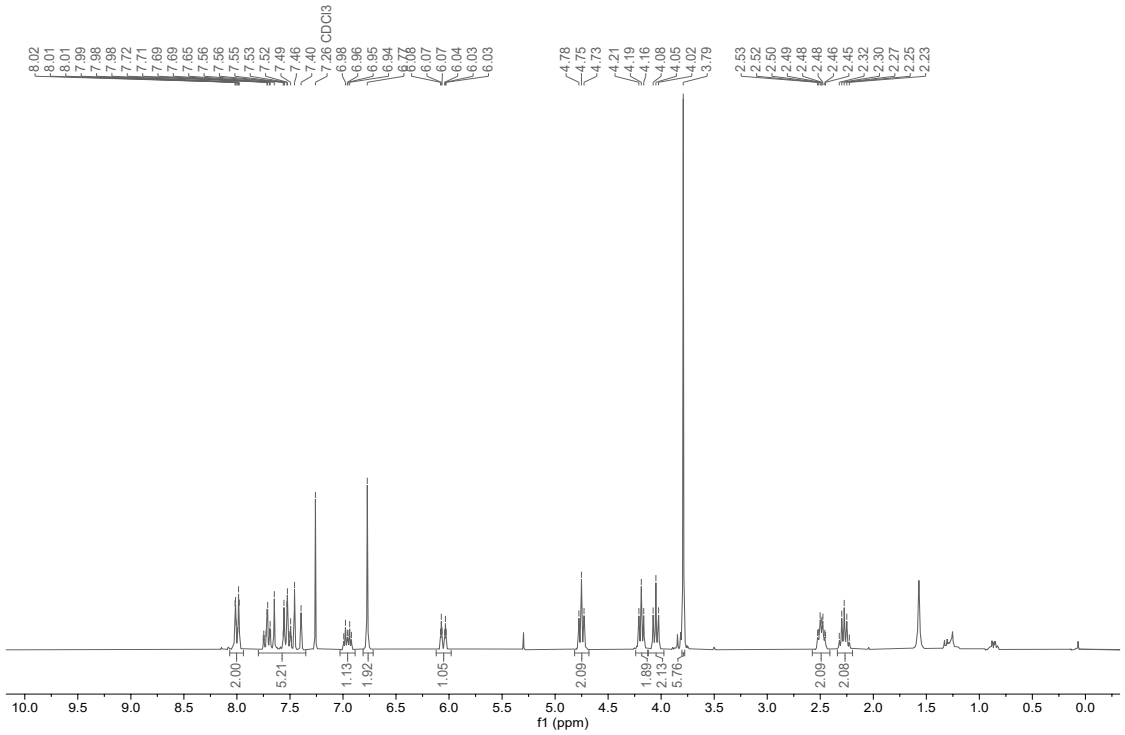


Figure S7. ^{1}H NMR spectrum of compound **7** (250 MHz, $CDCl_3$, 298 K).

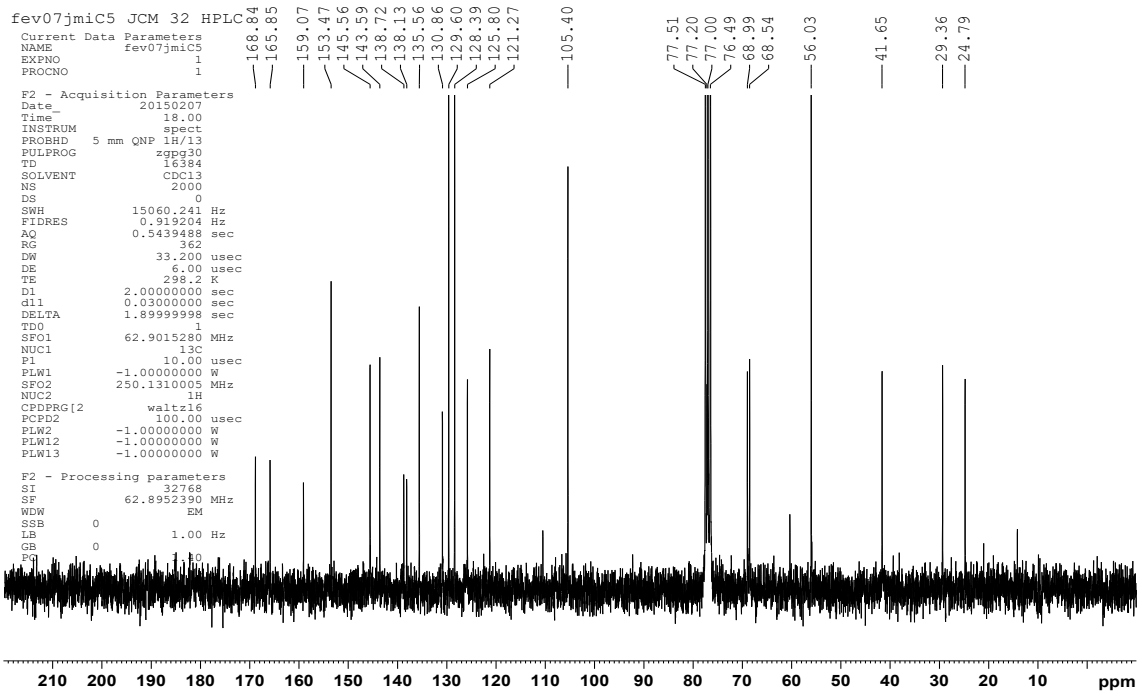


Figure S8. ^{13}C NMR spectrum of compound **7** (62.5 MHz, $CDCl_3$, 298 K).

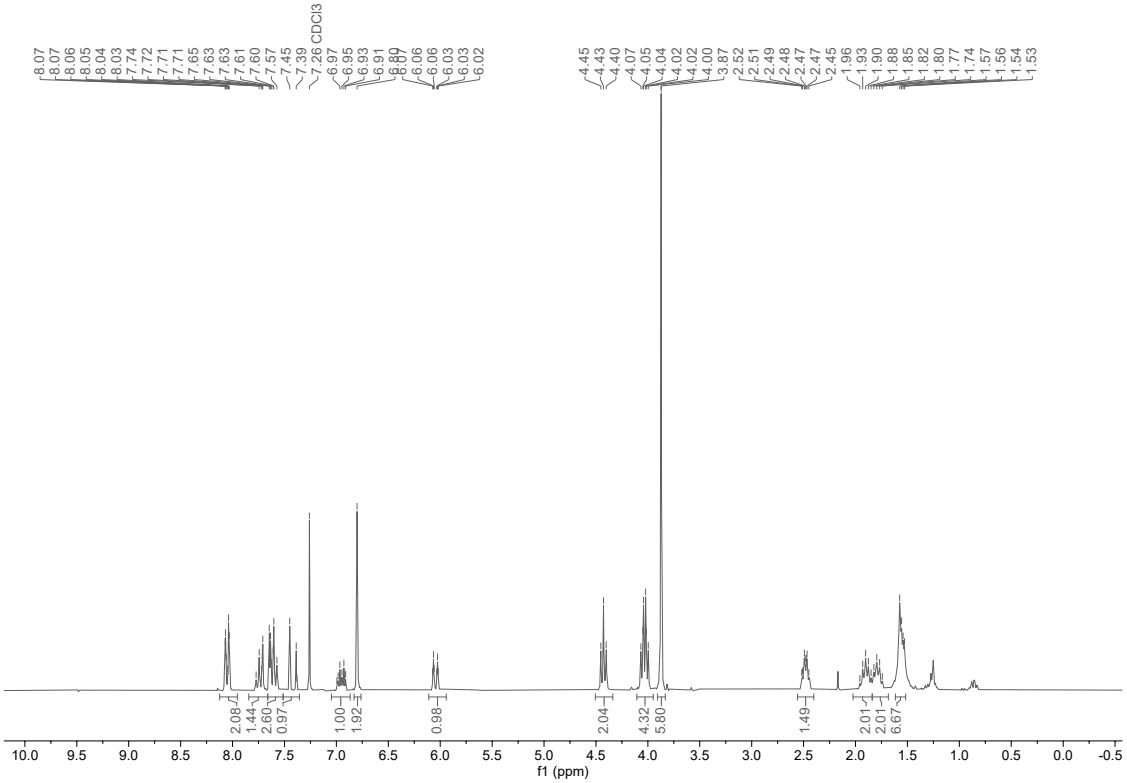


Figure S9. ^{1}H NMR spectrum of compound **8** (250 MHz, $CDCl_3$, 298 K).

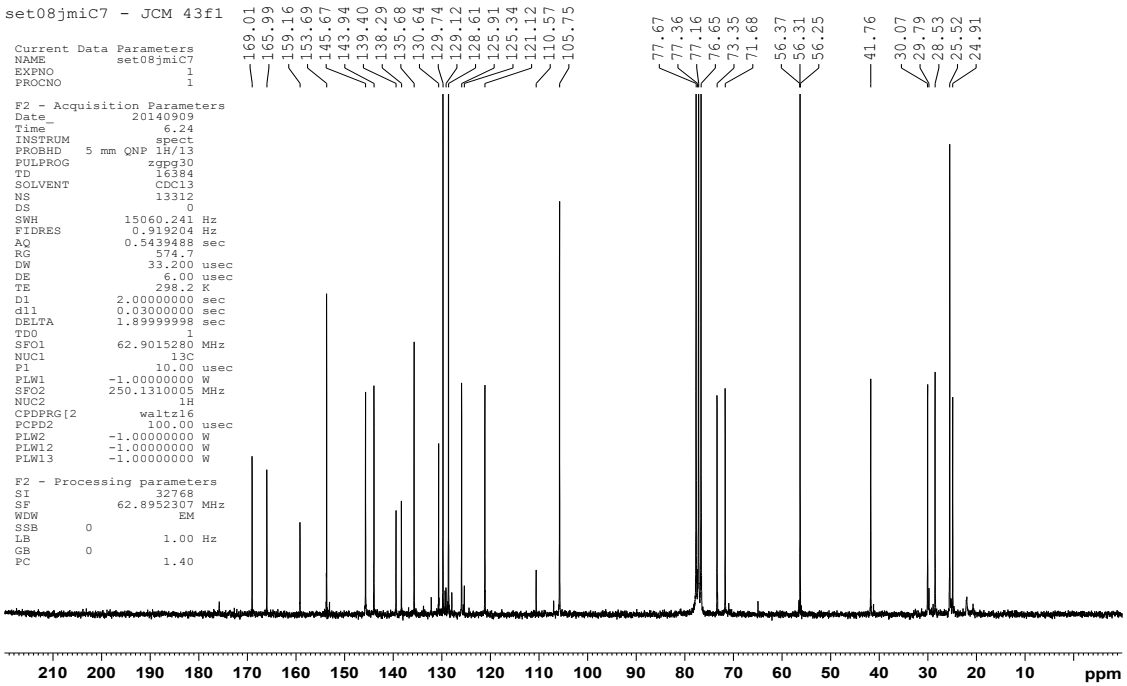


Figure S10. ^{13}C NMR spectrum of compound **8** (62.5 MHz, $CDCl_3$, 298 K).

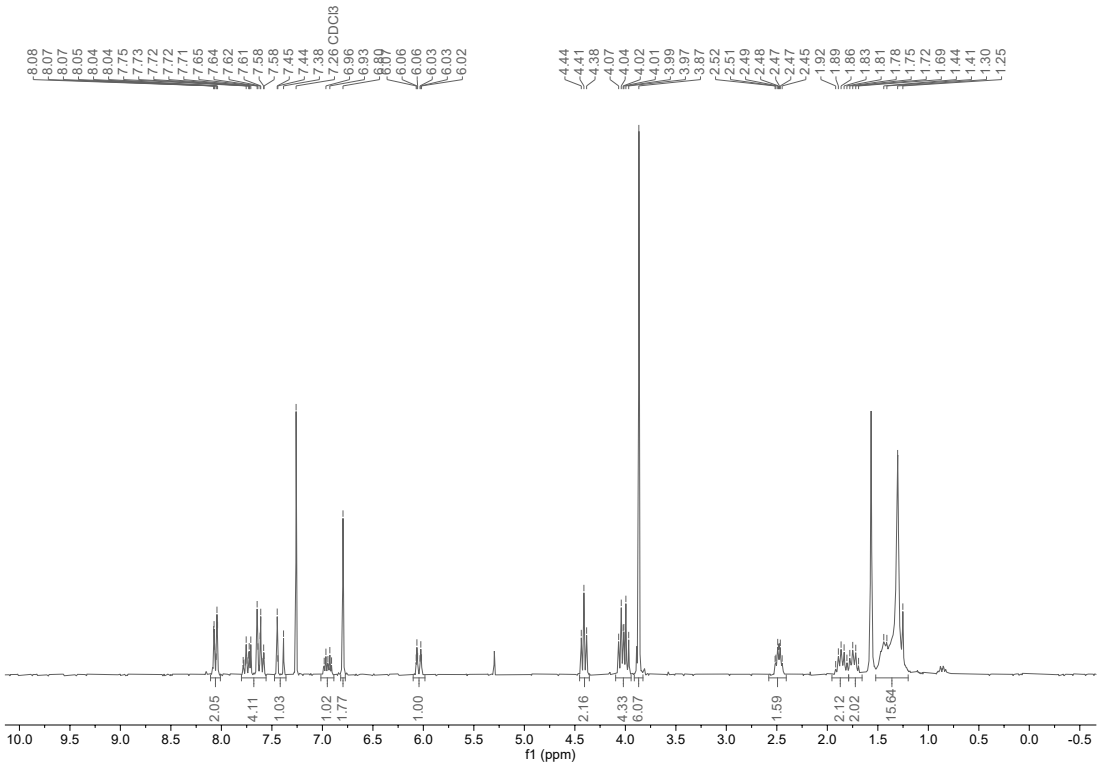


Figure S11. ^{1}H NMR spectrum of compound **9** (250 MHz, $CDCl_3$, 298 K).

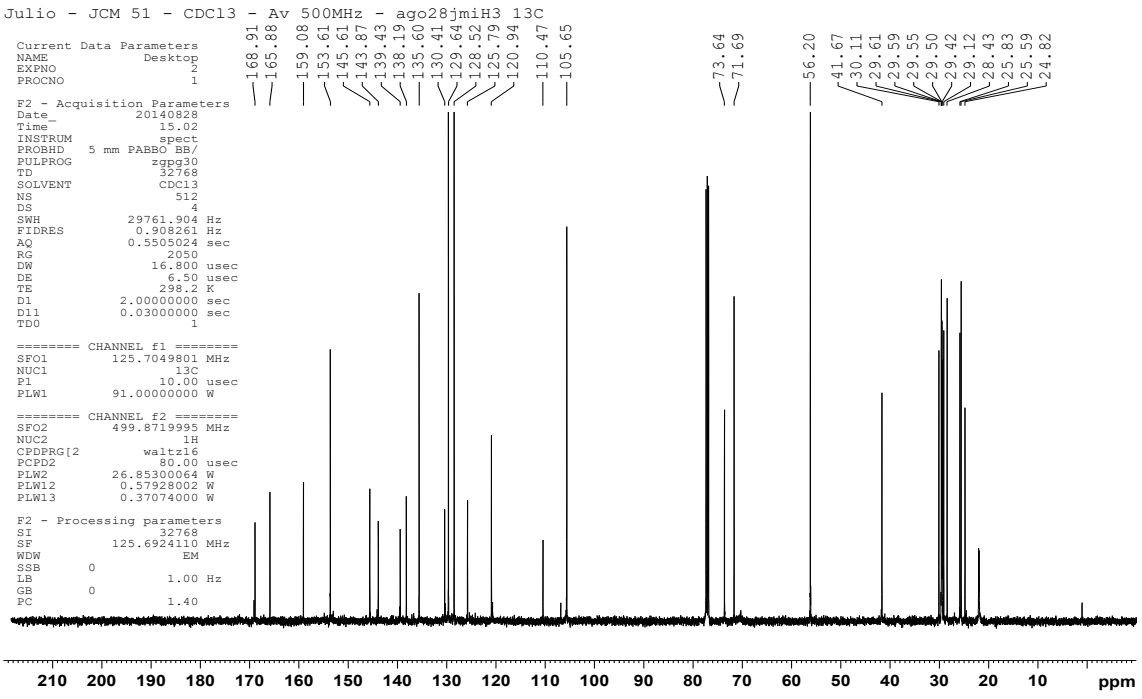


Figure S12. ^{13}C NMR spectrum of compound **9** (125 MHz, $CDCl_3$, 298 K).

A.2. HRMS spectra of compounds 4 – 9

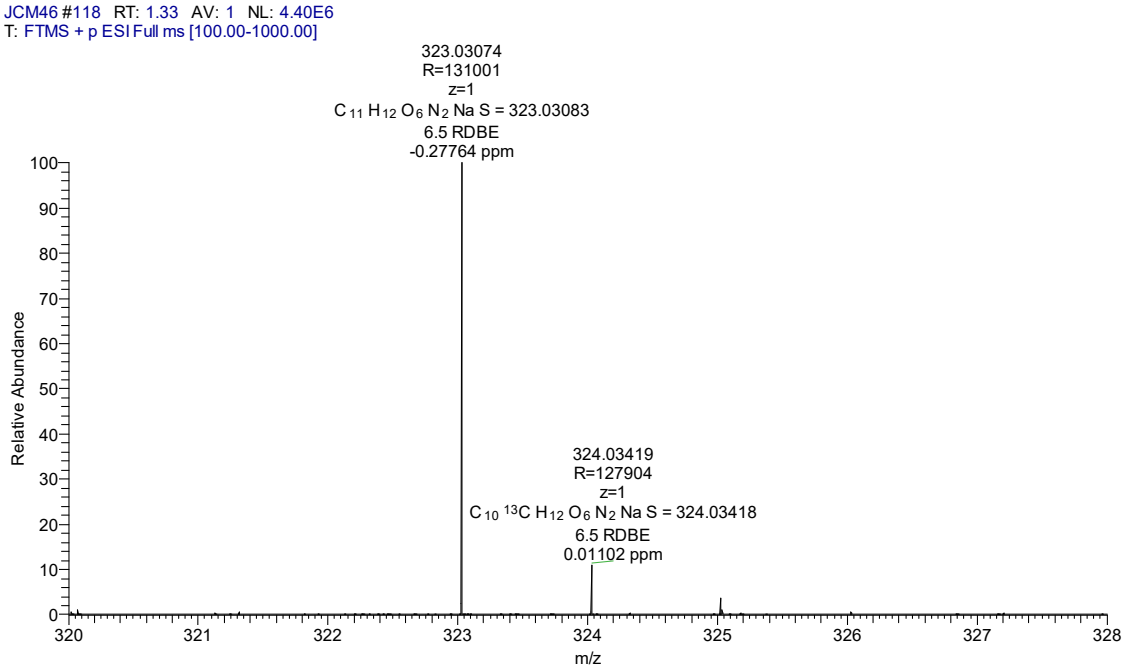


Figure S13. HRMS spectrum of compound **4**.

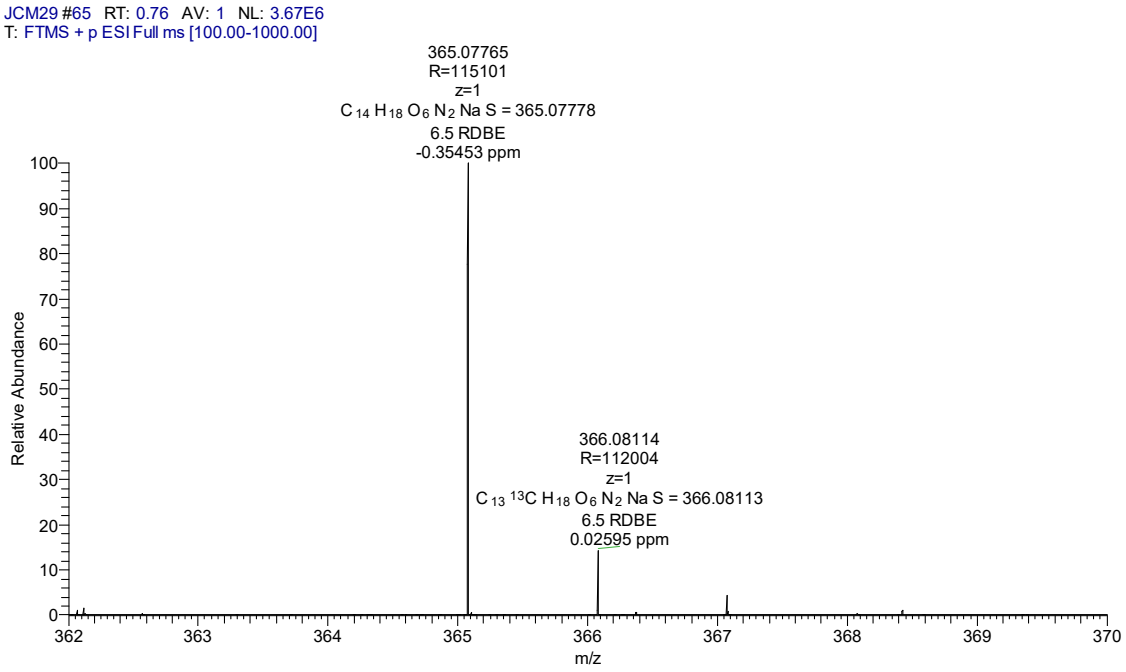


Figure S14. HRMS spectrum of compound **5**.

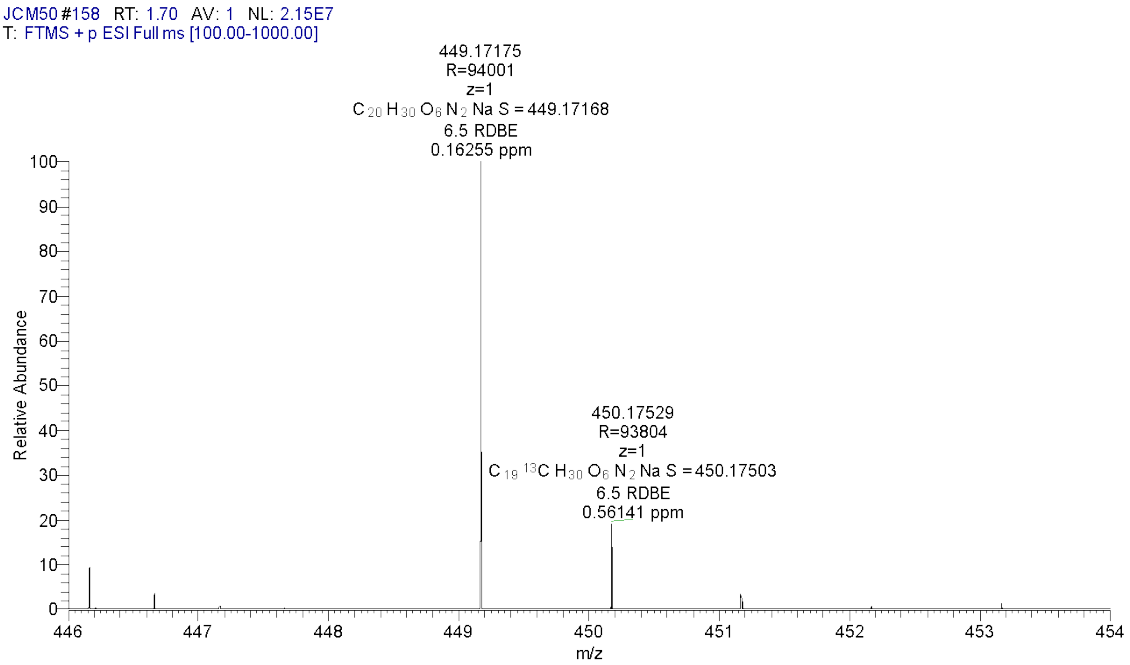


Figure S15. HRMS spectrum of compound **6**.

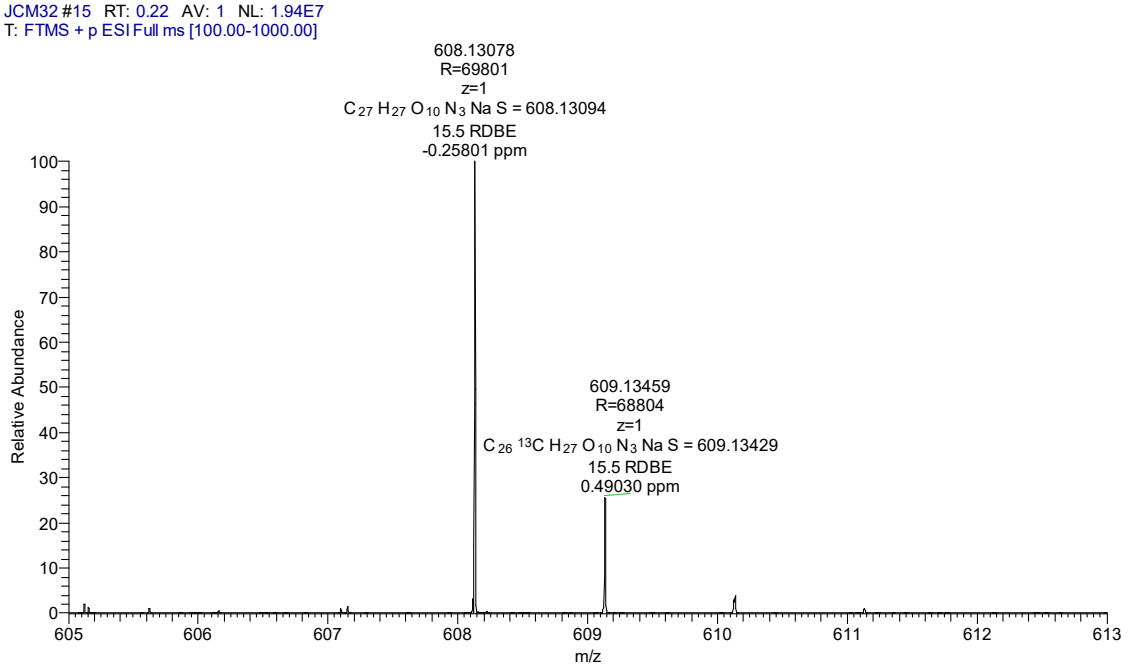


Figure S16. HRMS spectrum of compound **7**.

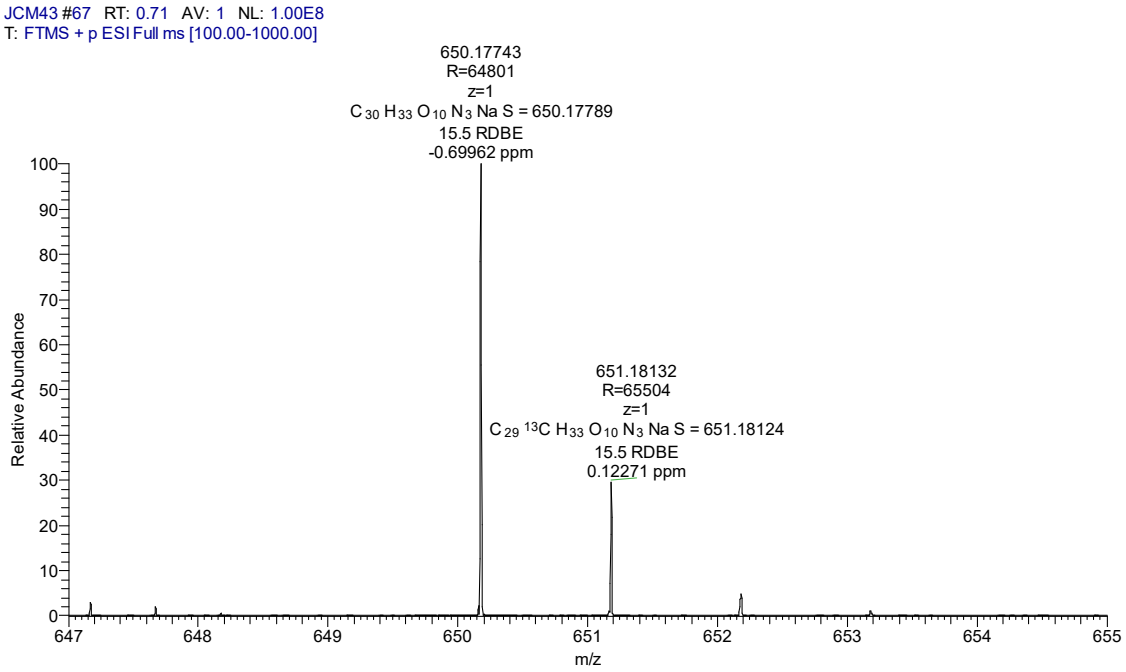


Figure S17. HRMS spectrum of compound **8**.

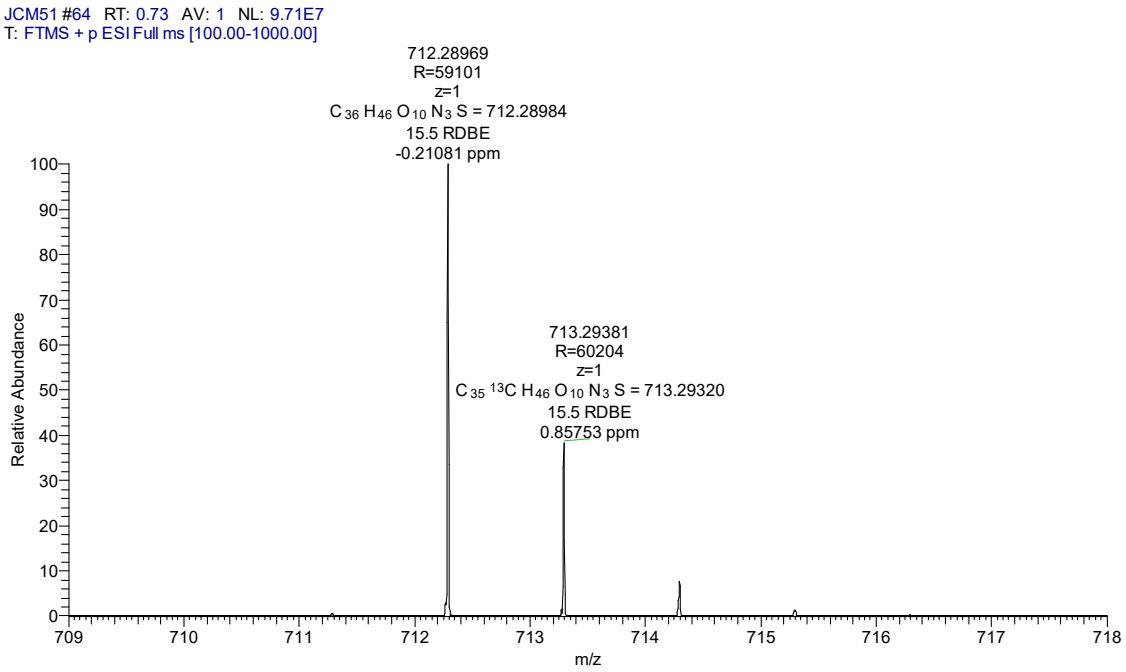


Figure S18. HRMS spectrum of compound **9**.

A.3. HPLC method conditions and chromatograms

- **Sample preparation:** The compounds were dissolved in DMSO (at 10 mM), and then diluted (1:10) in isopropanol.

- **Mobile Phase:** A) Hexane
  B) Isopropanol

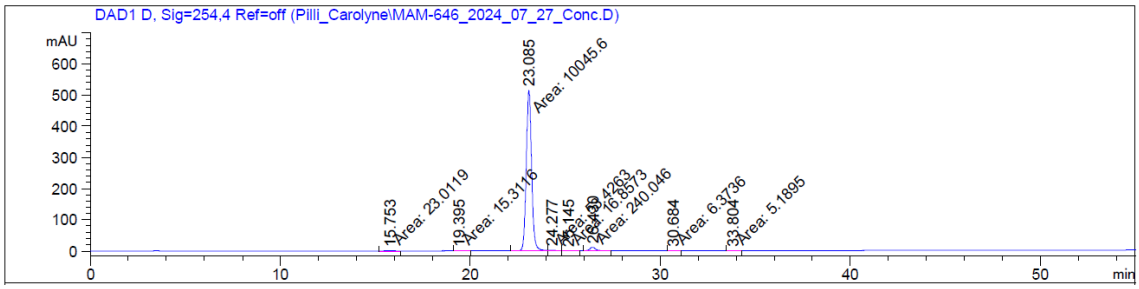
- **Chromatographic conditions:**

  System: Agilent 1260 Infinity
  Column: Kromasil® SILICA, 5µ 4.6 mm x 250 mm
  Column temperature: 25 ºC
  Flow rate: 1.0 mL/min
  Injection volume: 10 µL
  Wavelength: 254 nm

- **Gradient program:**

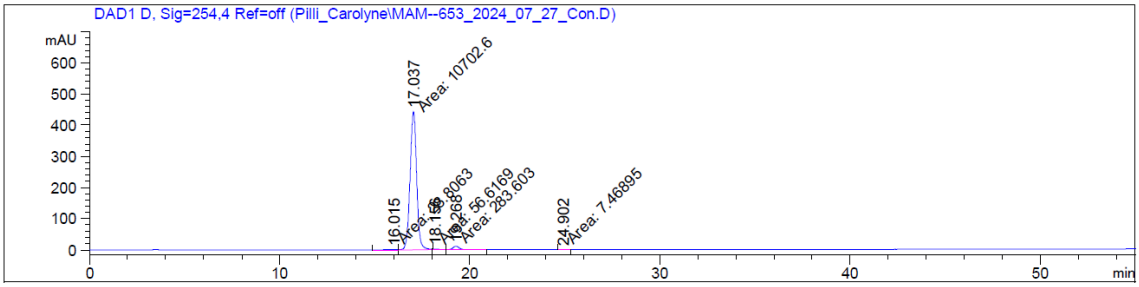
Time	%B
0.1	10
10	10
45	50
50	90

- **HPLC Chromatograms:**



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.753	MM	0.4605	23.01194	8.32845e-1	0.2211
2	19.395	MM	0.3730	15.31159	6.84085e-1	0.1471
3	23.085	MF	0.3262	1.00456e4	513.31067	96.5383
4	24.277	MF	0.4228	53.42625	2.10600	0.5134
5	25.145	FM	0.5071	16.85726	5.54062e-1	0.1620
6	26.430	MM	0.3479	240.04642	11.50004	2.3068
7	30.684	MM	0.3522	6.37360	3.01618e-1	0.0613
8	33.804	MM	0.3249	5.18950	2.66230e-1	0.0499

Figure S19. HPLC chromatogram of compound **7**.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.015	MF	0.4498	28.80630	1.06738	0.2600
2	17.037	MF	0.4024	1.07026e4	443.28036	96.6018
3	18.156	MF	0.4404	56.61694	2.14288	0.5110
4	19.268	FM	0.4033	283.60266	11.71914	2.5598
5	24.902	MM	0.2904	7.46895	4.28635e-1	0.0674

Figure S20. HPLC chromatogram of compound **9**.

B. Biological assays

B.1. PCR data

Table S1. Sequences of the primers used for amplification in real-time PCR.

Gene	Sequence	Reference
c-MYC	F: 5'AAGCTGAGGCACACAAAGA3' R: 5'GCTTGGACAGGTTAGGAGTAAA3'	NM_001354870.1
CCND1	F 5'- CCATAGCCTCTACTGCCACCATC-3' R 5'- GTCCAGCGACCTTCCTCATCCA-3'	NM_001291549.1
CDKN1A	F 5'- CCATAGCCTCTACTGCCACCATC-3' R 5'- GTCCAGCGACCTTCCTCATCCA-3'	NM_001291549.1
NRF2	F 5'- CAATGAGGTTTCTTCGGCTACG -3' R 5'- AAGACTGGGCTCTCGATGTG -3'	NM_006164.4
BAX	F:5'- TTCCTTACGTGTCTGATCAATCC-3' R:5'- GGGCAGAAGGCACTAATCAA -3'	NM_004324.3
BCL-2	F:5'- CAGAAGTCTGGGAATCGATCTG -3' R:5'- AATCTTCAGCACTCTCCAGTTATAG -3'	NM_000657.2
ACTB	F 5'- AGAGCTACGAGCTGCCTGAC-3' R 5'- AGCACTGTGTTGGCGTACAG-3'	NM_001101.3
GAPDH	F 5'- GGATTTGGTCGTATTGGGC-3' R 5'- TGGAAGATGGTGATGGGATT-3'	NM_002046.4
18srRNA	F 5'- GTAACCCGTTGAACCCCATT-3' R 5'- CCATCCAATCGGTAGTAGCG-3'	HQ387008.1

F = forward primer; R = reverse primer.

B.2. Western Blot data

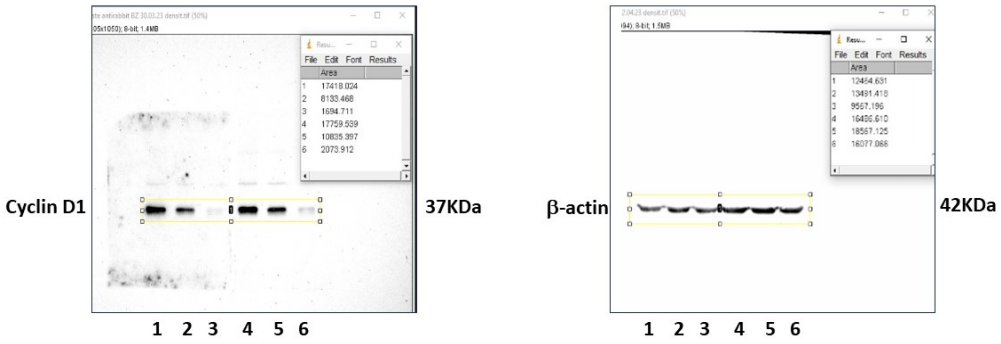


Figure S21. Protein expression of cyclin D determined by Western Blot.