

Supplementary material

Triazole derivative of guttiferone-A inhibits the proliferation of HepG2 cells by modulating MAPK/ERK signaling and expression profiles of regulators of G1/S transition

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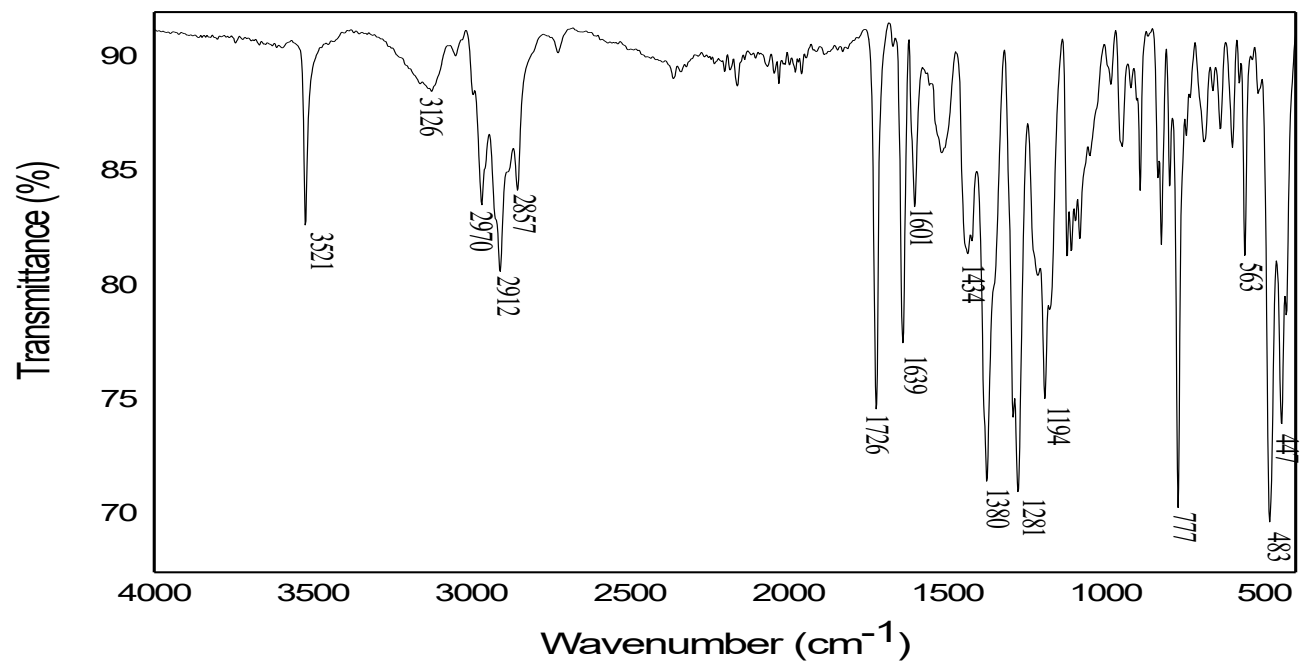


Fig. S1. FTIR (ATR) spectrum of guttiferone-A.

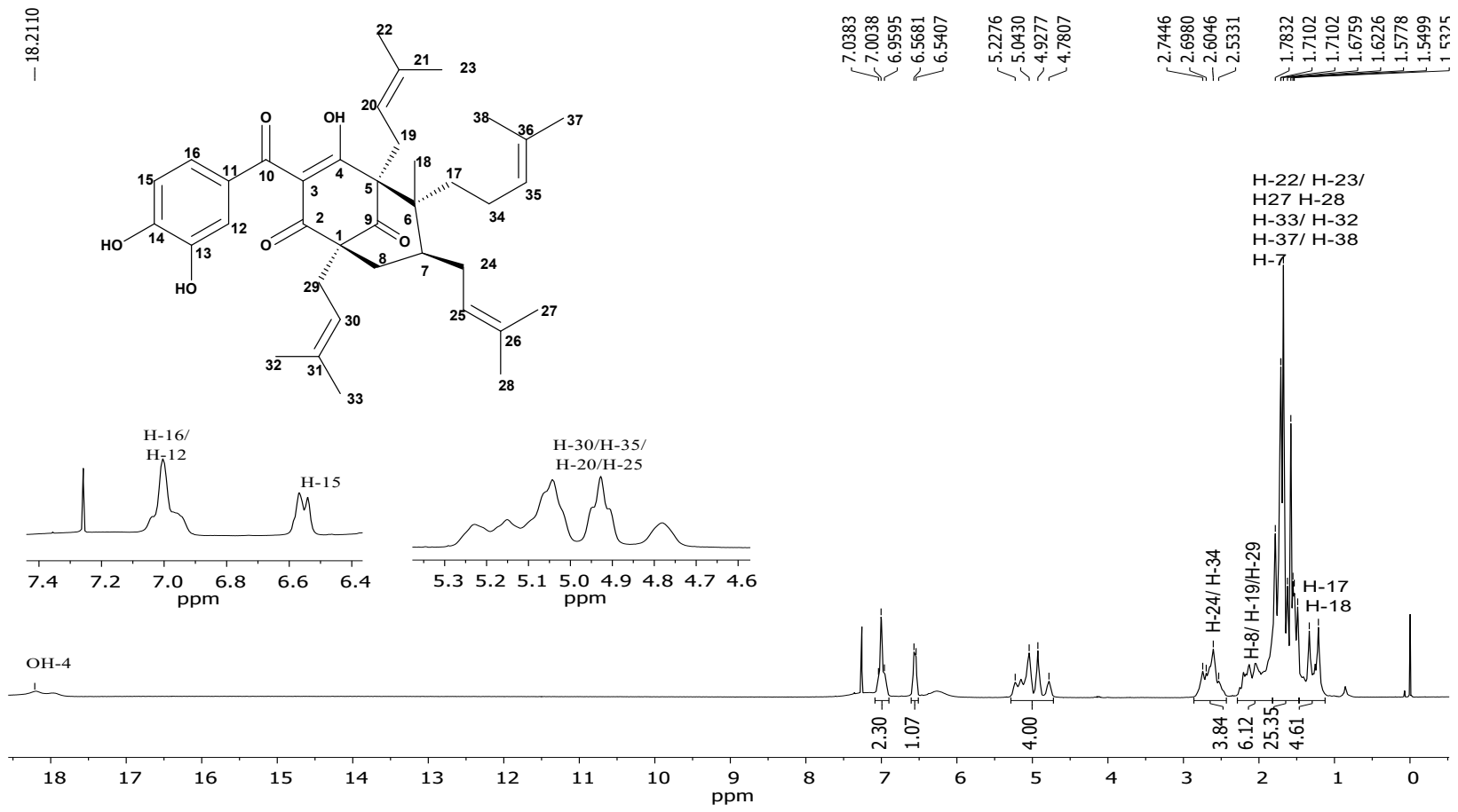


Fig. S2. ^1H NMR spectrum (300 MHz, CDCl_3) of guttiferone-A.

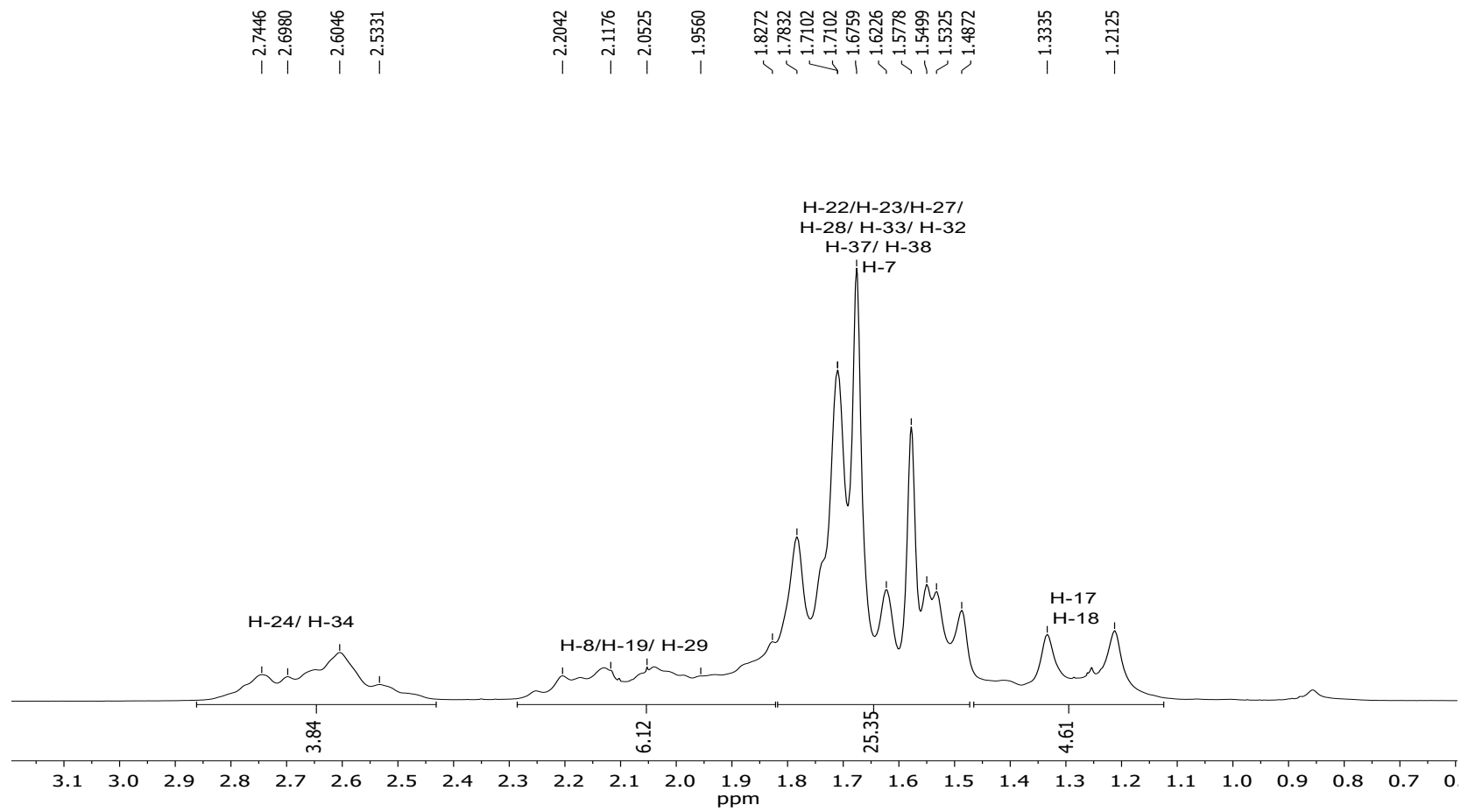


Fig. S3. Magnification of ^1H NMR spectrum (300 MHz, CDCl_3) from 1.0 to 2.8 ppm of guttiferone-A.

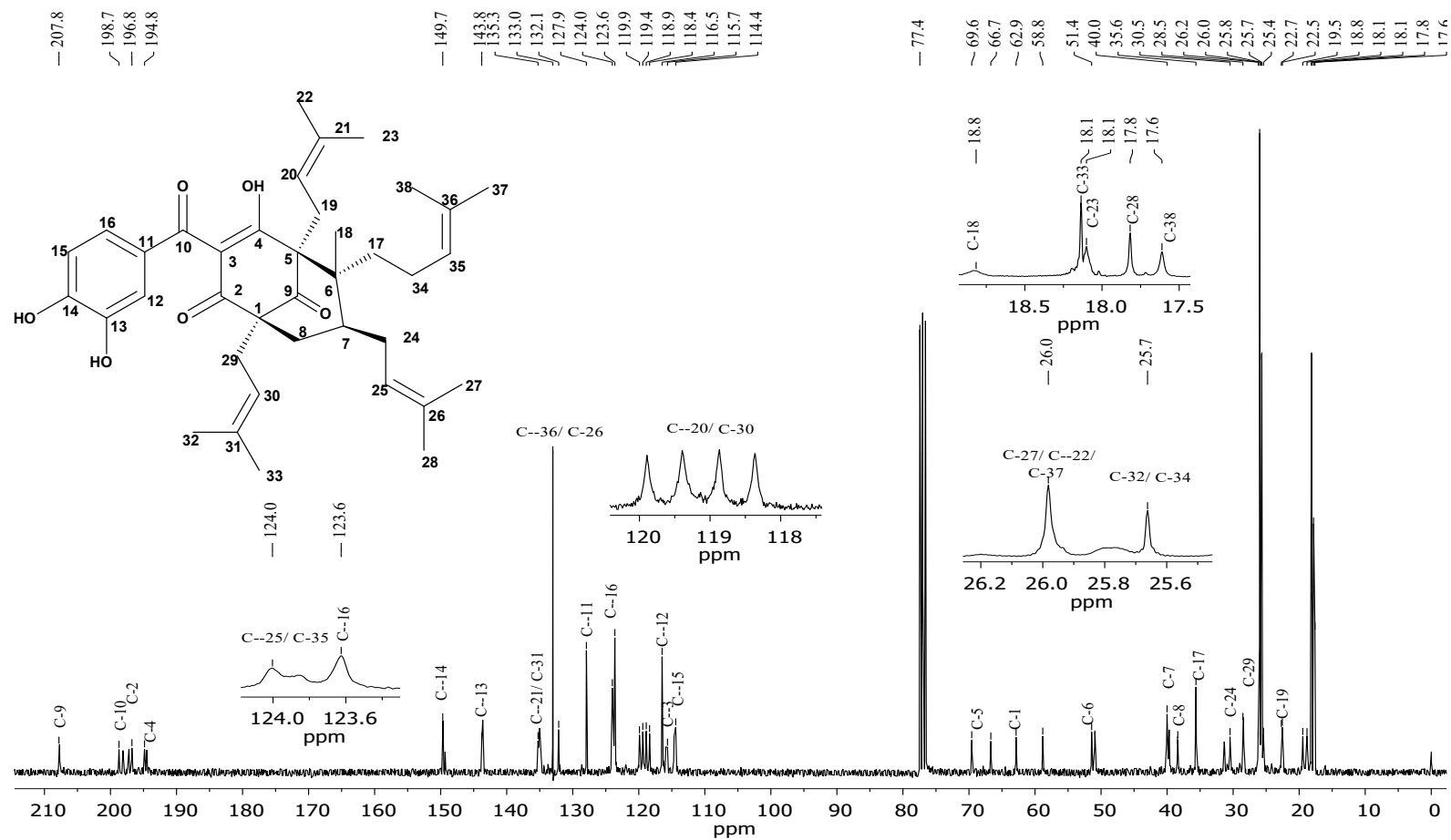


Fig. S4. ^{13}C NMR spectrum (75 MHz, CDCl_3) of guttiferone-A.

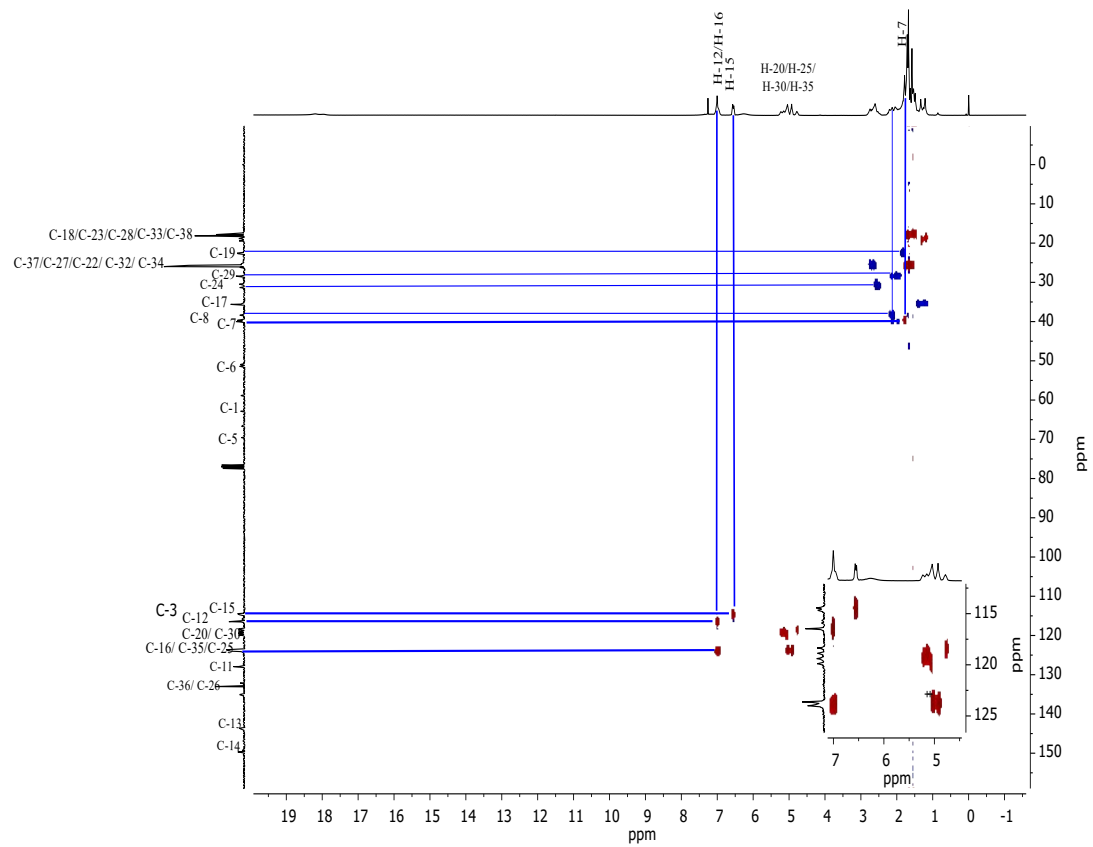


Fig. S5. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of guttiferone-A.

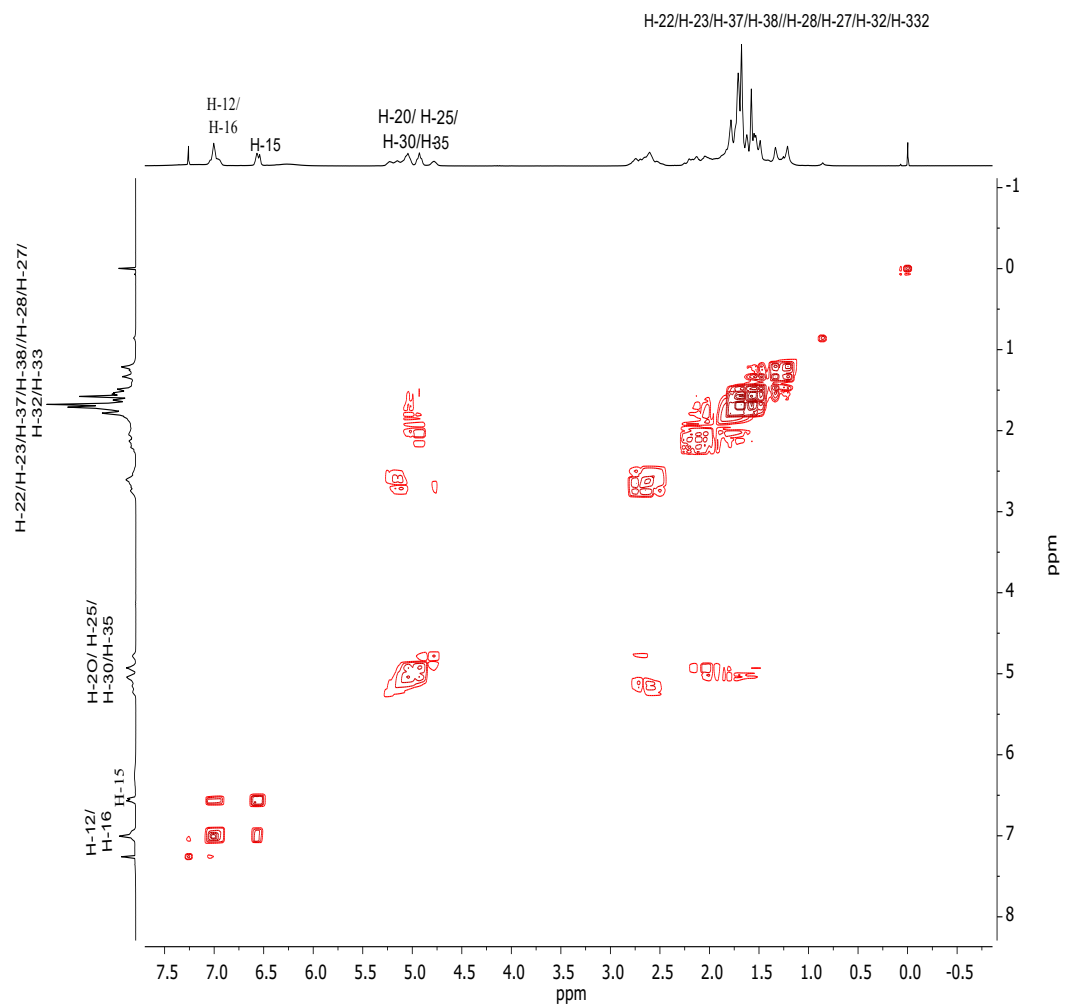


Fig. S6. COSY ^1H - ^1H NMR spectrum (300 MHz, CDCl_3) of guttiferone-A.

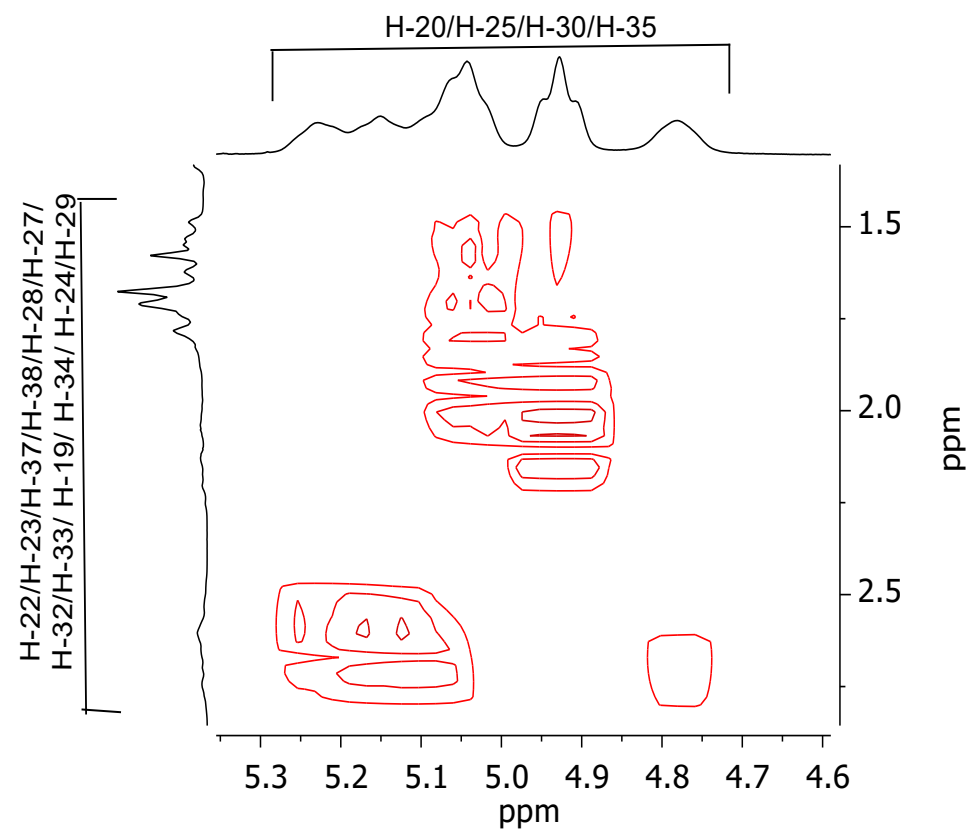


Fig. S7. Magnification of COSY ^1H - ^1H NMR spectrum (300 MHz, CDCl_3) of guttiferone-A.

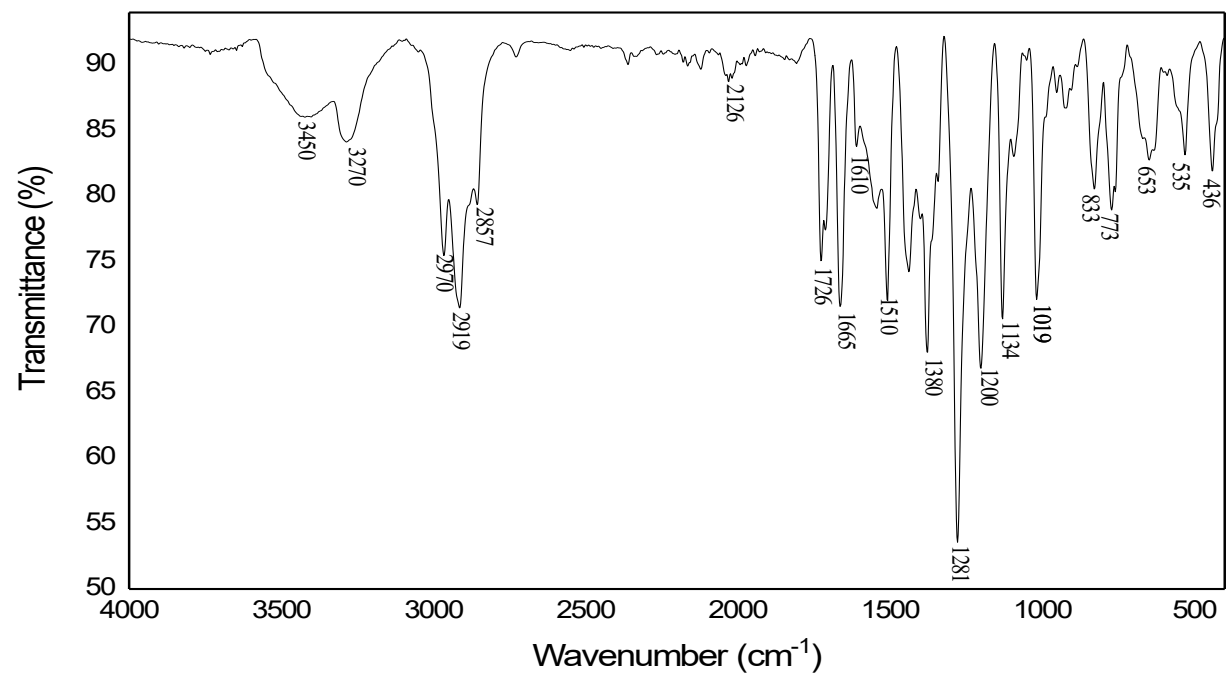


Fig. S8. FTIR (ATR) spectrum of compound **2**.

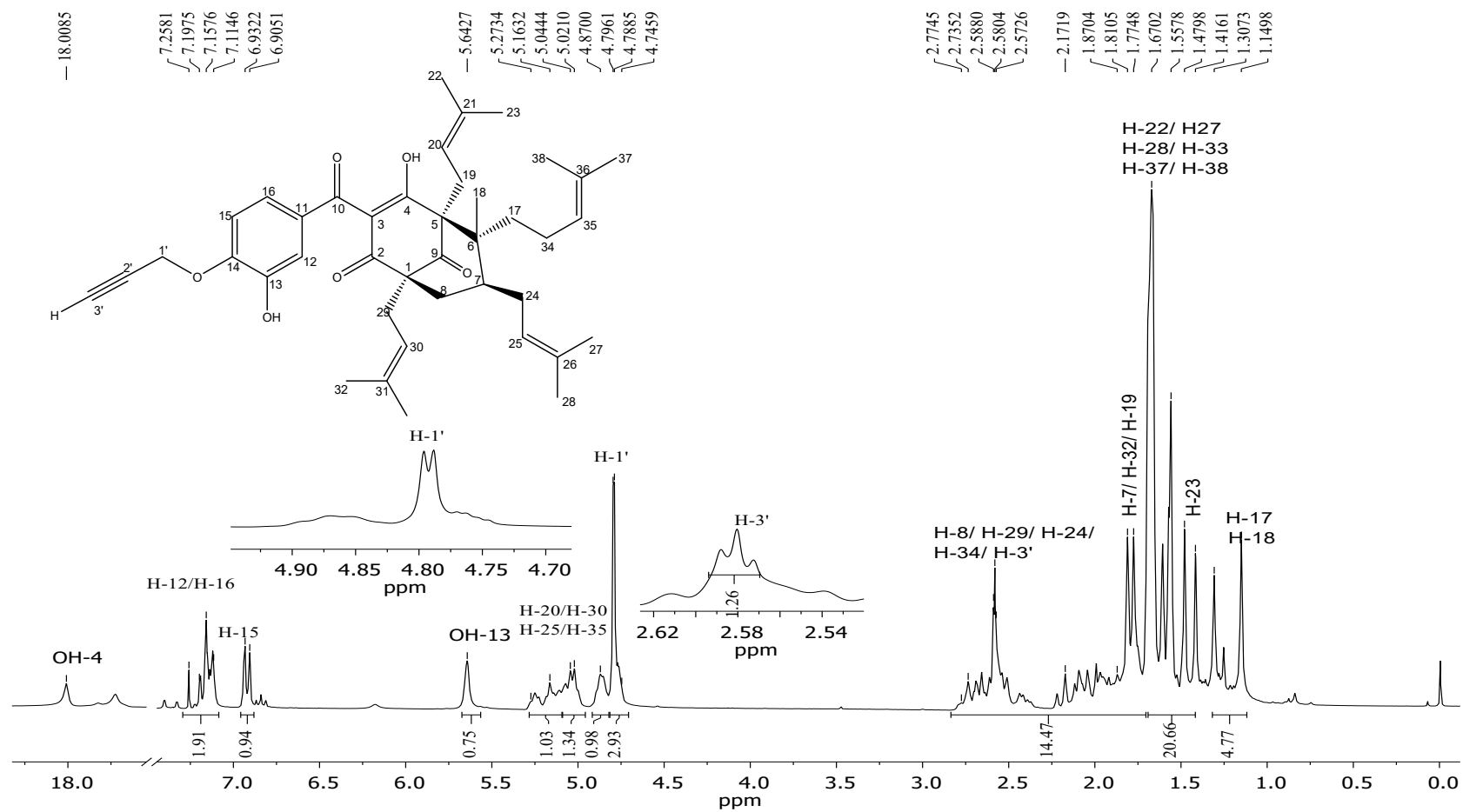


Fig. S9. ¹H NMR spectrum (300 MHz, CDCl₃) of compound **2**.

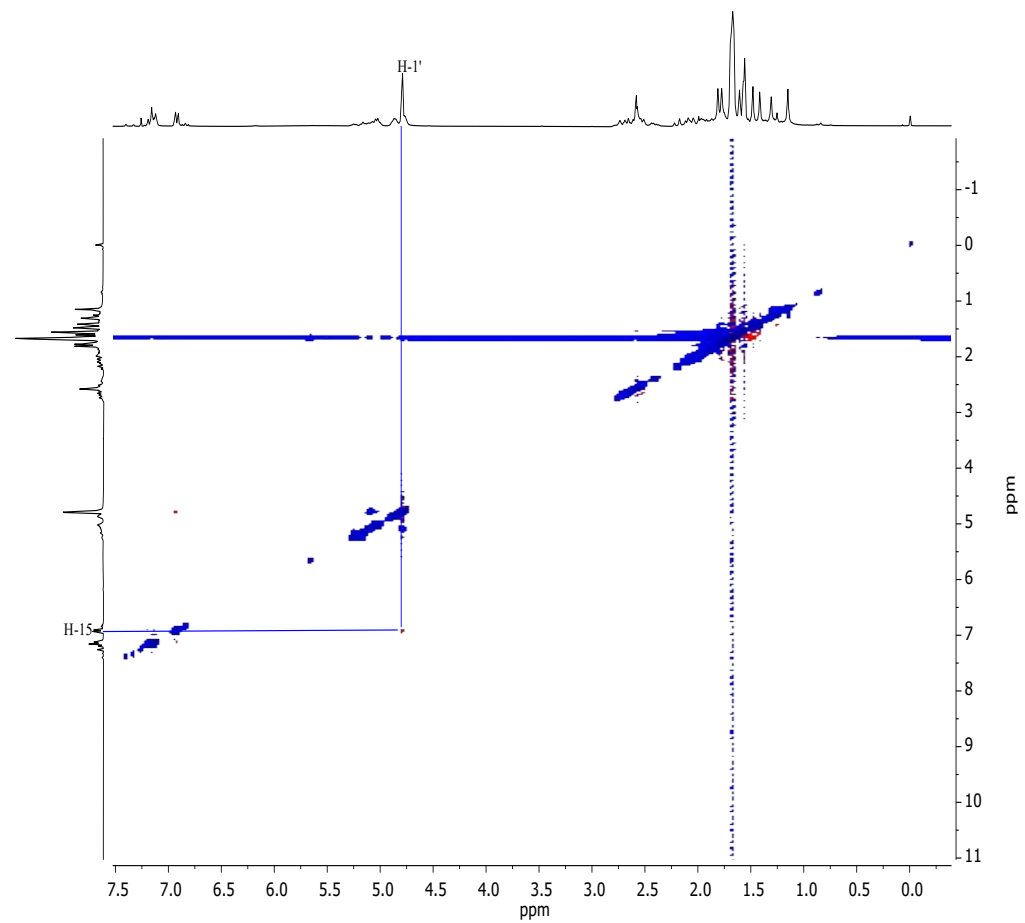


Fig. S10. NOESY ¹H-¹H NMR spectrum (300 MHz, CDCl₃) of compound **2**.

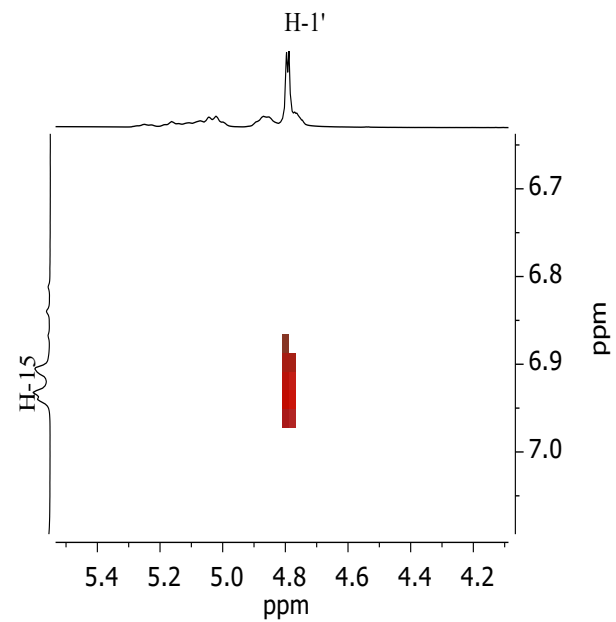


Fig. S11. Expansion of NOESY ^1H - ^1H NMR spectrum (300 MHz, CDCl_3) of compound **2**.

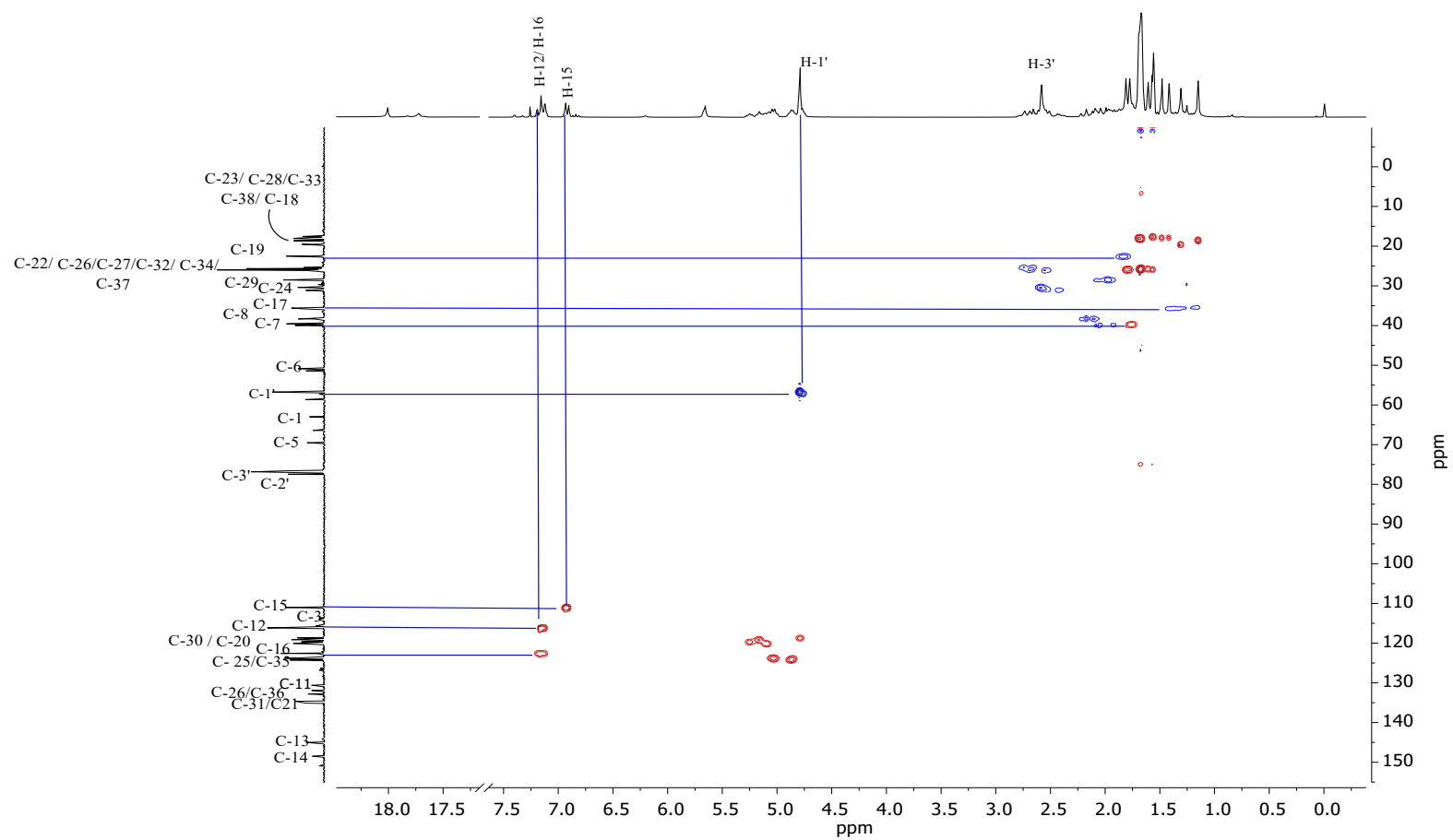


Fig. S12. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound **2**.

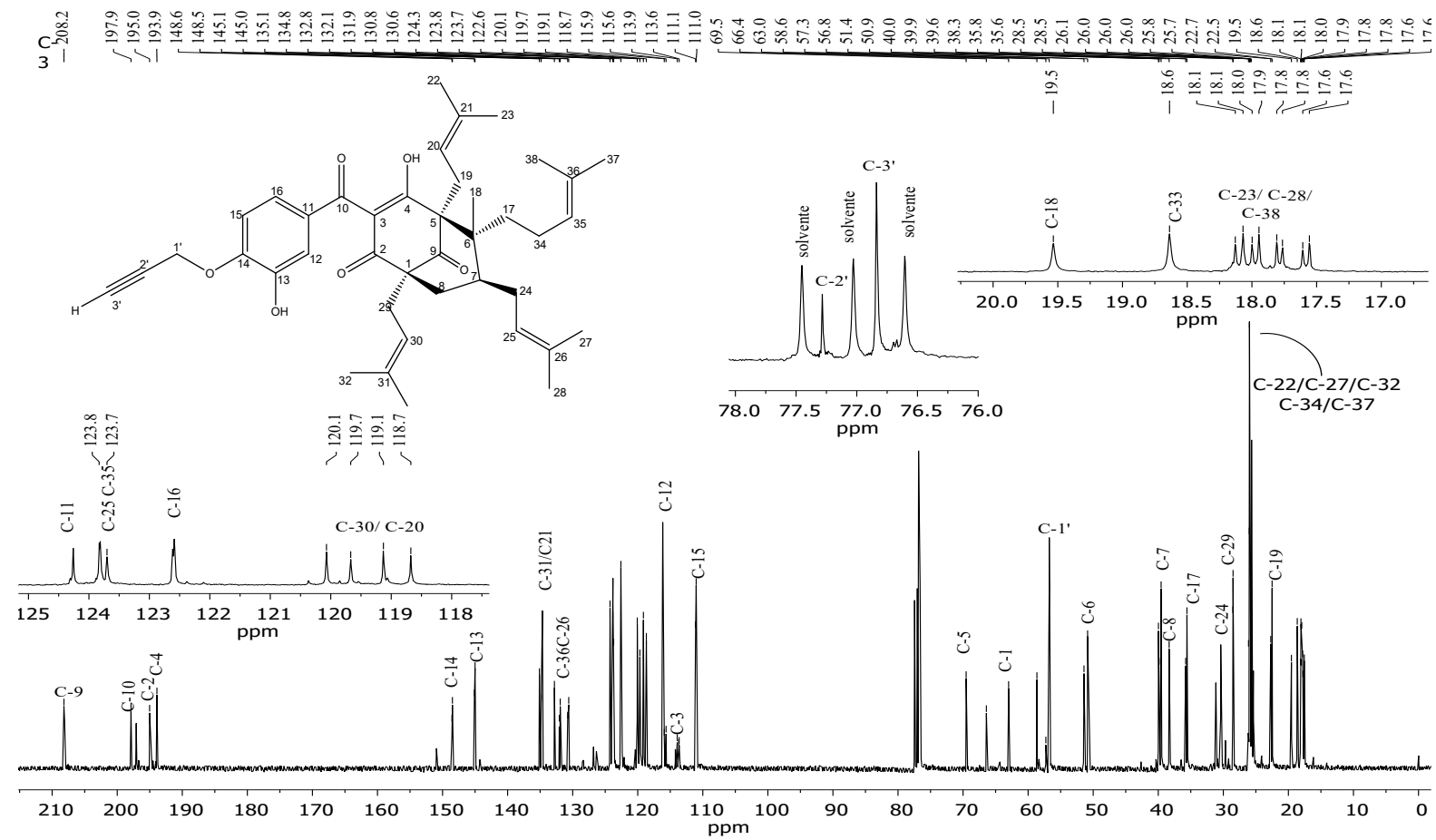


Fig. S13. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound **2**.

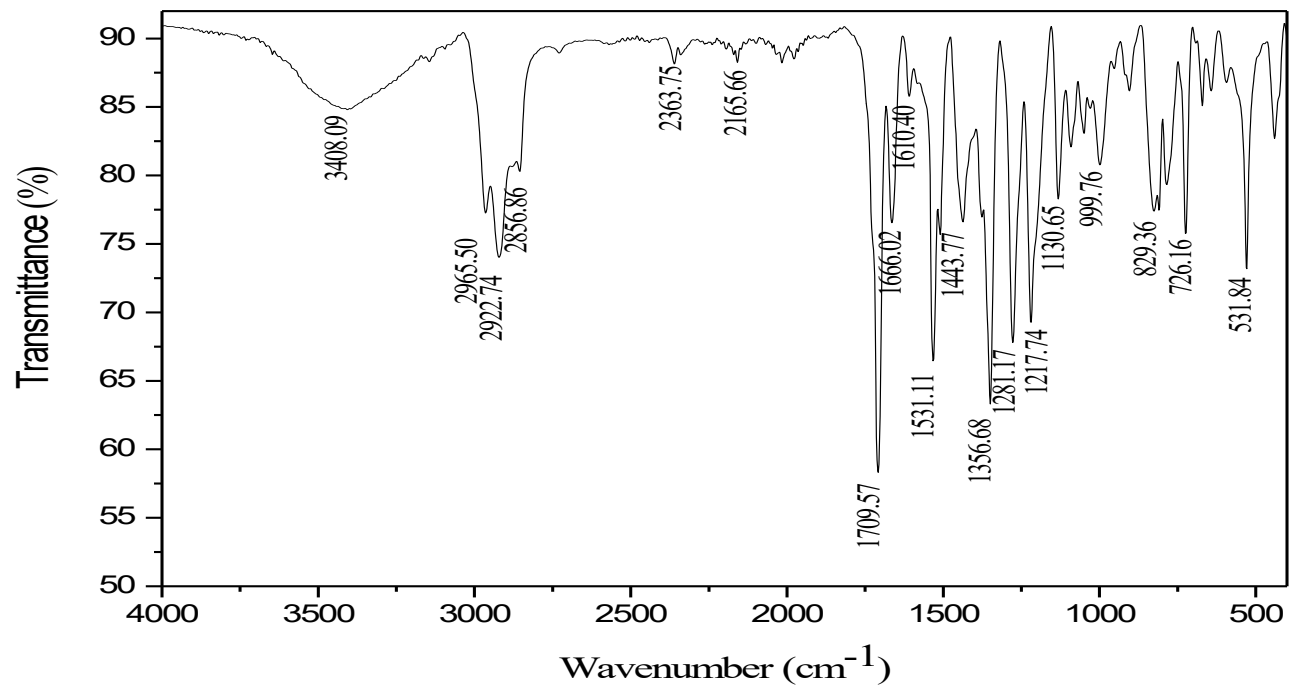


Fig. S14. FTIR (ATR) spectrum of compound 2.

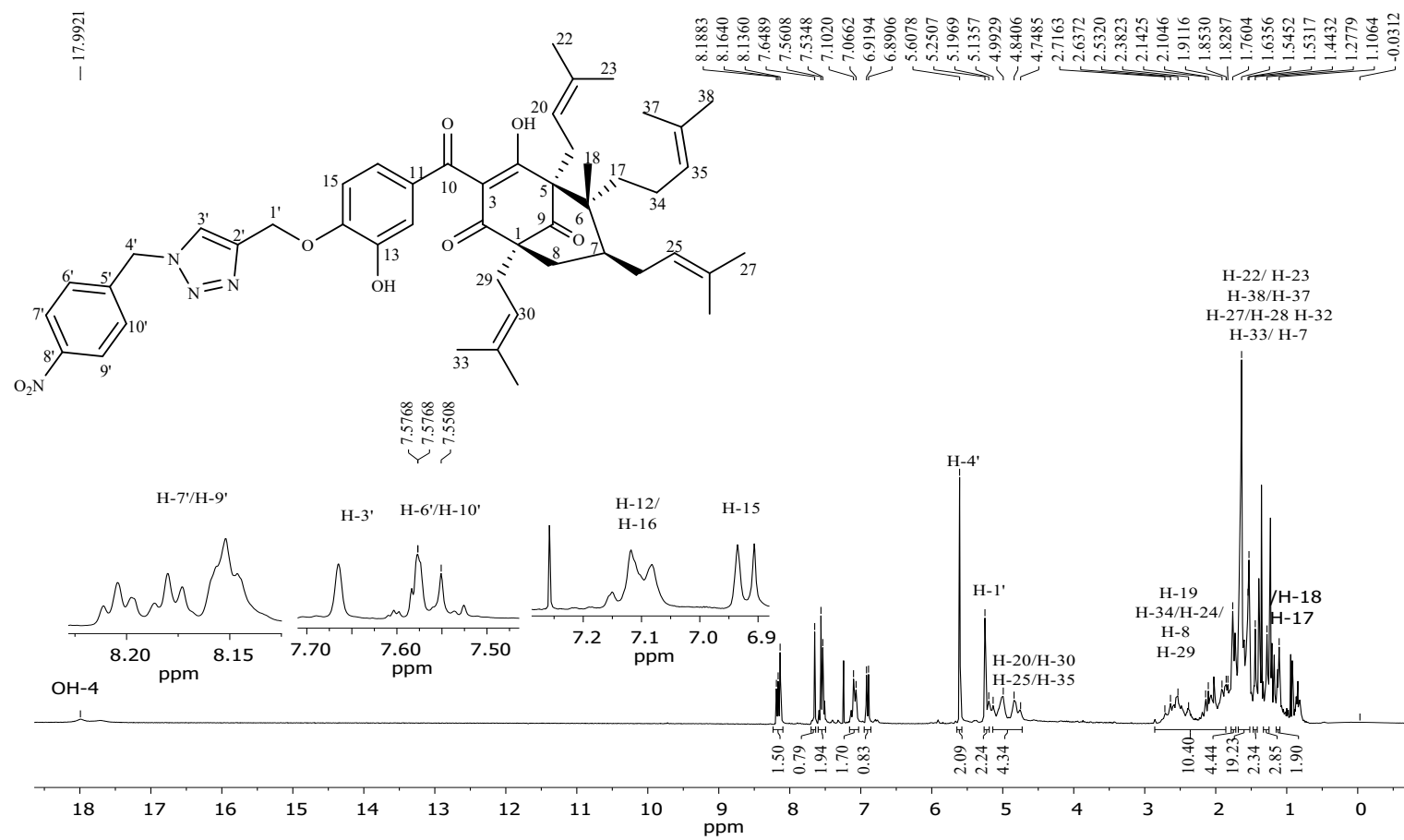


Fig. S15. ¹H NMR spectrum (300 MHz, CDCl₃) of compound **3**.

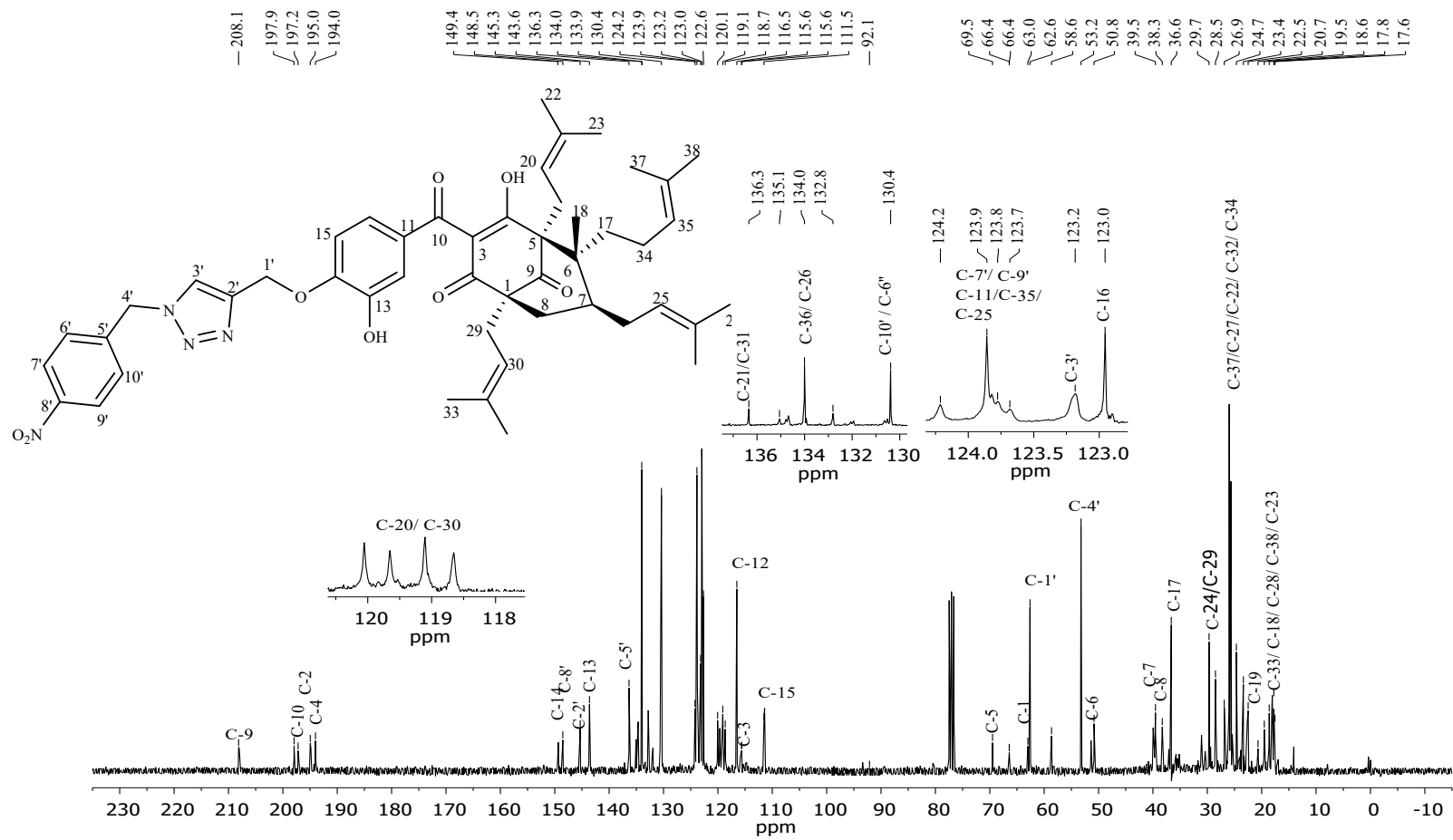


Fig. S16. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound **3**.

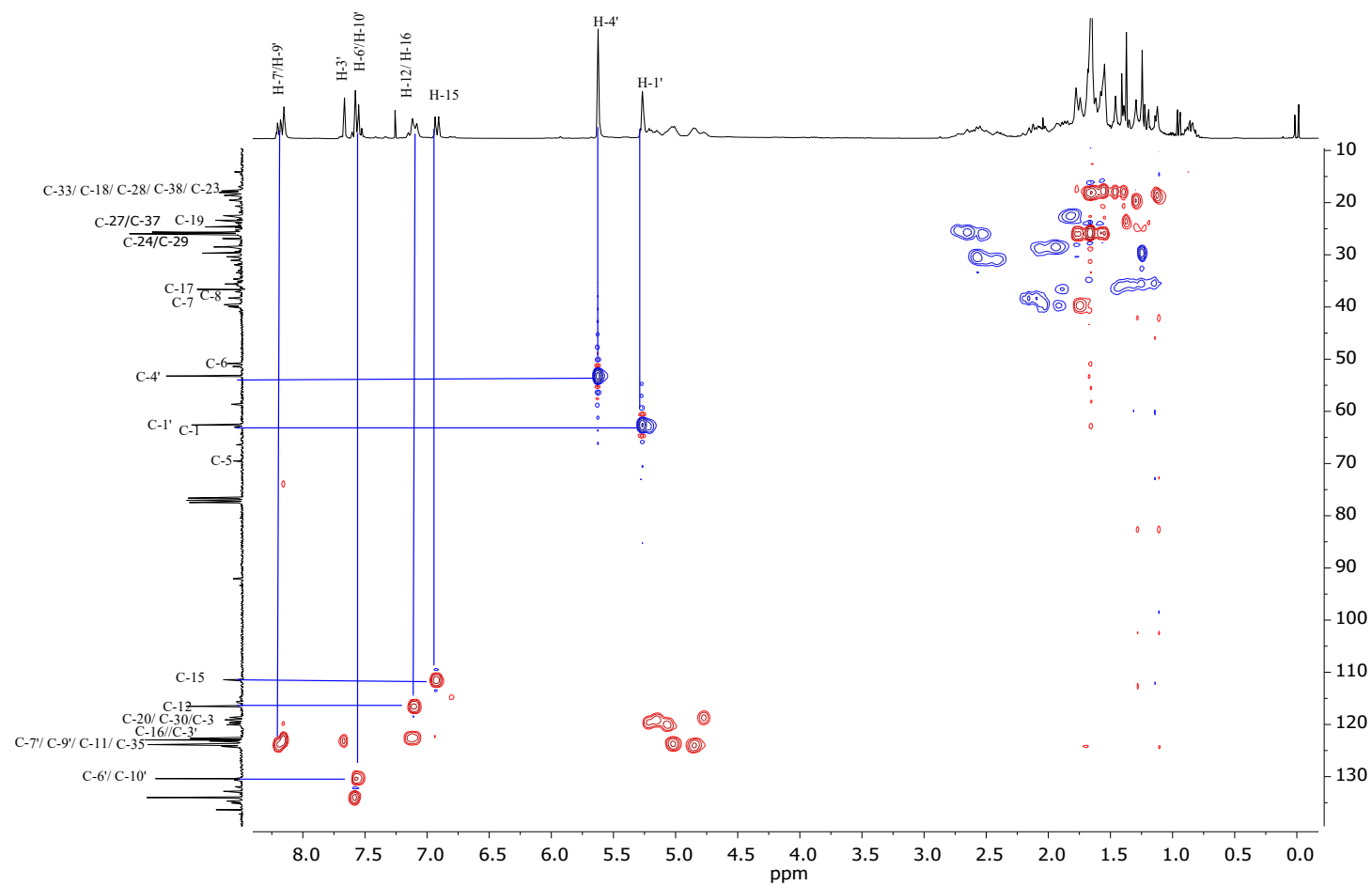


Fig. S17. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound **3**.

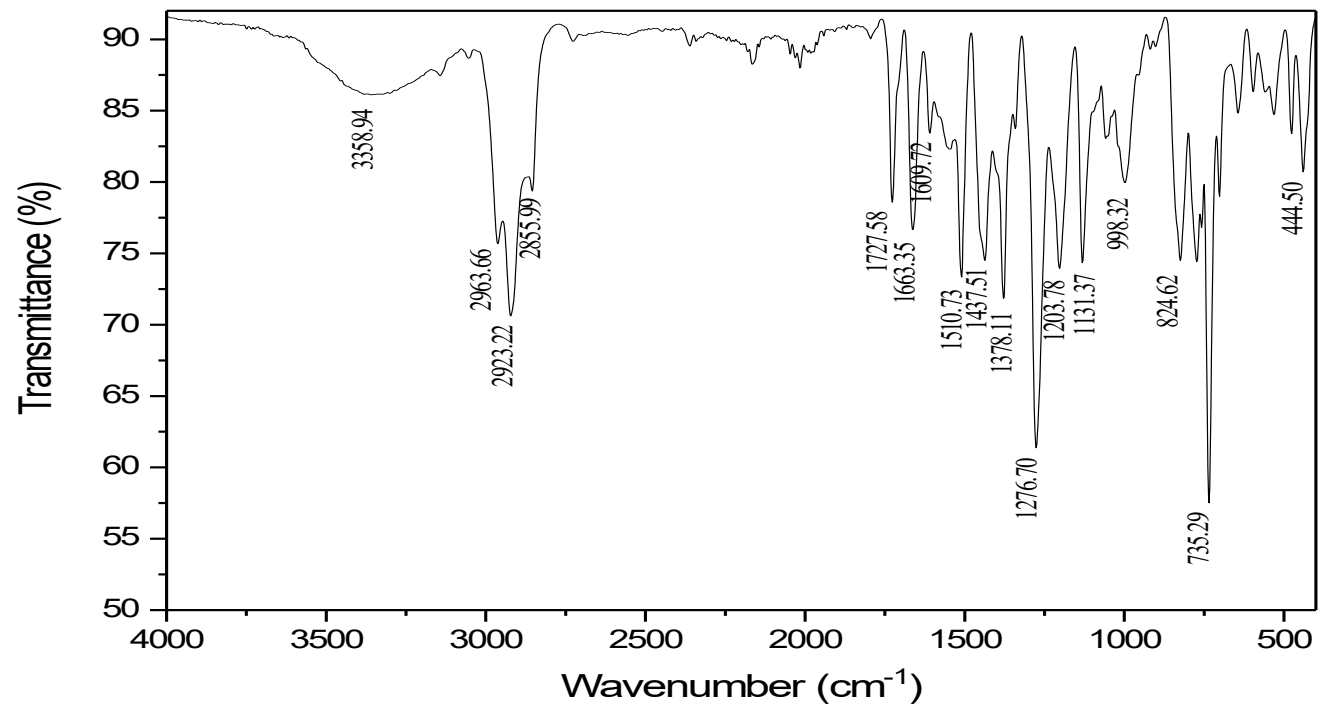


Fig. S18. FTIR (ATR) spectrum of compound **4**.

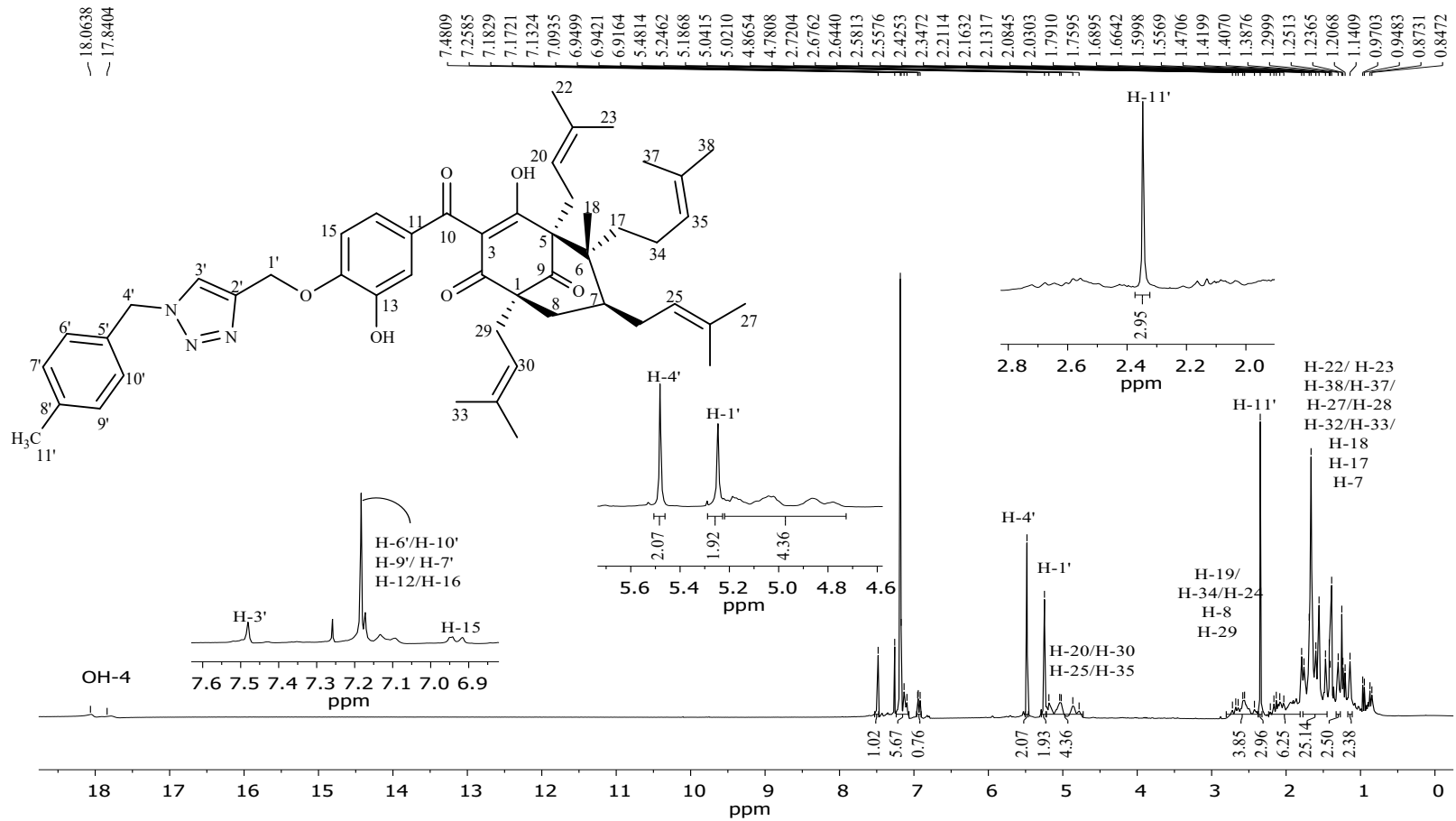


Fig. S19. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 4.

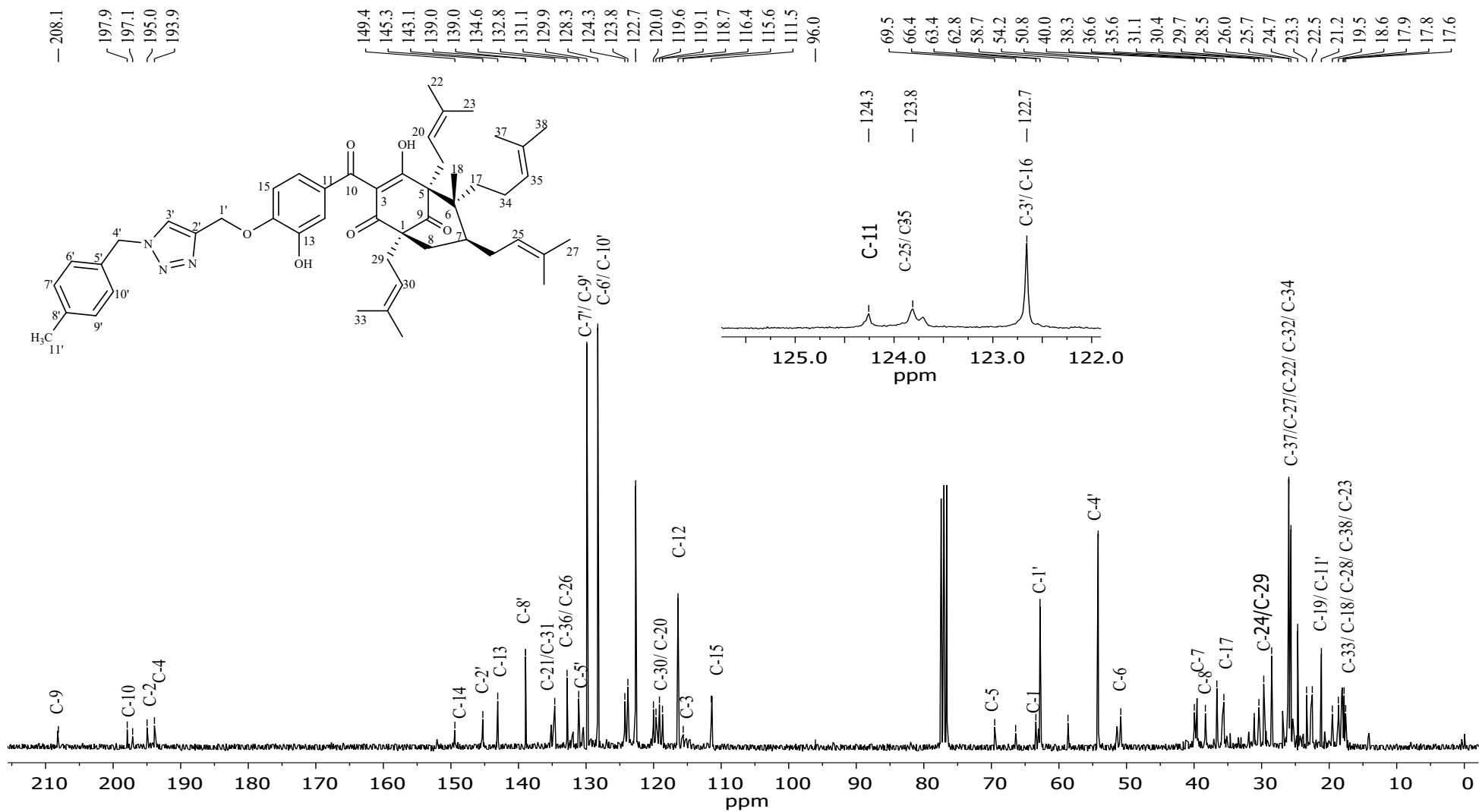


Fig. S20. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 4.

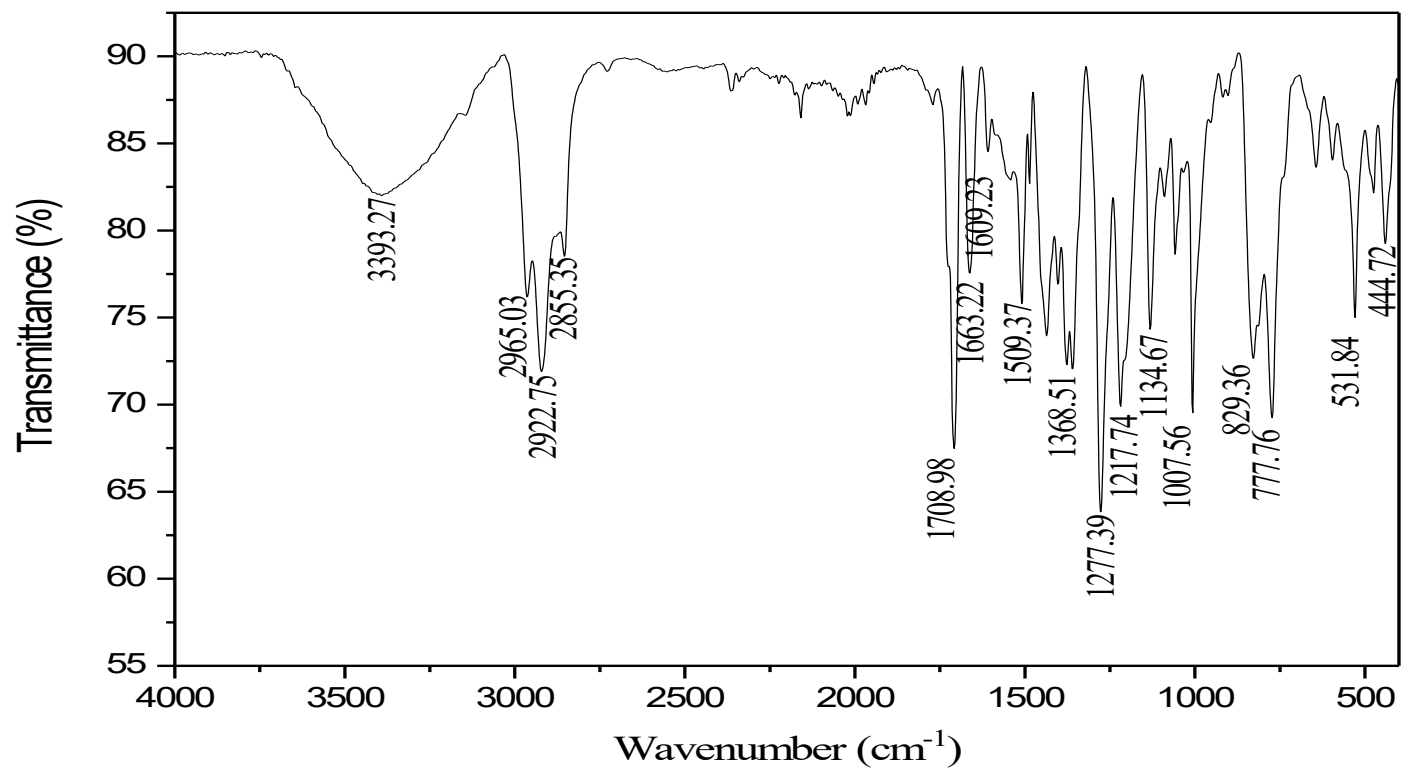


Fig. S21. FTIR (ATR) spectrum of compound **5**.

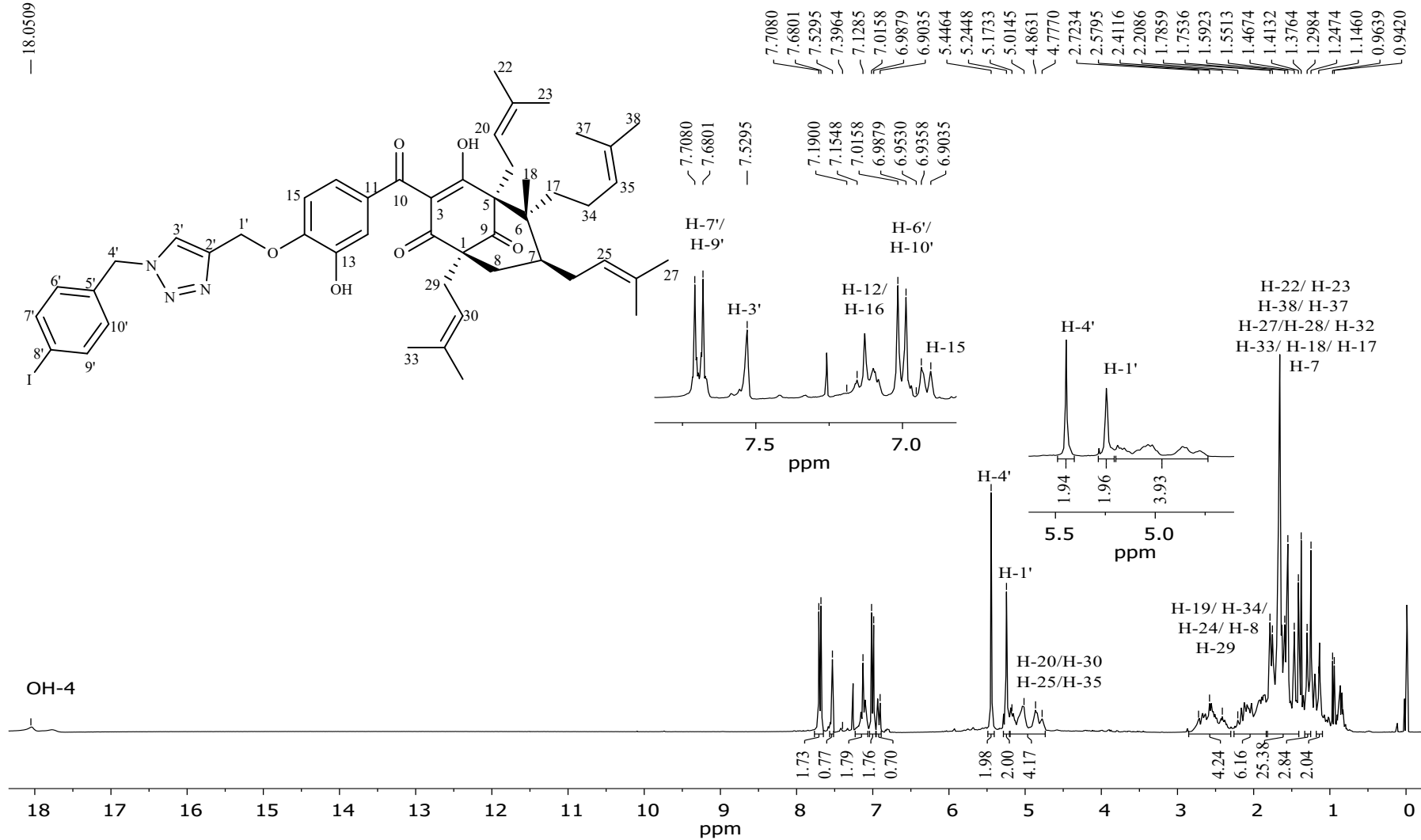


Fig. S22. ^1H NMR spectrum (300 MHz, CDCl_3) of compound 5.

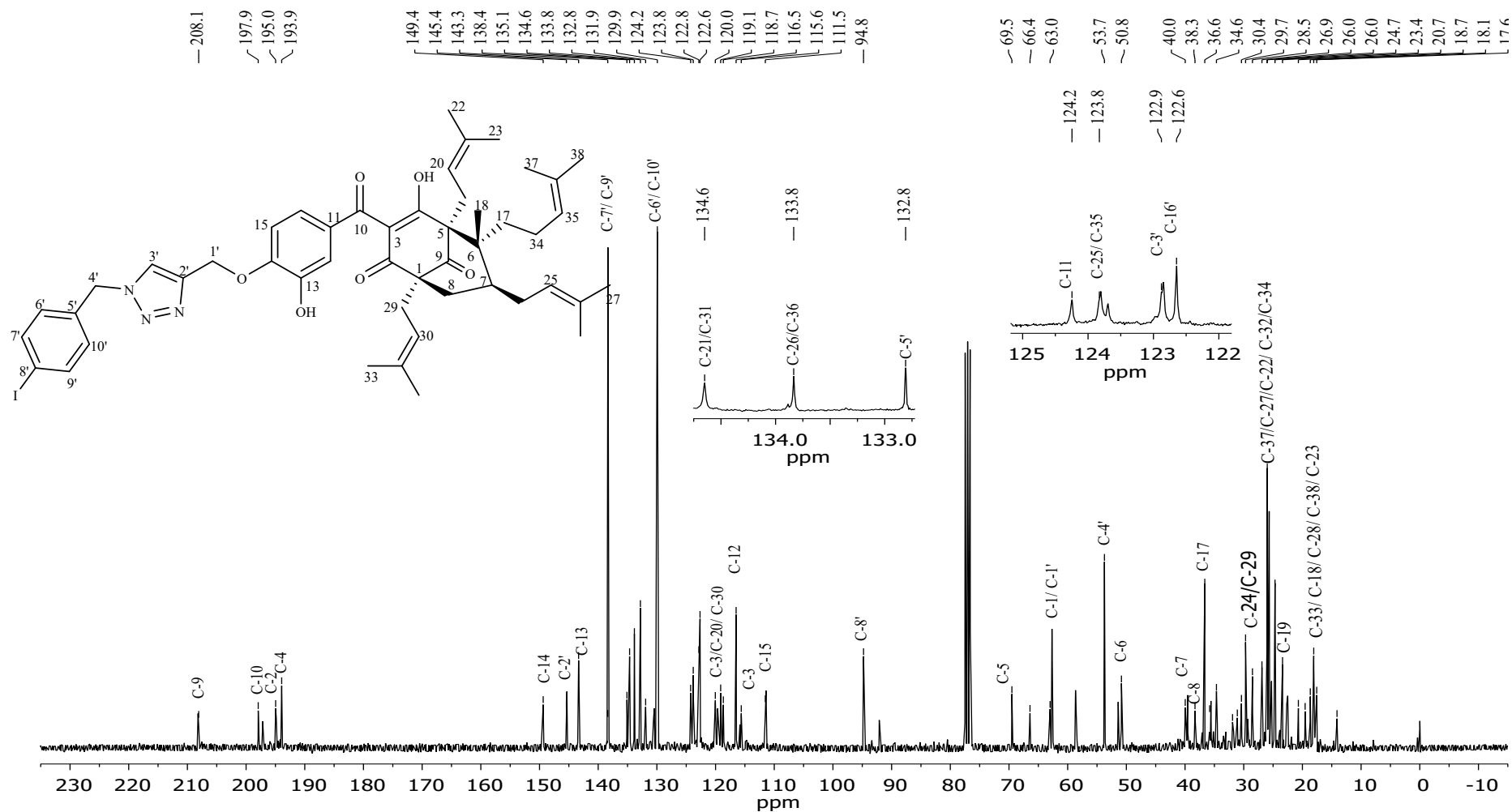


Fig. S23. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound 5.

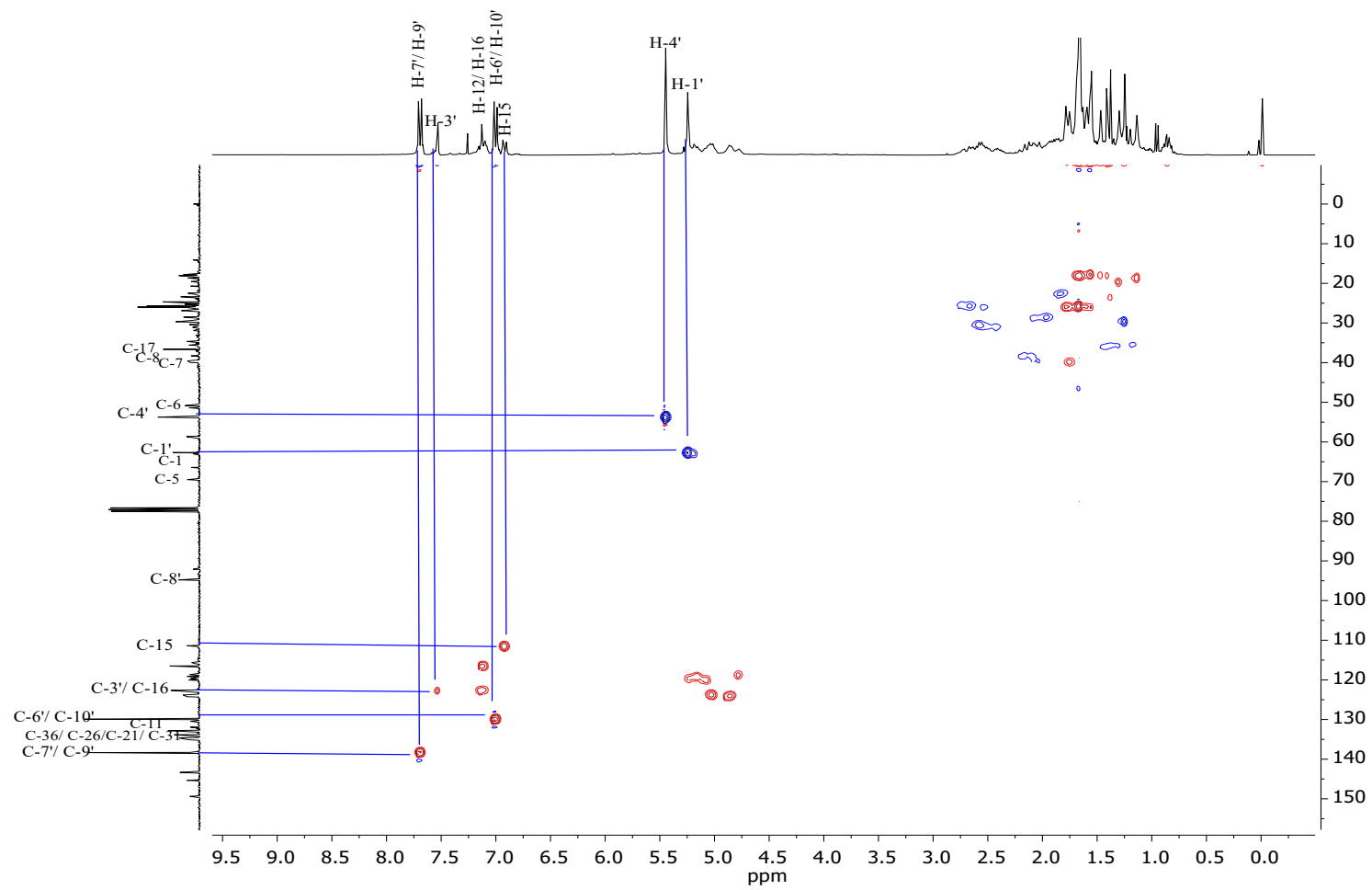


Fig. S24. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound **5**.

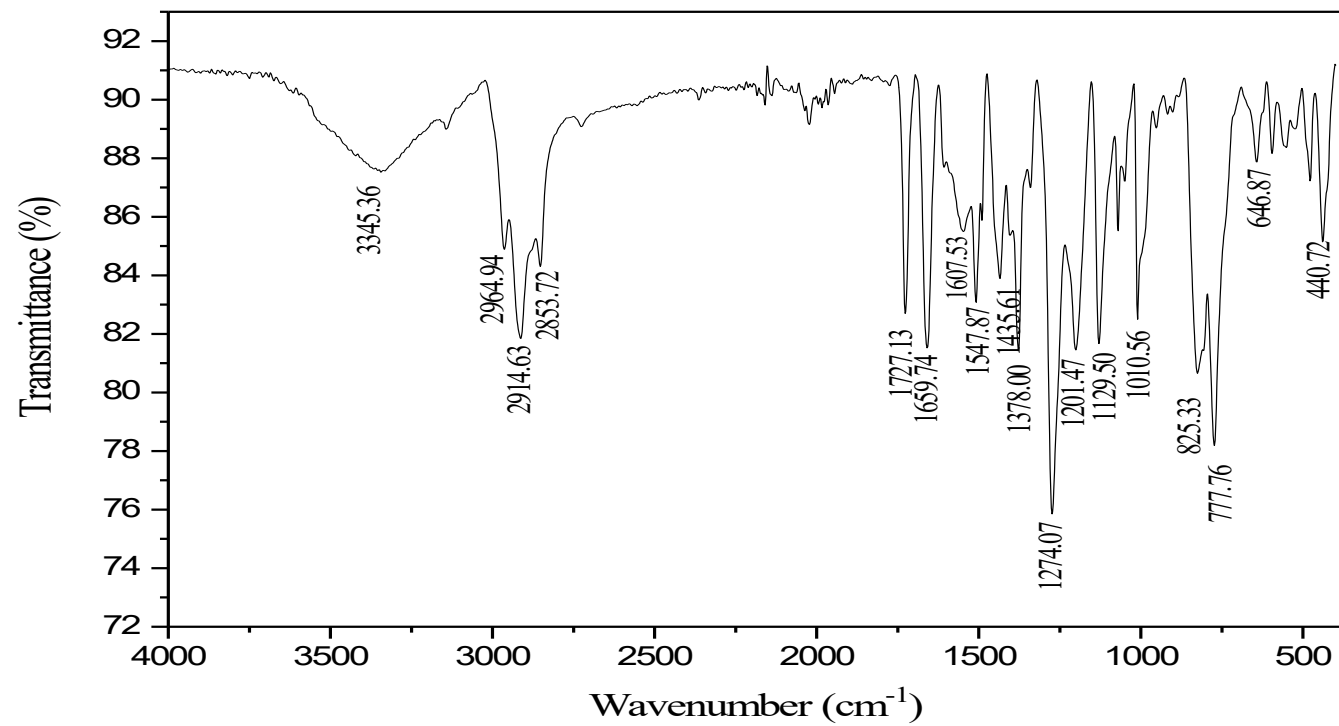


Fig. S25. FTIR (ATR) spectrum of compound 6.

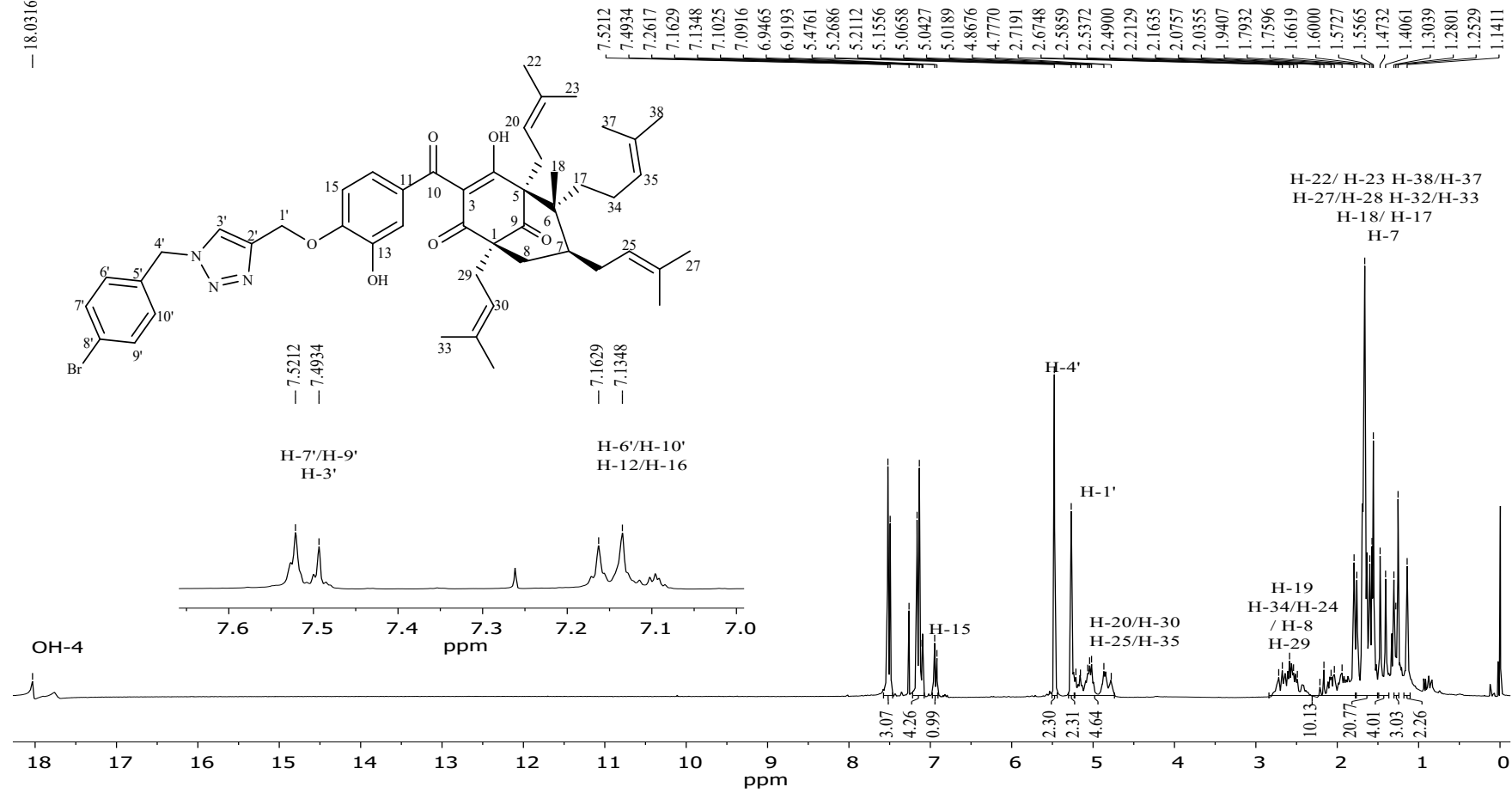


Fig. S26. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 6.

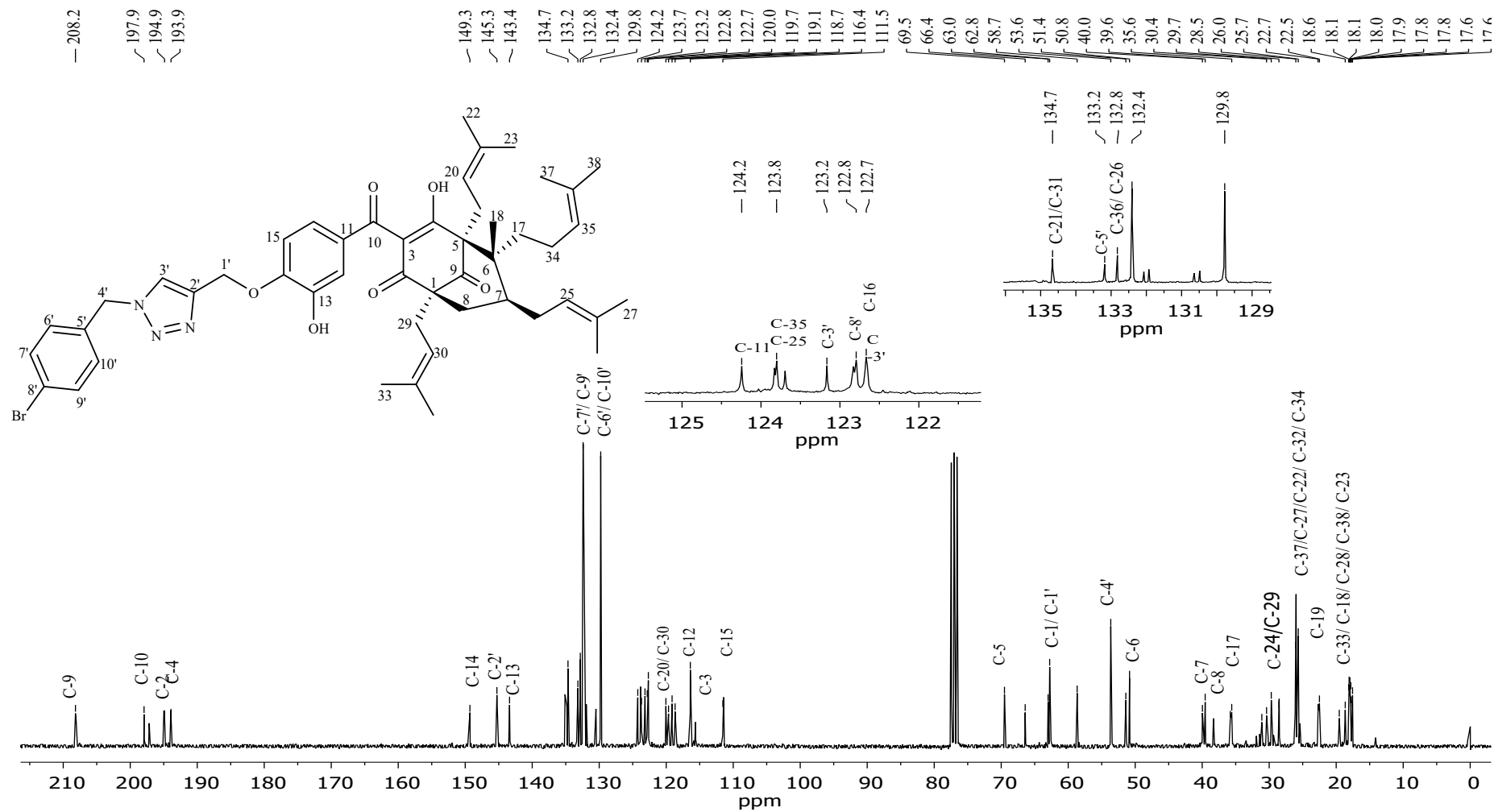


Fig. S27. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 6.

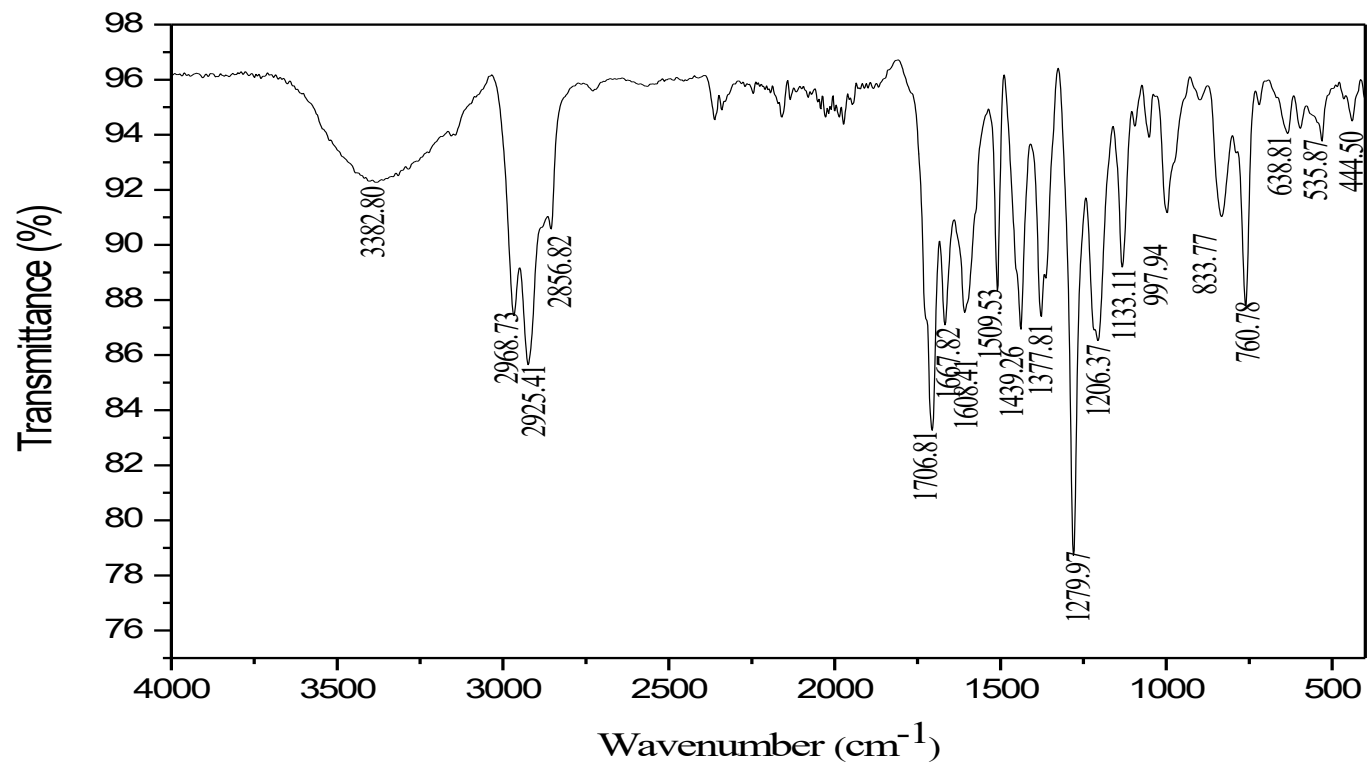


Fig. S28. FTIR (ATR) spectrum of compound 7.

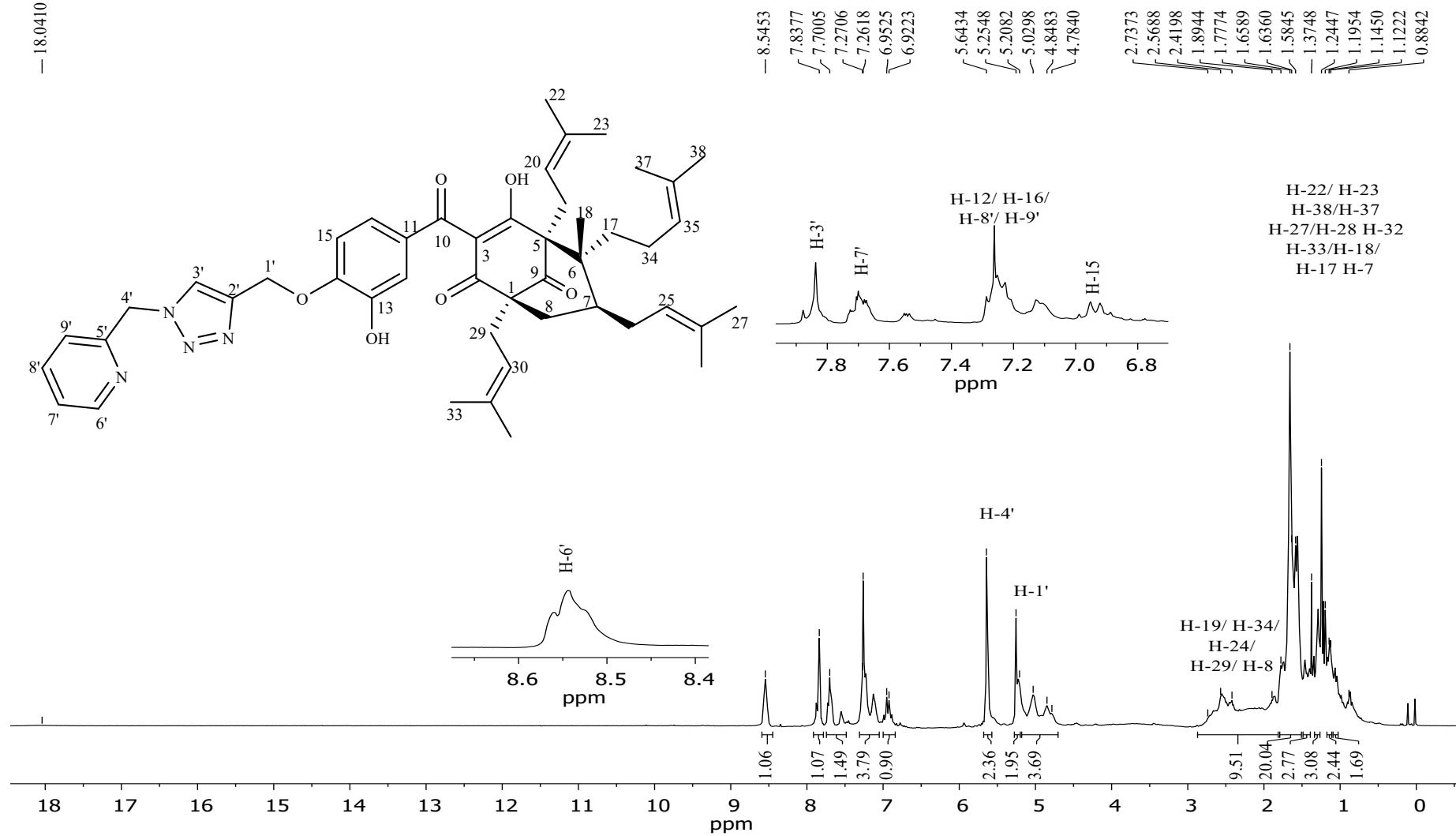


Fig. S29. ^1H NMR spectrum (300 MHz, CDCl_3) of compound 7.

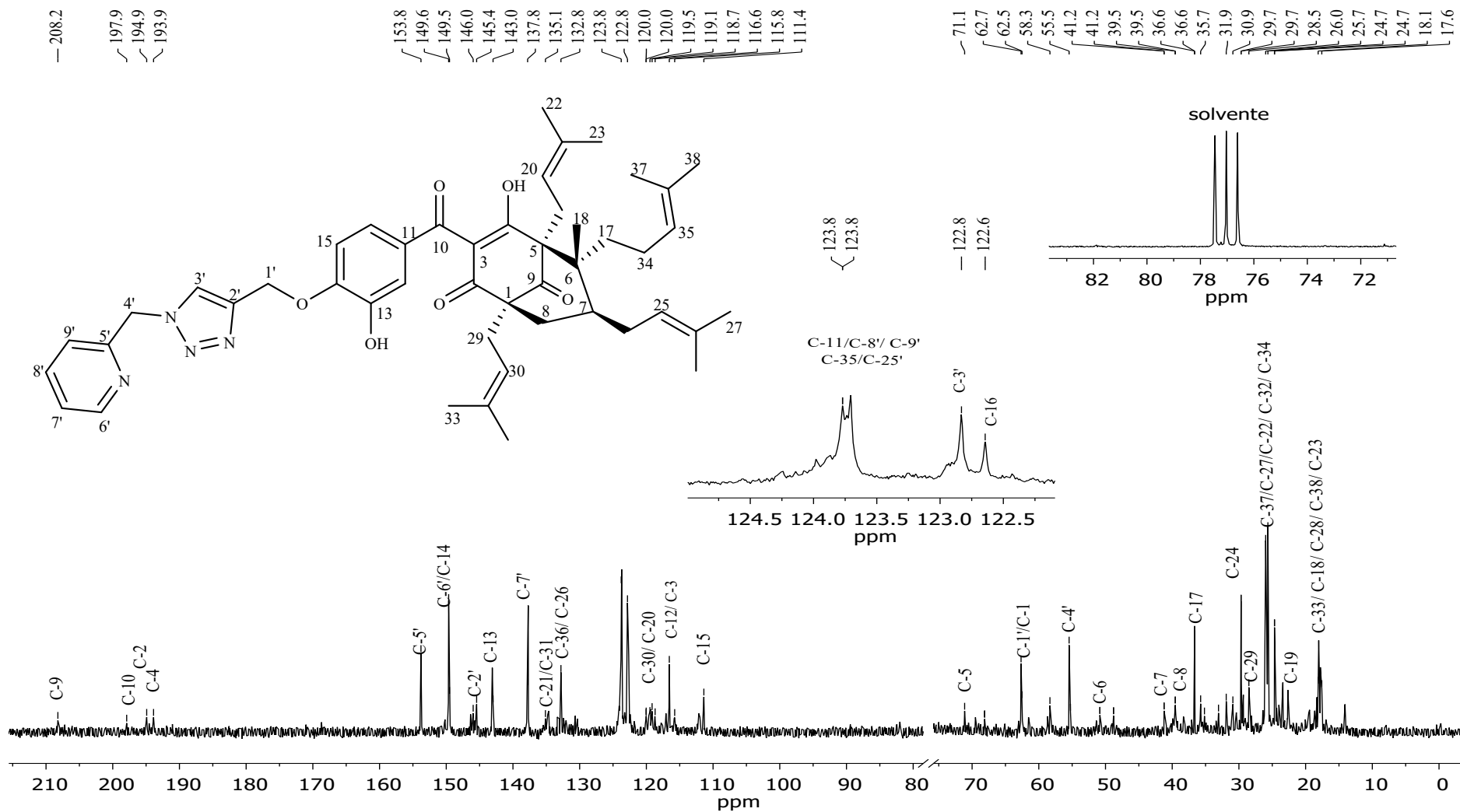


Fig. S30. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound 7.

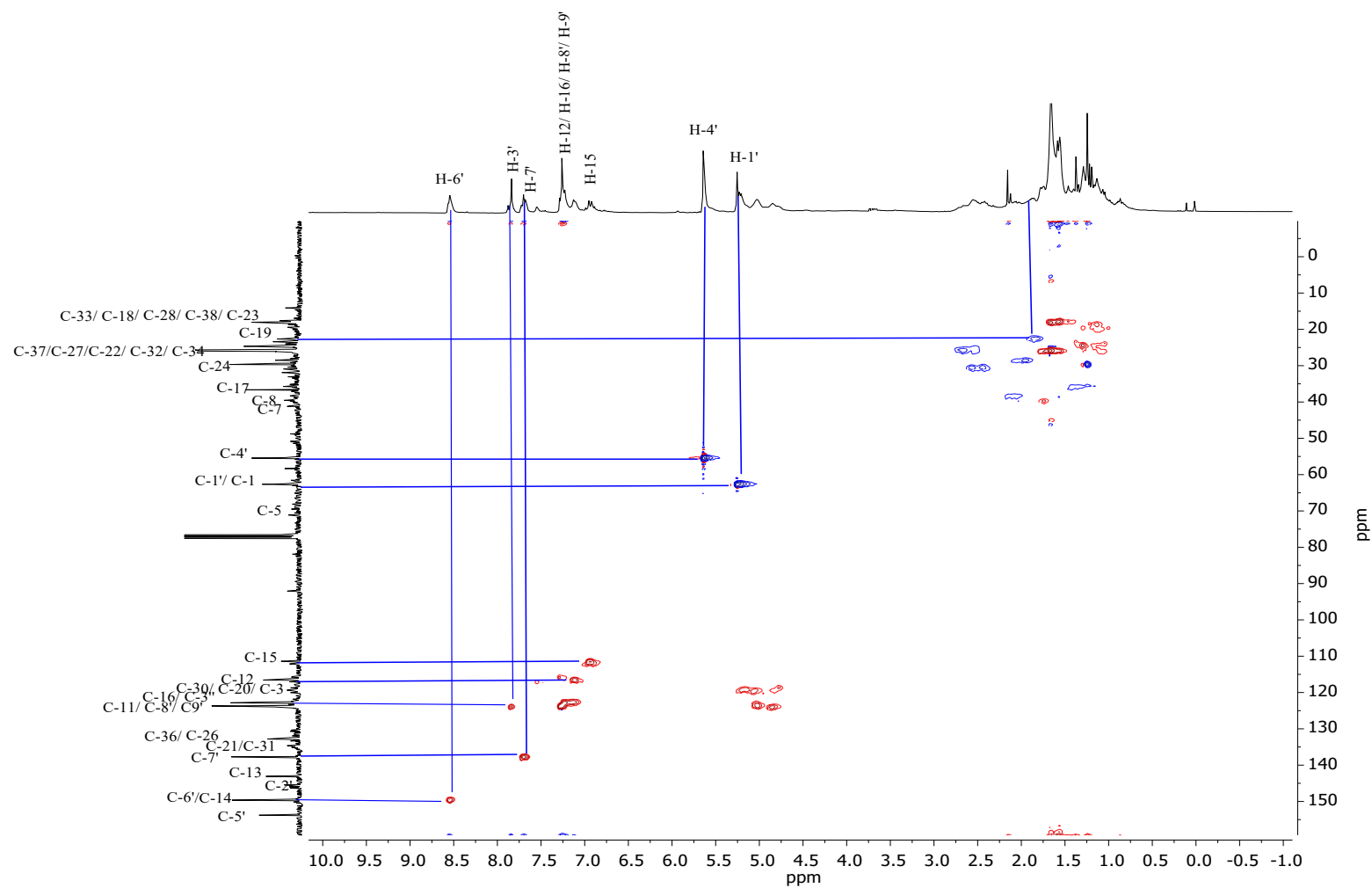


Fig. S31. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound 7.

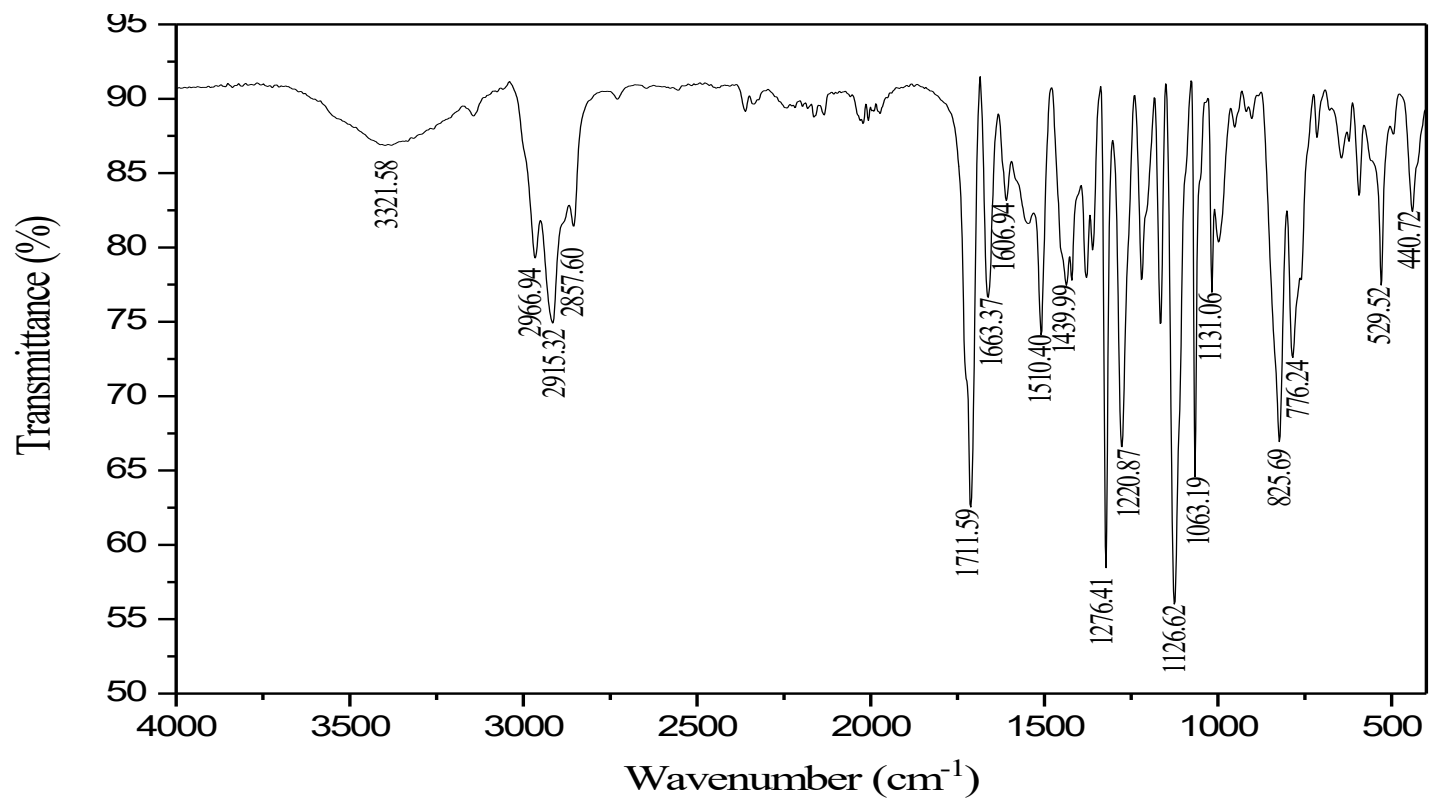


Fig. S32. FTIR (ATR) spectrum of compound **8**.

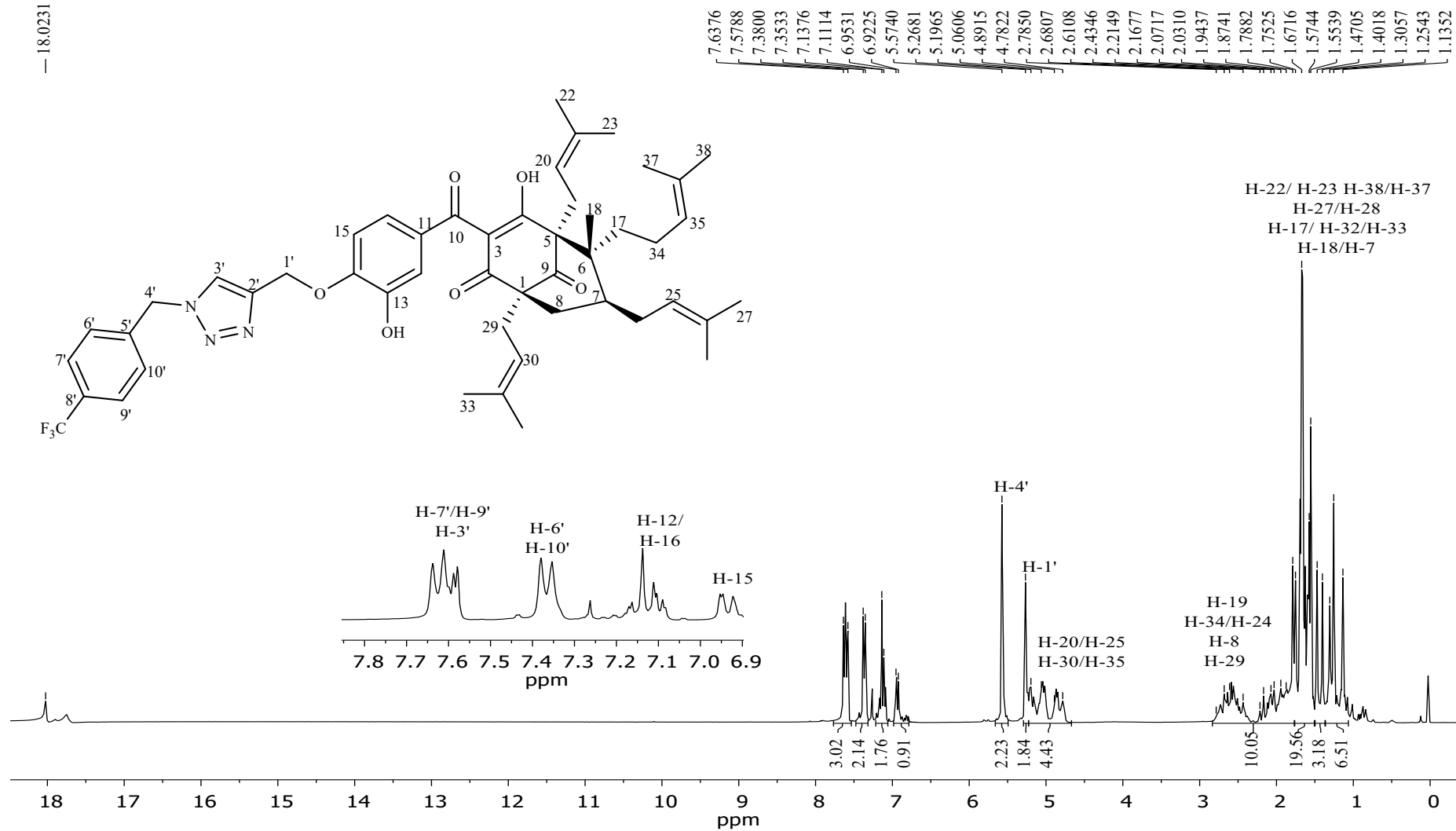


Fig. S33. ¹H NMR spectrum (300 MHz, CDCl₃) of compound **8**.

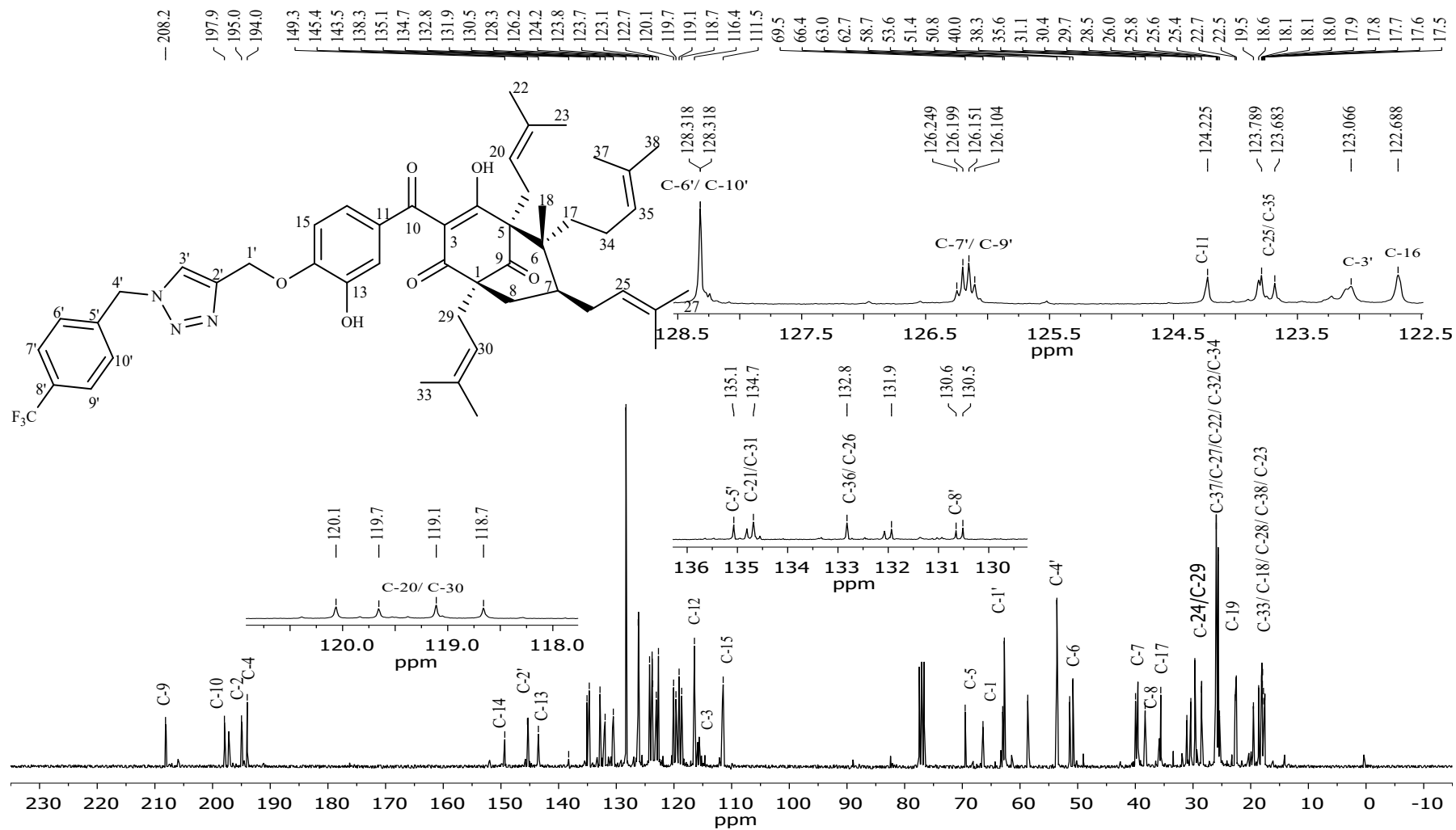


Fig. S34. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound **8**.

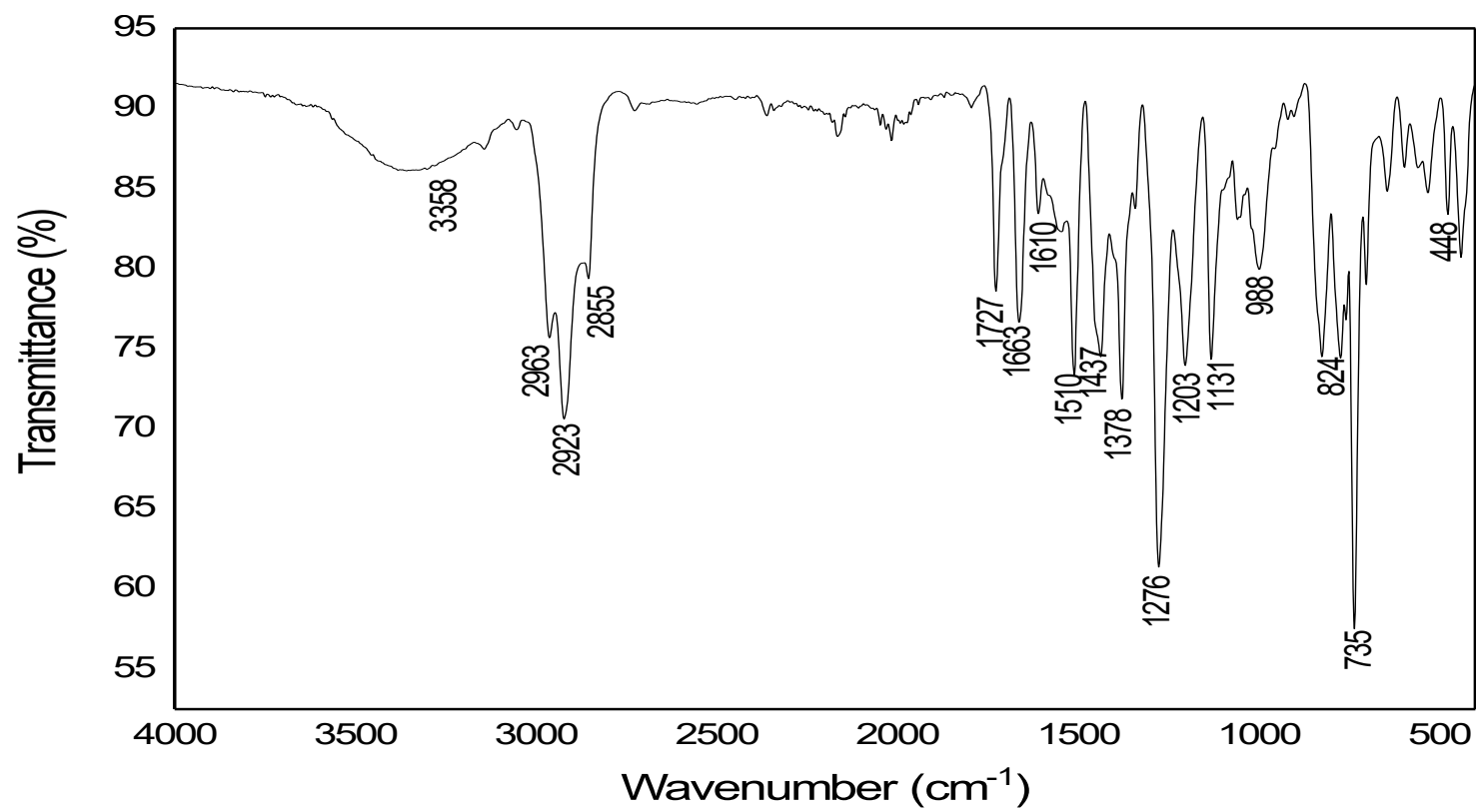


Fig. S35. FTIR (ATR) spectrum of compound 9.

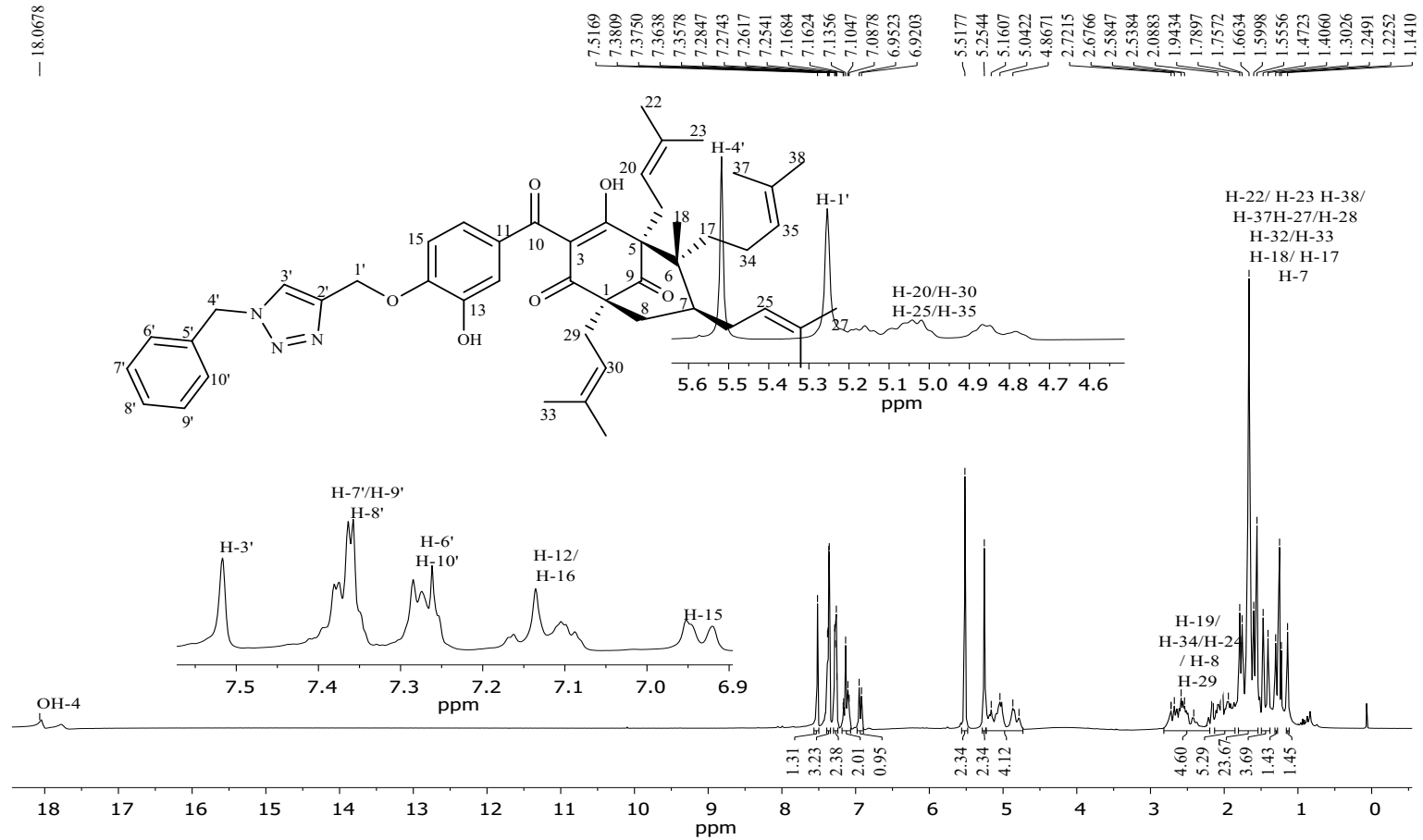


Fig. S36. ^1H NMR spectrum (300 MHz, CDCl_3) of compound 9.

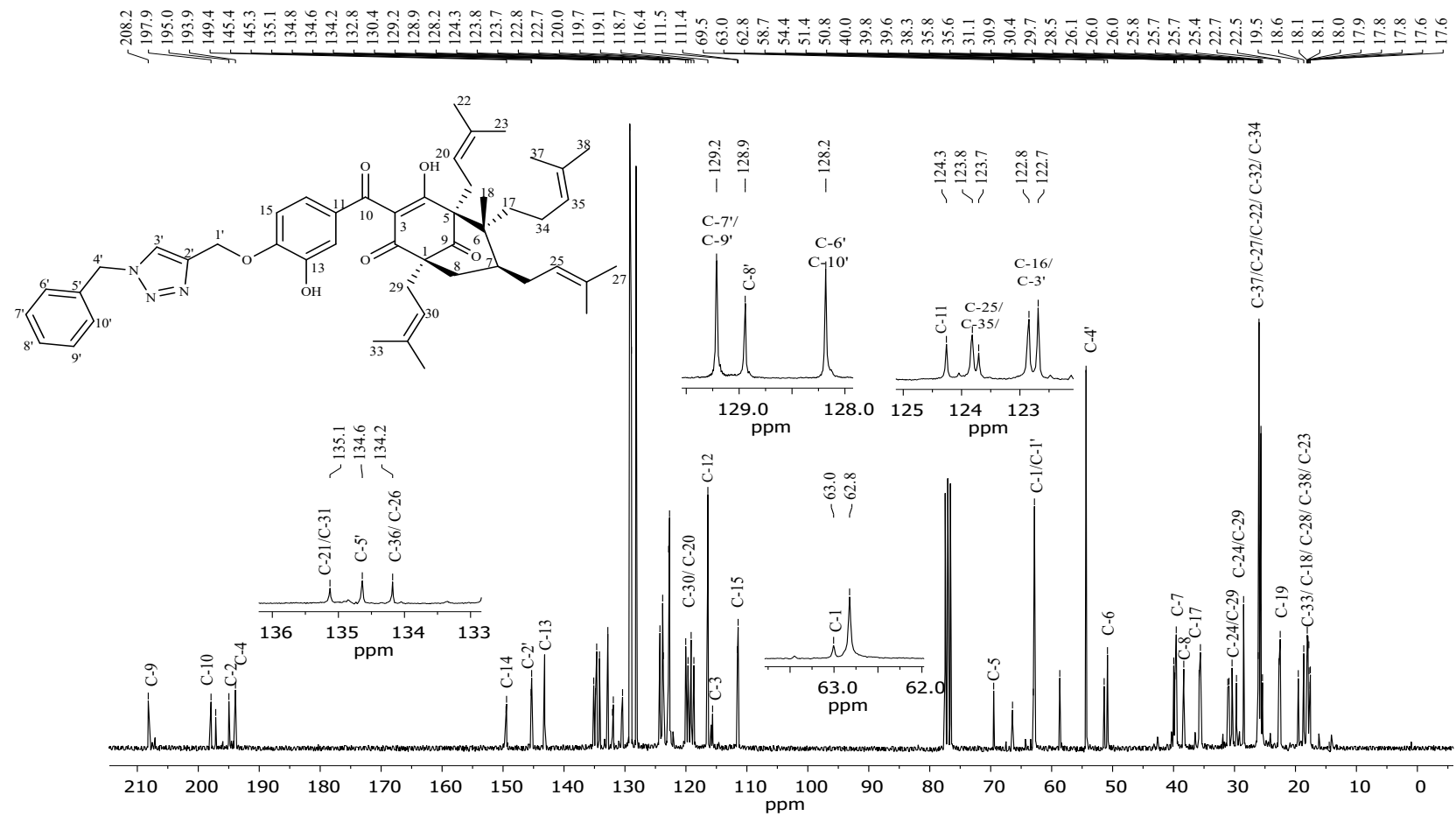


Fig. S37. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 9.

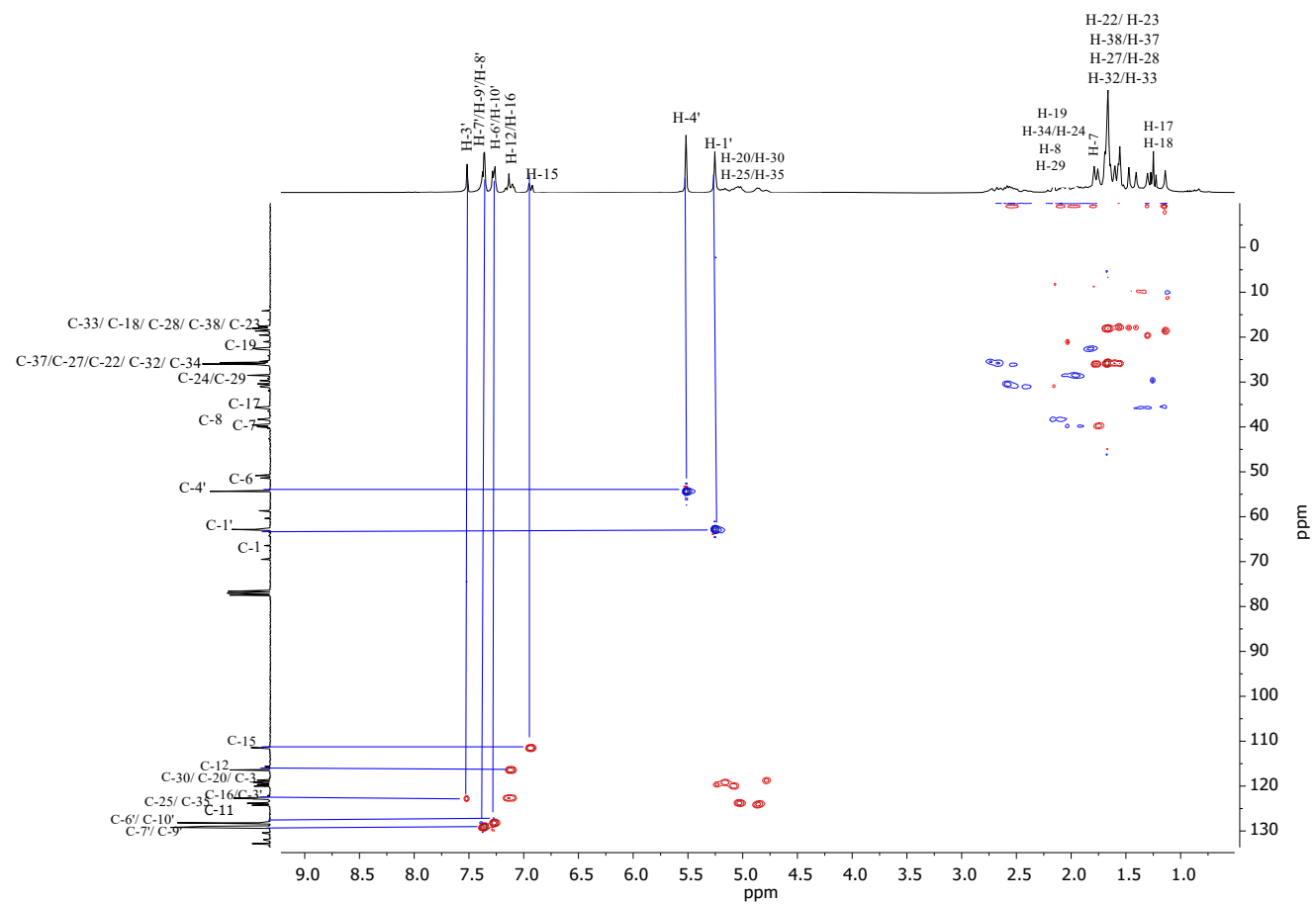


Fig. S38. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound **9**.

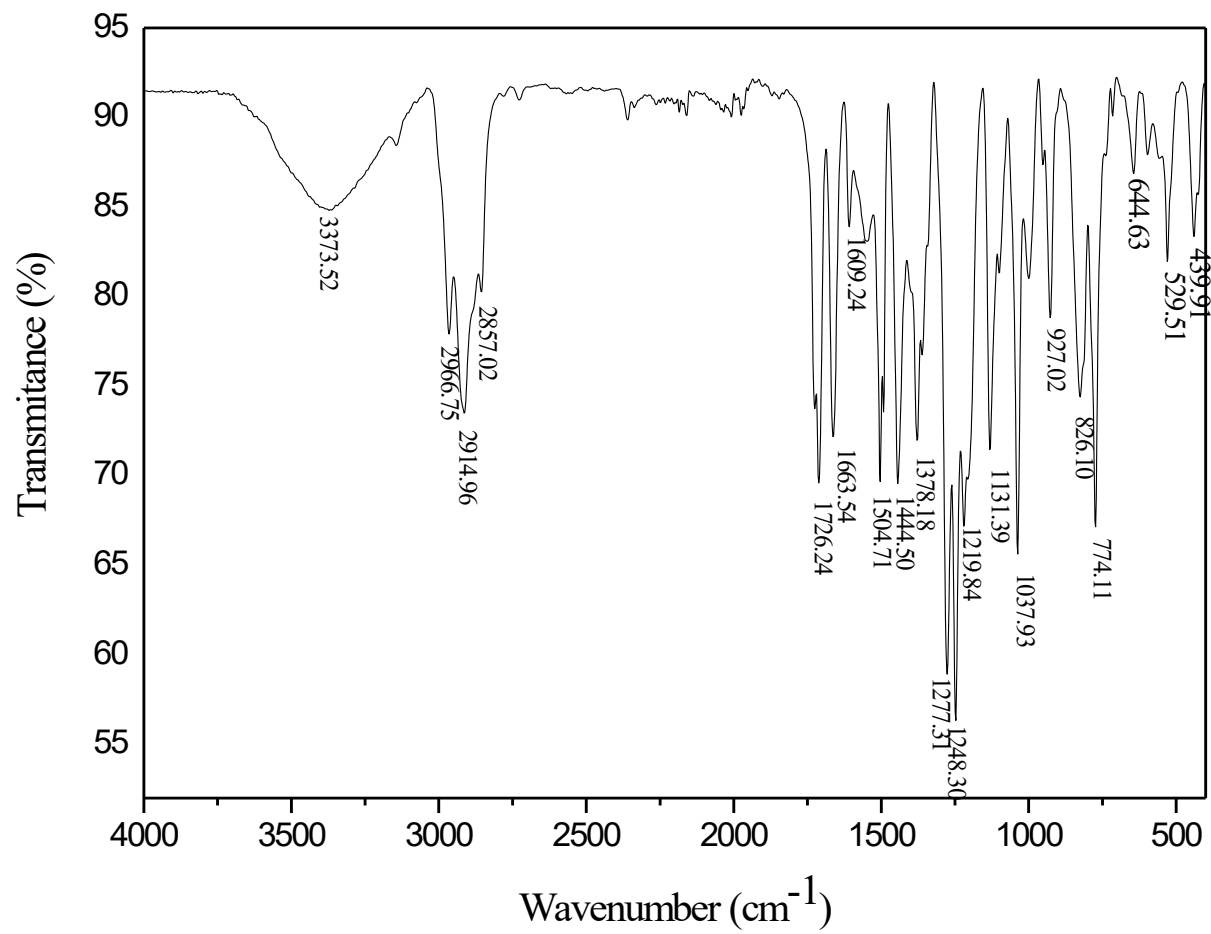


Fig. S39. FTIR (ATR) spectrum of compound **10**.

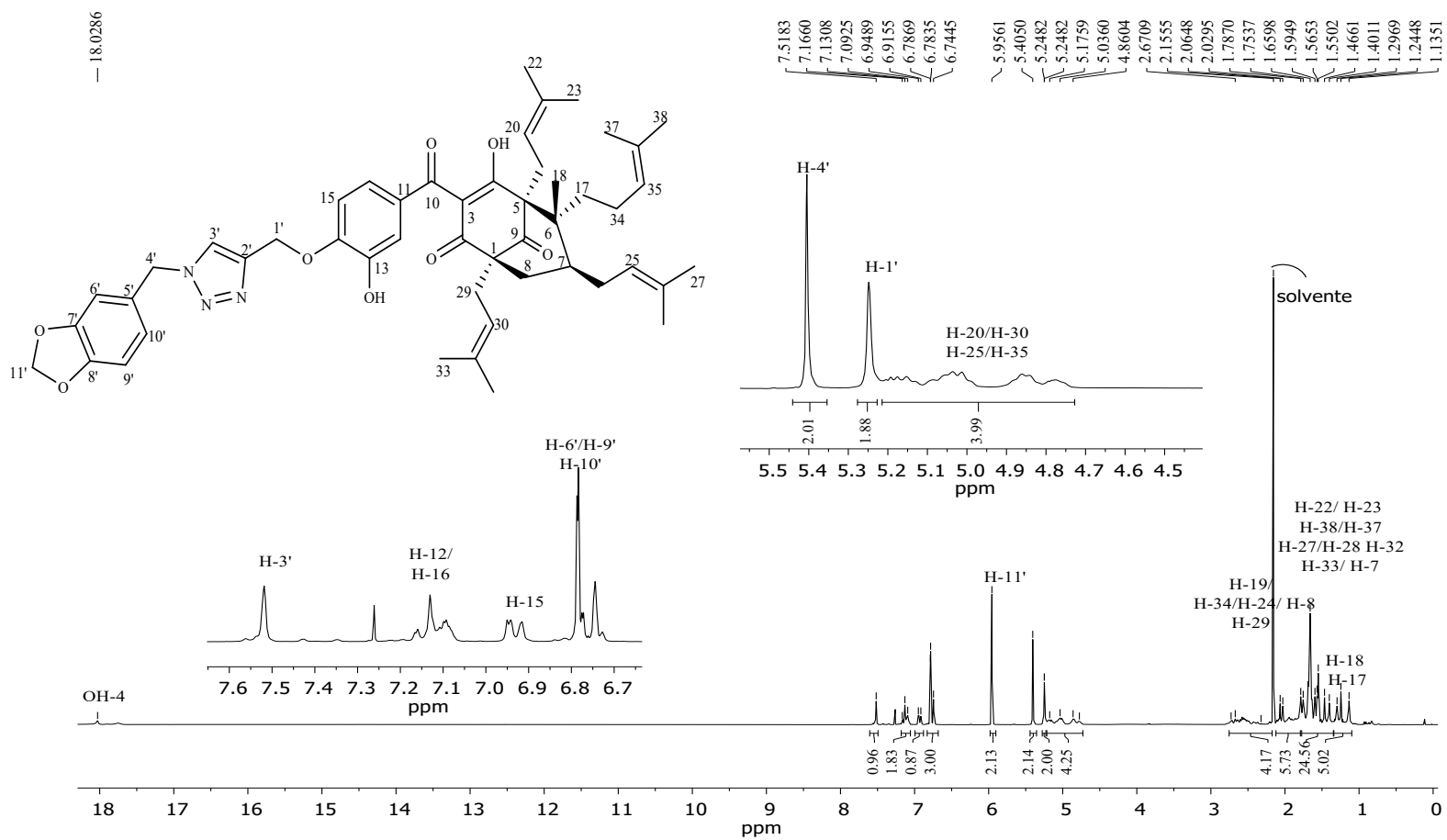


Fig. S40. ^1H NMR spectrum (300 MHz, CDCl_3) of compound 10.

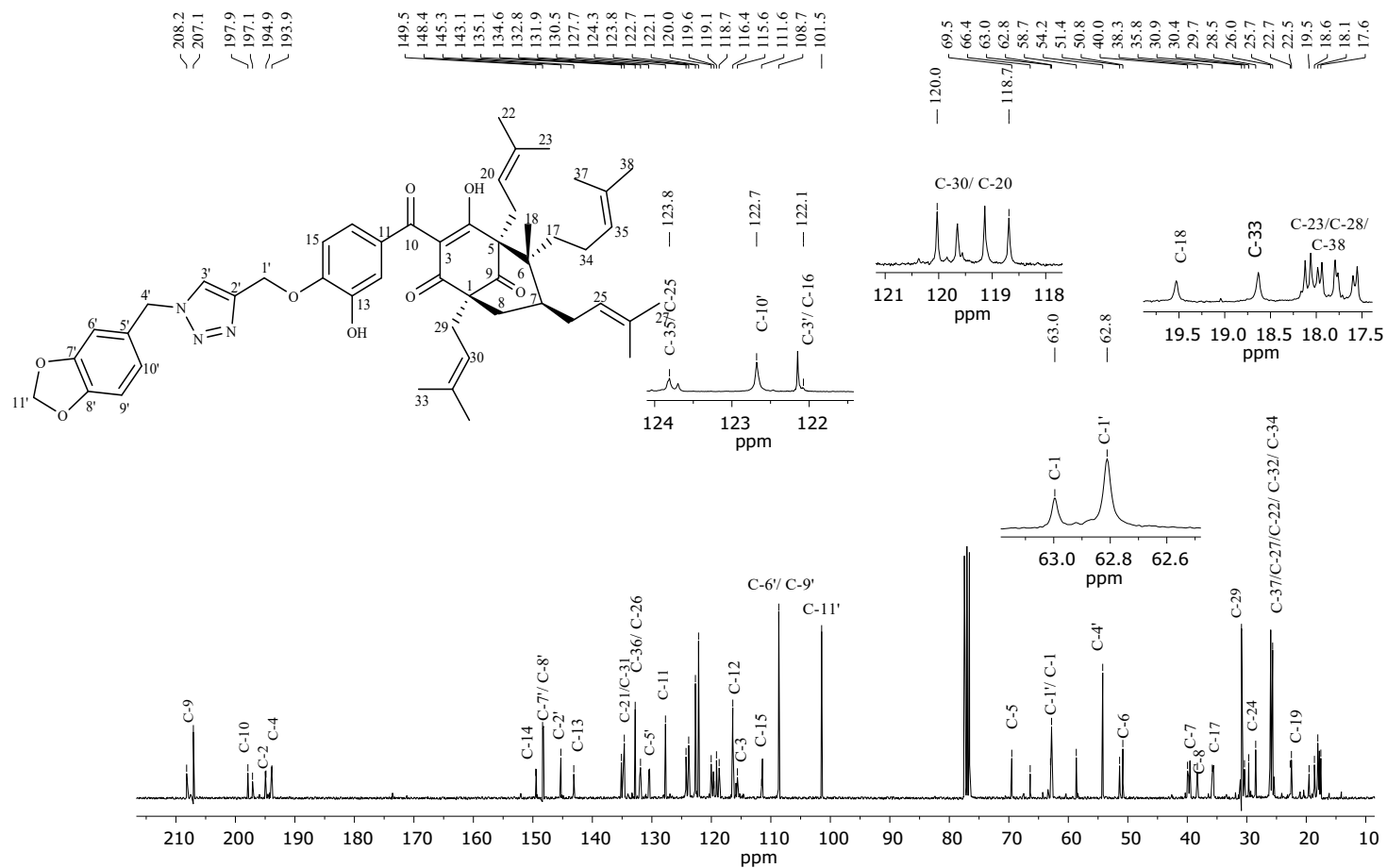


Fig. S41. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 10.

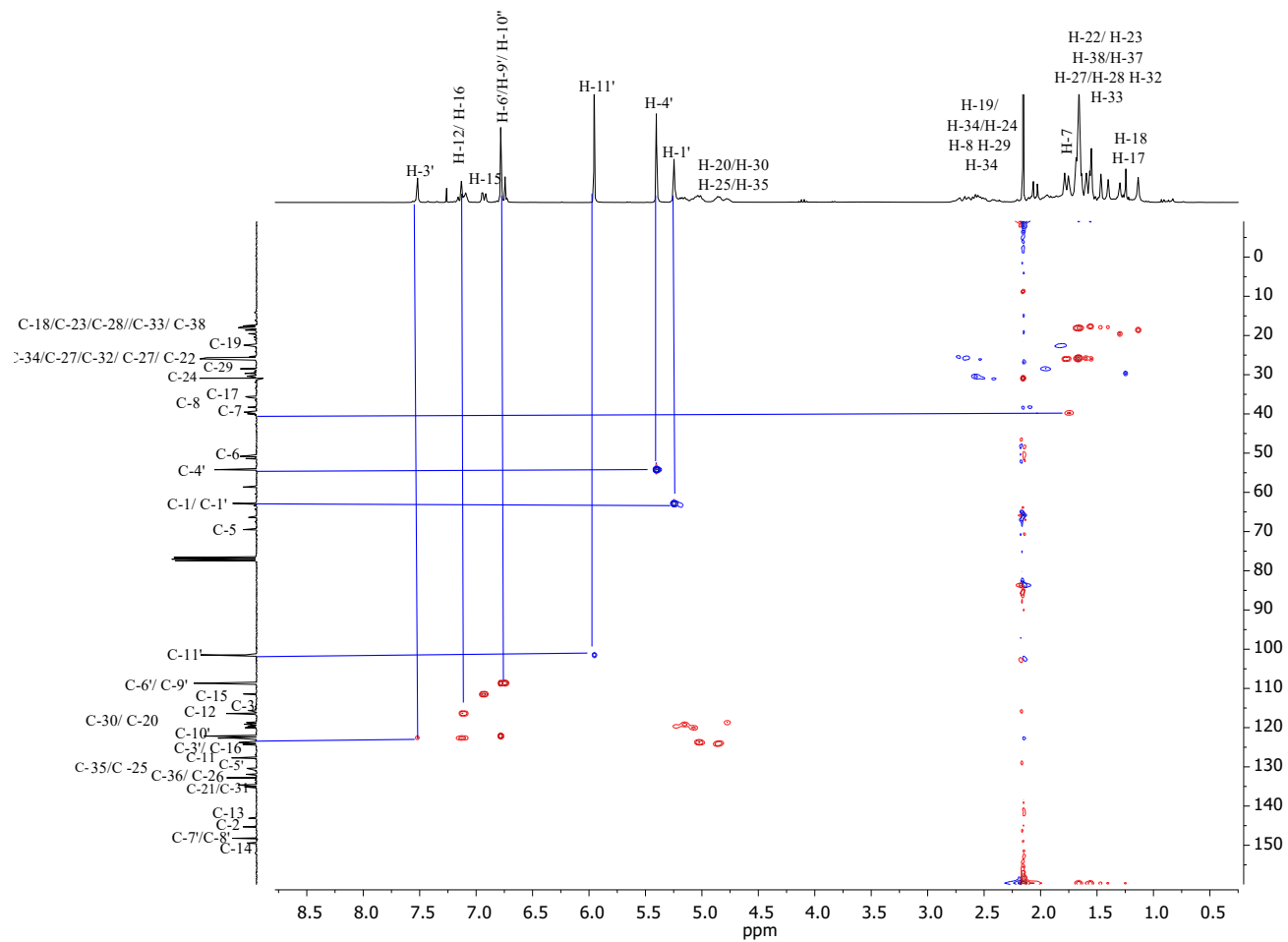


Fig. S42. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound **10**.

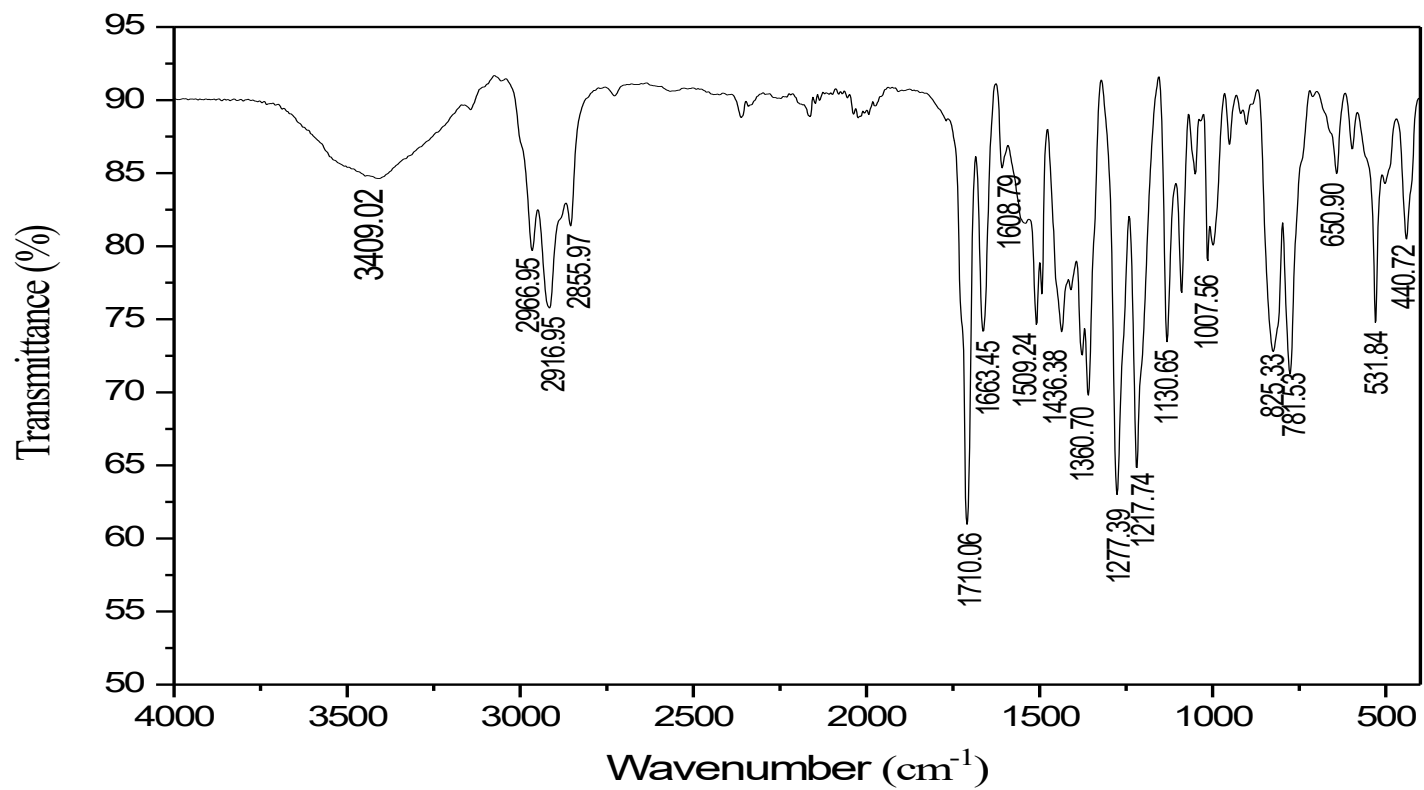


Fig. S43. FTIR (ATR) spectrum of compound 11.

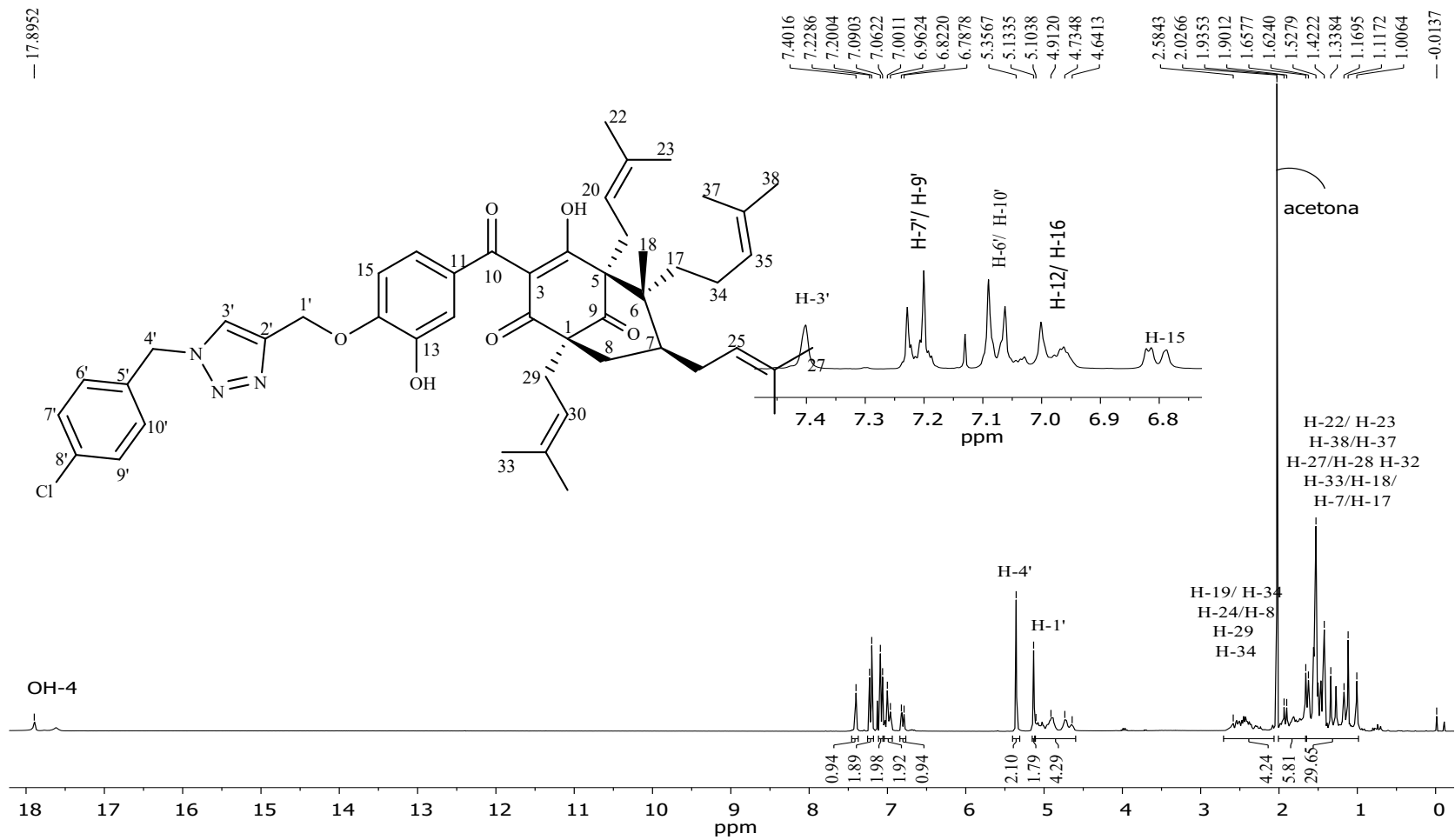


Fig. S44. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 11.

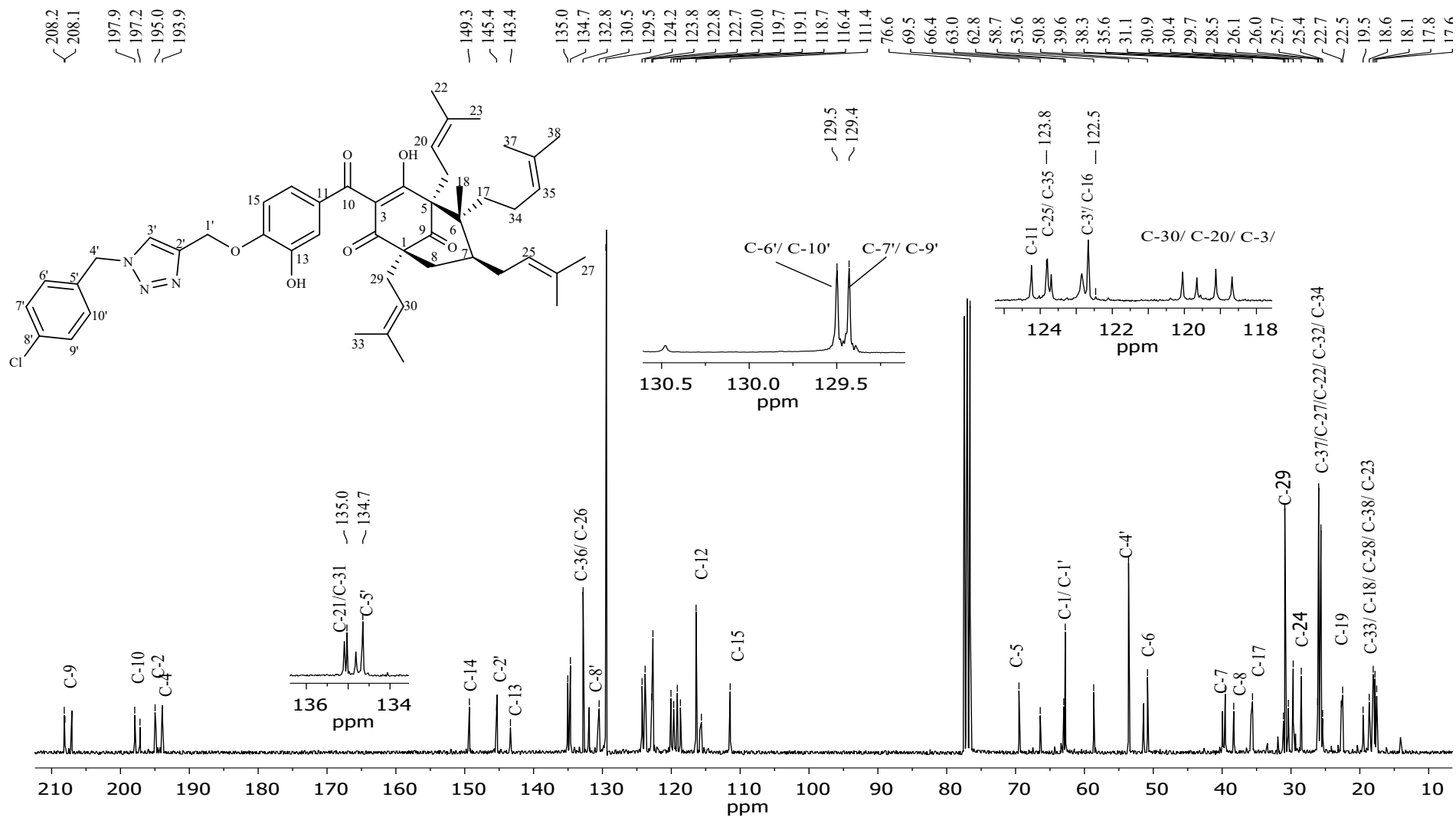


Fig. S45. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 11.

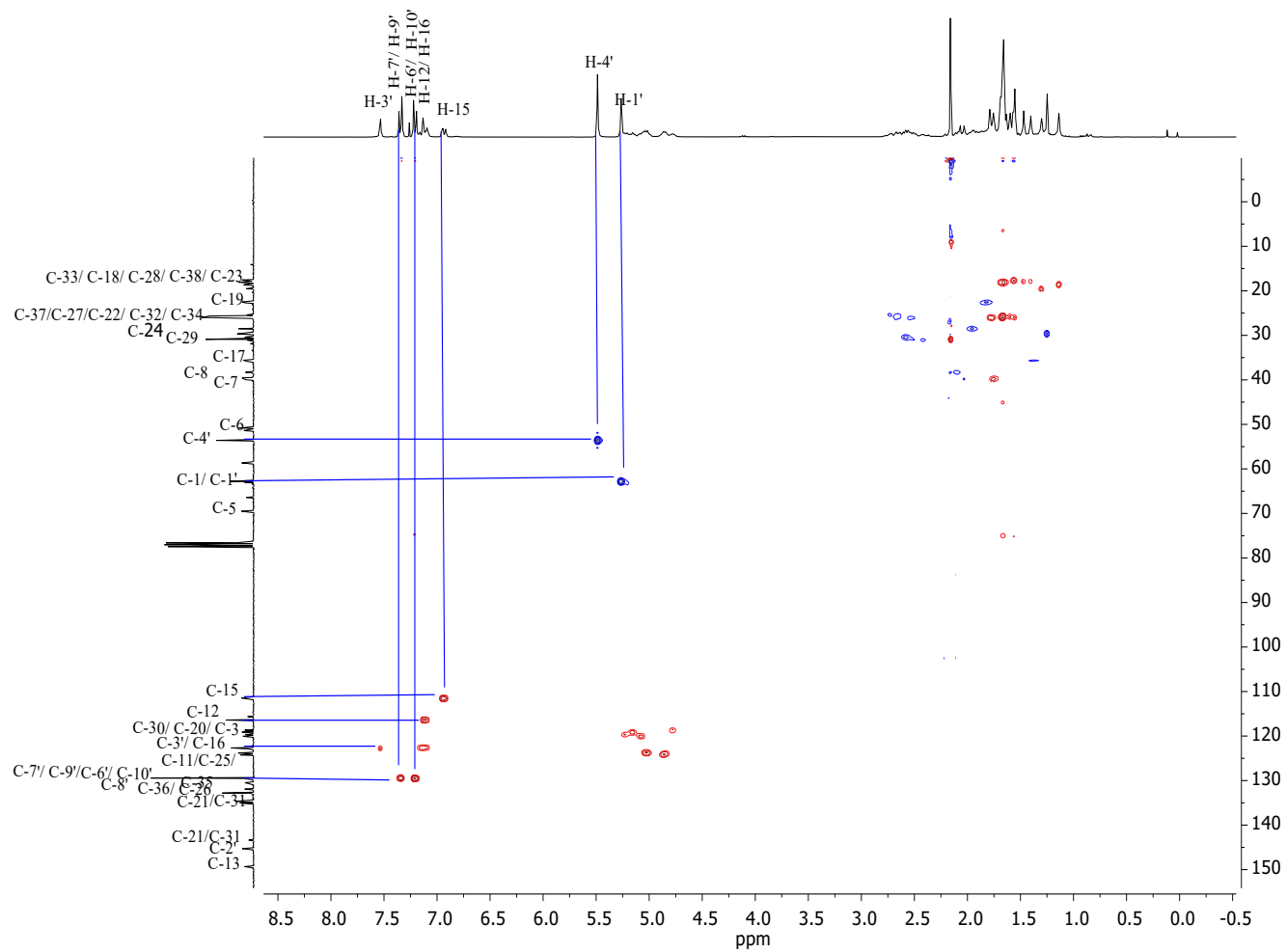


Fig. S46. HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of compound 11.

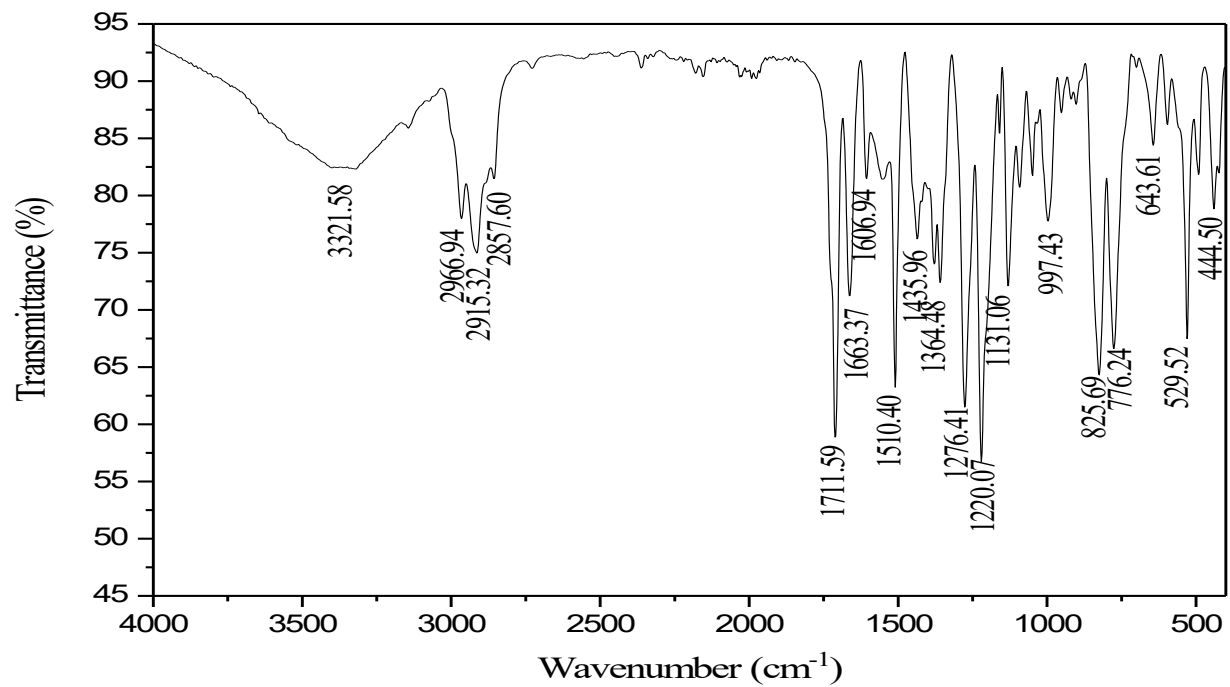


Fig. S47. FTIR (ATR) spectrum of compound 12.

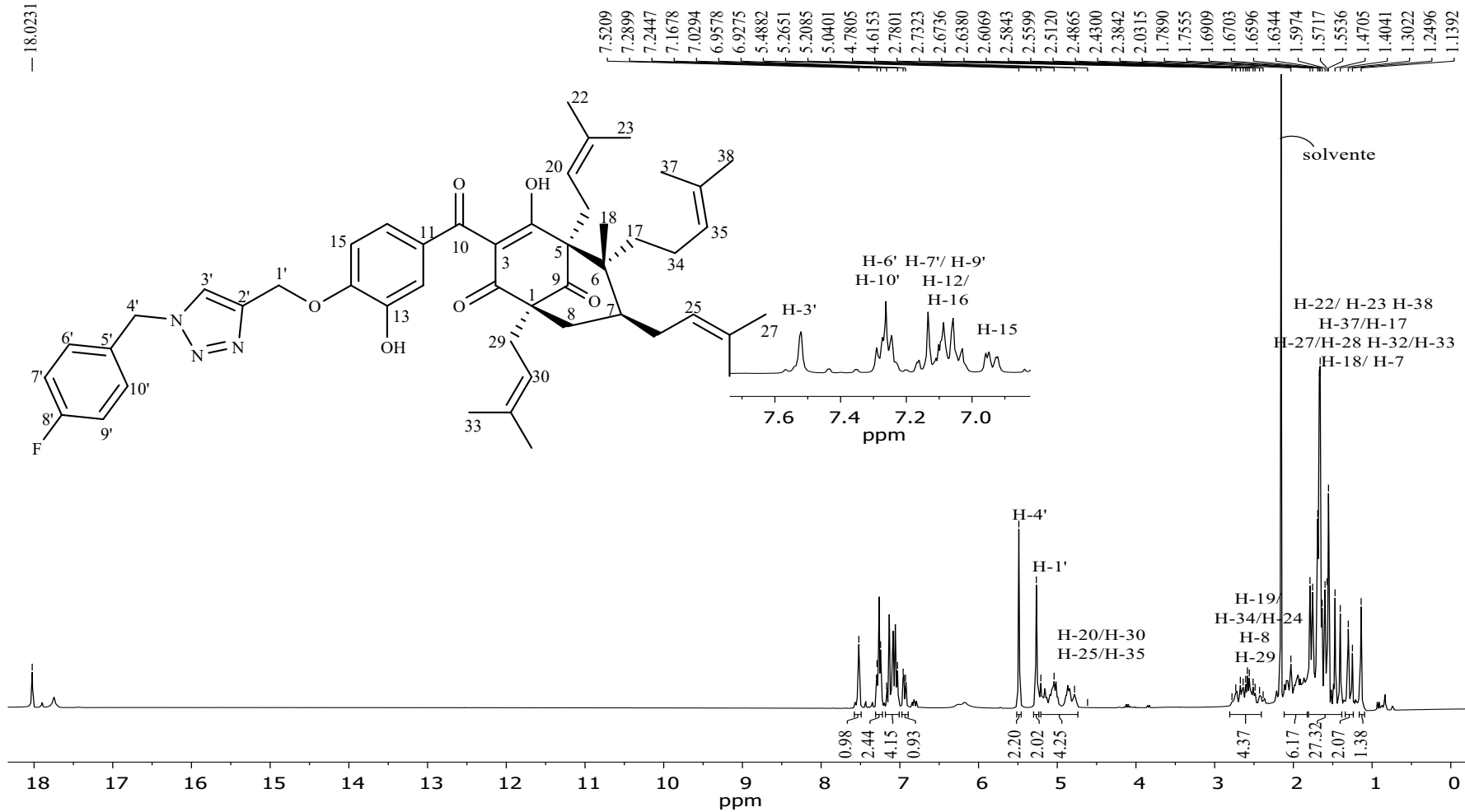


Fig. S48. ^1H NMR spectrum (300 MHz, CDCl_3) of compound 12.

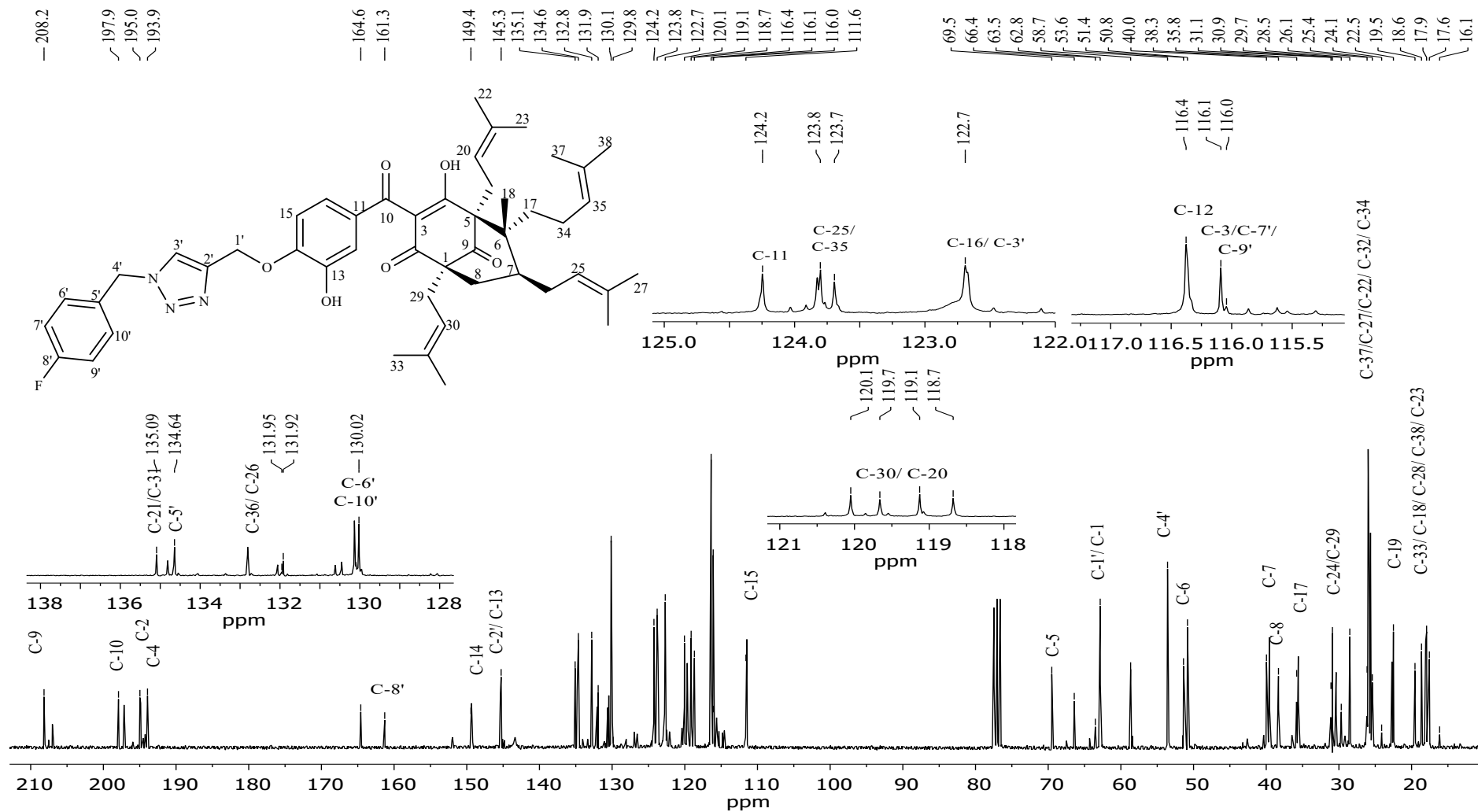


Fig. S49. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound 12.

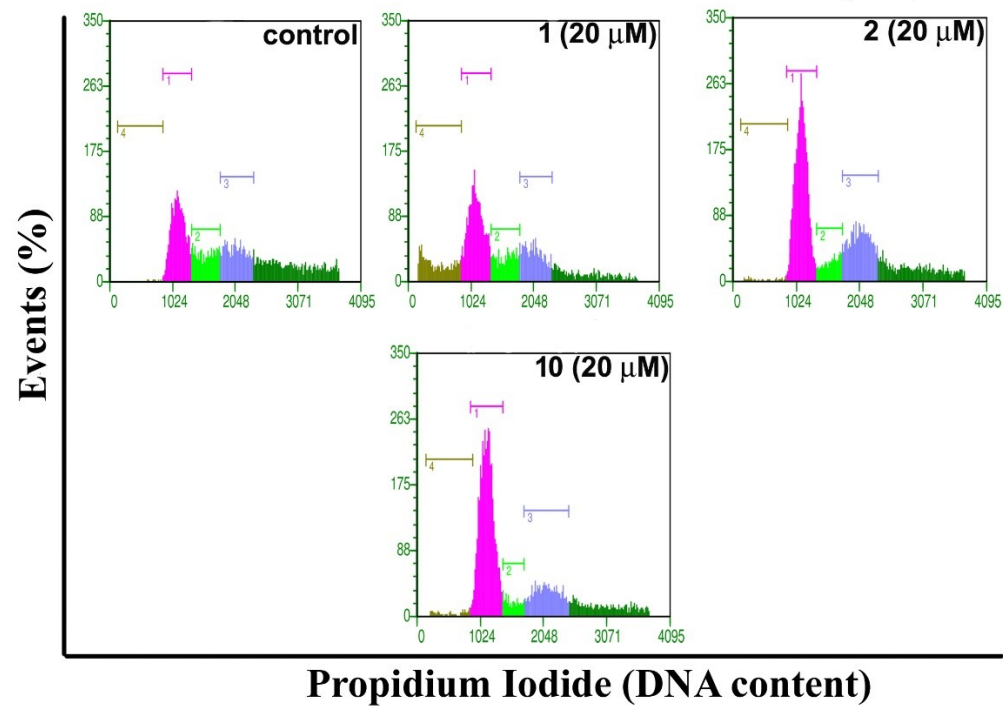


Fig. S50. Representative histograms showing DNA quantification performed by flow cytometry. HepG2 cells were treated for 48 h with guttiferone-A (**1**), alkyne (**2**), and compound **10** at 20 μ M Sub-G1 (brown), G0/G1 (pink), S (green), G2/M (blue), hypertetraploid population (dark green).

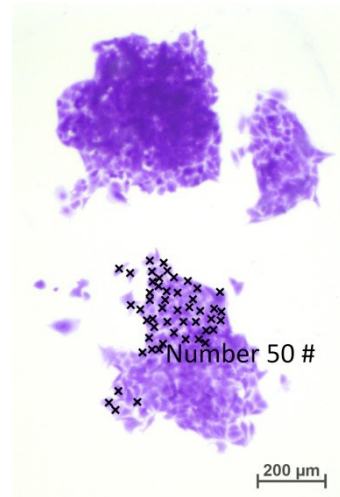


Fig. S51. Illustrative image evidencing the morphological features of the colonies visualized in a stereomicroscope. The software Zen Lite Zeiss 3.7 was used to select appropriately the colonies with, at least, 50 cells.

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

Gene	Sequence	Reference
<i>CDKN1A</i>	F 5'- CCATAGCCTCTACTGCCACCATC-3'	NM_001291549.1
	R 5'- GTCCAGCGACCTTCCTCATCCA-3'	
<i>CCND1</i>	F 5'- GGGTTGTGCTACAGATGATAGAG-3'	NM_053056.2
	R 5'- AGACGCCTCCTTTGTGTTAAT-3'	

<i>CCNE2</i>	F 5'- GGCTATGCTGGAGGAAGTAAAT-3' R 5'- GCTCTTCGGTGGTGTCATAAT-3'	NM_057749.2
<i>CDC25</i>	F 5'- TTTTCCAAGGTATGTGCGCTG-3' R 5'- TGGAACCTCCCCGACAGTAAGG-3'	XM_006714739.3
<i>CDK1</i>	F 5'- ATGAGGTAGTAACACTCTGG-3' R 5'- CCTATACTCCAAATGTCAACTG-3'	NM_001786.4
<i>CNNB1</i>	F 5'- GTACCCTCCAGAAATTGGTGA-3' R 5'- GACTACATTCTTAGCCAGGTG-3'	NM_031966.2
<i>ACTB</i>	F 5'- AGAGCTACGAGCTGCCTGAC-3' R 5'- AGCACTGTGTTGGCGTACAG-3'	NM_001101.3
<i>GAPDH</i>	F 5'- GGATTTGGTCGTATTGGGC-3' R 5'- TGGAAGATGGTGATGGGATT-3'	NM_002046.4
<i>18srRNA</i>	F 5'- GTAACCCGTTGAACCCCAT-3' R 5'- CCATCCAATCGGTAGTAGCG-3'	HQ387008.1

F = forward primer; R = reverse primer

Table S2: Specification of the antibody used in Western blotting

Antibody	Dilution	Manufacture
Anti-phosfo-ERK (Tyr204)	1:100	Santa Cruz
Anti-ERK1/2	1:200	Cell signaling
Anti-Cyclin D1	1:200	Santa Cruz

Anti-Cyclin E2	1:200	Santa Cruz
Anti-α-tubulin	1:100	Sigma
Anti-rabbit peroxidase-conjugated	1:2000	Cell signaling

Table S3. Raw data from clonogenic assay

	Number of colonies (% colonies)	
	Control	10 (20 μM)
Replicate 1	375 (98.08 %)	250 (65.39 %)
Replicate 2	390 (102.00 %)	263 (68.79 %)
Replicate 3	382 (99.91 %)	269 (70.35 %)