

Supplementary Materials

A novel HDAC6/HIF-1 α dual inhibitor halts malignant progression and reprograms immune microenvironment in triple-negative breast cancer

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S2.1 Literature analysis of the synergistic mechanisms of HDAC6 and HIF-1 α dual-targeting.

(1) HDAC6 regulates EGFR transport and degradation via α -tubulin deacetylation^[1,2], thereby promoting tumor cell proliferation and metastasis. HDAC6 inhibitors increase the acetylation of α -tubulin, disrupting mitosis and inducing apoptosis^[3,4];

(2) HDAC6 inhibition promotes the acetylation of HSP90, leading to the degradation of its client protein, HIF-1 α ^[4]. This creates a synergistic effect when combined with direct HIF-1 α inhibition, resulting in more potent suppression of the HIF-1 α /VEGF/VEGFR axis, metastasis, and angiogenesis^[5].

(3) Simultaneous targeting of HDAC6 and HIF-1 α yields a synergistic reduction in HIF-1 α protein levels. In the hypoxic environment of solid tumors, this significantly downregulates key glycolytic enzymes PKM2, HK2, and LDHA, reduces the glycolysis rate, and inhibits tumor glycolysis^[6-8];

(4) HDAC6 inhibition can reverse tumor immunosuppression, immune escape and resistance to immune checkpoint blockade (ICB)^[9,10];

(5) HIF-1 α inhibition suppresses the recruitment of regulatory T cells (Tregs)^[11], while enhancing the infiltration and activity of cytotoxic T cells and upregulating IFN γ levels^[12]. Furthermore, the inhibition of glycolysis reshapes the tumor and immune microenvironments^[13,14], which, in synergy with the aforementioned immunomodulatory effects, reactivates anti-tumor immunity.

(6) In vivo signaling pathways form a complex network characterized by extensive crosstalk. Our rationale for the dual-target design is supported by the observation that

HDAC6 and HIF-1 α are co-overexpressed in solid tumors, with HDAC6 further stabilizing HIF-1 α via HSP90. Therefore, concurrent inhibition disrupts this interaction, conducive to a synergistically enhanced anti-tumor effect.

We predict that the dual-target inhibitor 12e will exhibit comprehensive efficacy against tumor proliferation, metastasis, and angiogenesis, while reversing immunosuppression and immunoescape through the synergistic mechanisms described above.

Reference

- [1] Liu W, Fan LX, Zhou X, Sweeney WE Jr, Avner ED, Li X. HDAC6 regulates epidermal growth factor receptor (EGFR) endocytic trafficking and degradation in renal epithelial cells. *PLoS One*. 2012;7(11):e49418.
- [2] Wen J, Fu J, Ling Y, Zhang W. MIIP accelerates epidermal growth factor receptor protein turnover and attenuates proliferation in non-small cell lung cancer. *Oncotarget*. 2016;7(8):9118–9134.
- [3] Tao H, Chen YY, Sun ZW, et al. Silence of HDAC6 suppressed esophageal squamous cell carcinoma proliferation and migration by disrupting chaperone function of HSP90. *J Cell Biochem*. 2018;119:6623–6632.
- [4] Haakenson J, Wu JY, Xiang S, Williams KA, Bai W, Zhang X. HDAC6-dependent functions in tumor cells: crossroad with the MAPK pathways. *Crit Rev Oncog*. 2015;20:65–81.
- [5] Liu JJ, Liu XY, Nie JP, Jia MQ, Yu Y, Qin N, Duan HQ. Discovery of anti-metastatic chiral ionone alkaloid derivatives targeting HIF-1 α /VEGF/VEGFR2 pathway. *ChemMedChem*.

2021;16:1–17.

[6] Yi XL, Qi MX, Huang MX, Zhou S, Xiong J. Honokiol inhibits HIF-1 α -mediated glycolysis to halt breast cancer growth. *Front Pharmacol*. 2022;13:796763.

[7] Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, et al. AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. *Cell Metab*. 2013;17(1):113–124.

[8] Chaika NV, Gebregiworgis T, Lewallen ME, Purohit V, Radhakrishnan P, Liu X, et al. MUC1 mucin stabilizes and activates hypoxia-inducible factor 1 alpha to regulate metabolism in pancreatic cancer. *Proc Natl Acad Sci U S A*. 2012;109(34):13787–13792.

[9] Bae J, Hideshima T, Tai YT, Song Y, Richardson P, Raje N, et al. Histone deacetylase (HDAC) inhibitor ACY241 enhances antitumor activities of antigen-specific central memory cytotoxic T lymphocytes against multiple myeloma and solid tumors. *Leukemia*. 2018;32(9):1932–1947.

[10] Banik D, Noonepalle S, Hadley M, Palmer E, Gracia-Hernandez M, Zevallos-Delgado C, et al. HDAC6 plays a noncanonical role in the regulation of antitumor immune responses, dissemination, and invasiveness of breast cancer. *Cancer Res*. 2020;80:3649–3662.

[11] Ren L, Yu Y, Wang L, Zhu ZF, Lu R, Yao Z. Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. *Oncotarget*. 2016;7(46):75763–75773.

[12] Kheshtchin N, Arab S, Ajami M, Mirzaei R, Ashourpour M, Mousavi N, et al. Inhibition of HIF-1 α enhances anti-tumor effects of dendritic cell-based vaccination in a mouse model of breast cancer. *Cancer Immunol Immunother*. 2016;65(10):1159–1167.

[13] Jian SL, Chen WW, Su YC, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. *Cell Death Dis.* 2017;8(5):e2779.

[14] Li XY, Wenes M, Romero P, Huang CCC, Fendt SM, Ho PC, et al. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. *Nat Rev Clin Oncol.* 2019;16(7):425–441.

S2.2 Synthesis methods of intermediates and target compounds

Synthesis of Intermediate 6:

Weigh (+)-ABA (3 g, 11.35 mmol, 1.0 eq.) and dissolve it in an appropriate amount of DCM. Then N-Boc-1, 2-ethylenediamine (1.8 g, 11.35 mmol, 1.0 eq.), EDCI (2.40 g, 12.5 mmol, 1.1 eq.), and HOBt (1.69 g, 12.5 mmol) were added successively. 1.1 eq.) and triethylamine (4.3 mL, 28.37 mmol, 2.5 eq.) were stirred at room temperature for 12 hours, and the reaction was monitored by TLC. Add 10% citric acid, saturated NaHCO₃ solution and saturated brine in sequence for extraction, combine organic substances, dry with anhydrous magnesium sulfate, filter and concentrate to obtain the crude product 6.

Synthesis of Intermediate 7:

Weigh compound 6 (4.25 g, 10.45 mmol, 1.0 eq.) and dissolve it in an appropriate amount of methanol. Slowly add 4M HCl a.q. (7.84 mL, 31.36 mmol, 3.0 eq.) under stirring in an ice bath. React at room temperature for 5 hours. Then the pH of reaction solution was adjusted to 8-9 with 1.0 mol/L NaOH solution, then concentrated under reduced pressure and dried to obtain 3.19 g of light yellow powder. Add water and EA for extraction, combine

the organic phase for vacuum concentration, and dry to obtain 7.

The general method for the synthesis of 8a-8b:

Weigh the corresponding bromobenzaldehyde (1.0 eq.) and disperse it in an appropriate amount of distilled water. Add Na_2CO_3 (3.0 eq.) dissolved in water, acrylic acid (1.5 eq.), and PdCl_2 (0.01 eq.) dispersed in water. Stir at room temperature for 15 minutes and then raise to reflux temperature for reaction. The TLC monitoring reaction was complete. The heating was removed, the reaction liquid was cooled to room temperature and then filtered. The filtrate was adjusted to pH 1-2 with dilute hydrochloric acid in an ice bath and filtered again to obtain the filter cake. After drying, to obtain 8a-8b.

The general method for the synthesis of 9a-9b:

Weigh 8a or 8b (1.0 eq.), add an appropriate amount of DCM to dissolve it, then add EDCI (1.1 eq.), HOBt (1.1 eq.) and triethylamine (2.5 eq.), react at room temperature for 1 hour, and then add O-(tetrahydropyran-2-yl) hydroxylamine (0.9 eq.) dissolved in DCM to continue the reaction. The TLC monitoring reaction was complete. Add 30 mL of DCM for quenching reaction, then successively add 10% citric acid, saturated NaHCO_3 solution and saturated brine for extraction. concentrate under reduced pressure and dry to obtain the intermediate 9a-9b.

The general method for the synthesis of 10a-10b:

Weigh compound 7 (1.0 eq.) and dissolve it in an appropriate amount of MeOH. Add compound 9a or 9b(0.9 eq.), stir at room temperature for 6 hours, then drop in NaCNBH_3 (1.5 eq.) dissolved in MeOH. Continue the reaction overnight. TLC monitoring shows that the reaction is complete. Methanol was evaporated under reduced pressure,

water and EA were added for extraction, and the intermediate 10a-10b was obtained through filtration and concentration.

The general method for the synthesis of 11a-11r:

Weigh compound 10a or 10b (1.0 eq.) and the corresponding substituted benzaldehyde and dissolve them in an appropriate amount of MeOH. Stir at room temperature for 6 hours, then add sodium cyanoborohydride (1.5 eq.) dissolved in MeOH. Continue the reaction overnight. TLC monitoring indicates that the reaction is complete. Methanol was evaporated under reduced pressure, add water and EA for extraction, and concentrate to obtain 11a-11r.

The general method for the synthesis of compounds 12a-12r:

Using 11a-11r as raw material, an appropriate amount of methanol was added and dissolved in the reaction flask. After stirring, dilute hydrochloric acid (0.1eq.) was dropped and reacted at room temperature for 12 hours. After the reaction was monitored by TLC, the pH was adjusted to 7 with 1.0 mol/L NaOH solution, the solvent was concentrated under reduced pressure, water and EA were added for extraction, and the organic phase was combined and concentrated under reduced pressure. The target compounds 12a – 12r were obtained through drying.

S2.3 Chemical data of derivatives 12a-12r

12a yellow oil (48.2% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.75 (br s, 1H), 9.04 (br s, 1H), 7.84 (m, 2H), 7.41 (m, 10H), 6.43 (d, J = 15.6 Hz, 1H), 6.06 (d, J = 16.4 Hz, 1H), 5.80 (s, 1H), 5.68 (s, 1H), 5.13 (s, 1H), 4.11 (d, J = 16.4 Hz, 1H), 3.56 (d, J = 13.6 Hz, 4H),

3.21 (m, 6H), 2.08 (d, $J = 16.4$ Hz 1H), 1.91 (s, 3H), 1.81 (s, 3H), 0.95 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 163.2, 163.5, 162.7, 143.6, 140.8, 138.7, 138.0, 135.1, 133.5, 131.0, 130.7, 129.0, 127.9, 127.3, 125.7, 121.4, 119.8, 118.5, 78.3, 57.2, 56.8, 52.6, 49.4, 48.6, 36.2, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 650.2238 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{BrN}_3\text{O}_5$, 650.2229. HPLC purity: 95.7%.

12b yellow oil (47.6% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.79 (br s, 1H), 9.04 (br s, 1H), 7.88 (m, 2H), 7.51 (m, 5H), 7.20 (dd, $J_1 = 8.0$ Hz, $J_2 = 11.2$ Hz, 4H), 6.45 (d, $J = 16.0$ Hz, 1H), 6.06 (d, $J = 16.0$ Hz, 1H), 5.78 (m, 1H), 5.68 (s, 1H), 5.14 (s, 1H), 4.12 (d, $J = 4.8$ Hz, 1H), 3.54 (d, $J = 14.4$ Hz, 4H), 3.23 (m, 6H), 2.27 (s, 3H), 2.08 (d, $J = 16.8$ Hz, 1H), 1.91 (s, 3H), 1.81 (s, 3H), 0.93 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.2, 163.5, 162.7, 143.6, 141.0, 136.0, 135.8, 135.1, 133.5, 129.0, 128.5, 127.9, 127.3, 125.7, 121.4, 118.5, 78.3, 57.3, 57.3, 57.1, 52.4, 49.4, 48.6, 36.2, 24.1, 23.1, 20.8, 20.7, 18.9. HR-ESIMS: m/z 586.3287 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_5$, 586.3281. HPLC purity: 96.2%.

12c yellow oil (47.6% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.79 (br s, 1H), 9.04 (br s, 1H), 7.88 (m, 2H), 7.51 (m, 5H), 7.20 (dd, $J_1 = 8.0$ Hz, $J_2 = 11.2$ Hz, 4H), 6.45 (d, $J = 16.0$ Hz, 1H), 6.06 (d, $J = 16.0$ Hz, 1H), 5.78 (m, 1H), 5.68 (s, 1H), 5.14 (s, 1H), 4.12 (d, $J = 4.8$ Hz, 1H), 3.54 (d, $J = 14.4$ Hz, 4H), 3.23 (m, 6H), 2.27 (s, 3H), 2.08 (d, $J = 16.8$ Hz, 1H), 1.91 (s, 3H), 1.81 (s, 3H), 0.93 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.2, 163.5, 162.7, 143.6, 141.0, 136.0, 135.8, 135.1, 133.5, 129.0, 128.5, 127.9, 127.3, 125.7, 121.4, 118.5, 78.3, 57.3, 57.3, 57.1, 52.4, 49.4, 48.6, 36.2, 24.1, 23.1, 20.8, 20.7, 18.9.

HR-ESIMS: m/z 586.3287 $[M+H]^+$, calcd for $C_{35}H_{44}N_3O_5$, 586.3281. HPLC purity: 96.2%.

12d yellow oil (35.6% yield). 1H NMR (400 MHz, DMSO- d_6): δ 10.79 (br s, 1H), 9.04 (br s, 1H), 7.90 (m, 2H), 7.41 (m, 10H), 6.45 (d, J = 12.0 Hz, 1H), 6.06 (dd, J_1 = 3.6 Hz, J_2 = 7.2 Hz, 1H), 5.79 (s, 1H), 5.66 (s, 1H), 5.37 (d, J = 2.4 Hz, 1H), 5.13 (s, 1H), 3.82 (m, 1H), 3.44 (d, J = 14 Hz, 1H), 3.25 (m, 4H), 2.09 (d, J = 9.6 Hz, 1H), 1.91 (d, J = 5.6 Hz, 3H), 1.81 (d, J = 7.2 Hz, 3H), 0.93 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.6, 165.4, 165.3, 163.5, 162.2 (d, J = 243.1 Hz), 162.6, 143.9 (d, J = 6.6 Hz), 143.6, 139.1, 137.0 (d, J = 7.7 Hz), 135.2, 133.9, 130.7 (d, J = 8.4 Hz), 129.0, 128.5, 127.9, 127.2 (d, J = 11 Hz), 125.8, 123.5, 121.5, 118.8, 115.7, 115.5, 114.4 (d, J = 21 Hz), 78.3, 57.6, 54.9, 52.2, 50.9, 49.4, 48.0, 36.1, 24.1, 23.1, 20.8, 20.7, 19.0, 18.9. HR-ESIMS: m/z 590.3036 $[M+H]^+$, calcd for $C_{34}H_{41}FN_3O_5$, 590.3030. HPLC purity: 95.2%.

12e yellow oil (47.7% yield). 1H NMR (400 MHz, DMSO- d_6): δ 10.75 (br s, 1H), 9.04 (br s, 1H), 7.84 (m, 2H), 7.41 (m, 8H), 6.87 (d, J = 8.0 Hz, 2H), 6.42 (d, J = 16.0 Hz, 1H), 6.06 (d, J = 16.0 Hz, 1H), 5.80 (s, 1H), 5.68 (s, 1H), 5.13 (s, 1H), 4.11 (d, J = 5.2 Hz, 1H), 3.72 (s, 3H), 3.53 (d, J = 20.0 Hz, 4H), 3.23 (m, 5H), 2.08 (d, J = 16.4 Hz, 1H), 1.91 (s, 3H), 1.81 (s, 3H), 0.93 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.2, 163.5, 158.2, 143.6, 141.1, 138.2, 135.1, 133.4, 130.8, 129.7, 129.0, 128.3, 127.9, 127.3, 125.7, 121.4, 118.4, 113.5, 78.3, 57.3, 57.0, 56.9, 52.4, 49.4, 48.6, 36.2, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 602.3233 $[M+H]^+$, calcd for $C_{35}H_{44}N_3O_6$, 602.3230. HPLC purity: 94.8%.

12f yellow oil (39.8% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.74 (br s, 1H), 9.01 (br s, 1H), 7.83 (m, 5H), 7.54 (m, 8H), 7.42 (m, 8H), 6.43 (d, J = 15.8 Hz, 1H), 6.06 (d, J = 16.1 Hz, 1H), 5.79 (s, 1H), 5.68 (s, 1H), 5.12 (s, 1H), 3.62 (d, J = 14.9 Hz, 4H), 3.23 (d, J = 4.6 Hz, 2H), 3.17 (d, J = 4.0 Hz, 2H), 2.06 (d, J = 16.6 Hz, 1H), 1.92 (s, 3H), 1.81 (s, 3H), 0.92 (m, 6H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 165.3, 163.5, 145.6, 143.7, 135.2, 132.1, 129.3, 129.1, 127.9, 127.4, 125.8, 121.4, 118.9, 118.6, 109.5, 78.3, 57.4, 57.2, 52.8, 49.4, 48.6, 36.2, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 595.2946 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{41}\text{N}_4\text{O}_5$, 595.2921. HPLC purity: 94.0%.

12g yellow oil (28.9% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.75 (br s, 1H), 9.04 (br s, 1H), 7.85 (m, 2H), 7.65 (d, J = 7.36 Hz, 1H), 7.49 (d, J = 7.96 Hz, 1H), 7.41 (m, 3H), 7.29 (m, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 6.43 (d, J = 15.8 Hz, 1H), 6.06 (d, J = 16.2 Hz, 1H), 5.79 (s, 1H), 5.68 (s, 1H), 5.12 (s, 1H), 3.68 (s, 2H), 3.65 (s, 2H), 3.26 (dd, J_1 = 12.48 Hz, J_2 = 12.48 Hz, 2H), 3.17 (m, 2H), 2.07 (d, J = 16.6 Hz, 1H), 1.91 (s, 3H), 1.81 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 165.37, 165.3, 163.5, 143.6, 136.6, 135.2, 132.9, 132.92, 130.6, 129.1, 129.0, 128.4, 128.0, 127.3, 127.0, 125.8, 121.4, 118.6, 78.3, 49.4, 41.3, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 606.2748 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{ClN}_3\text{O}_5$, 606.2744. HPLC purity: 95.0%.

12h yellow oil (36.8% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.77 (br s, 1H), 9.05 (br s, 1H), 7.80 (m, 2H), 7.66 (m, 2H), 7.39 (m, 6H), 6.42 (d, J = 15.8 Hz, 1H), 6.05 (d, J = 16.2 Hz, 1H), 5.80 (s, 1H), 5.57 (s, 1H), 5.13 (s, 1H), 3.76 (d, J = 14.12 Hz, 1H), 3.57 (d,

$J = 14.12$ Hz, 1H), 3.17 (m, 2H), 2.07 (d, $J = 16.6$ Hz, 1H), 1.88 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.2, 143.8, 135.3, 132.7, 129.1, 127.9, 127.3, 123.7, 78.3, 49.4, 41.3, 24.1, 23.1, 20.7, 18.9. HR-ESIMS: m/z 650.2249 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{BrN}_3\text{O}_5$, 650.2229. HPLC purity: 95.5%.

12i yellow oil (48.9% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.78 (br s, 1H), 9.03 (br s, 1H), 7.87 (m, 2H), 7.47 (m, 8H), 6.43 (d, $J = 16.6$ Hz, 1H), 6.08 (d, $J = 16.2$ Hz, 1H), 5.80 (s, 1H), 5.64 (s, 1H), 5.15 (s, 1H), 3.77 (d, $J = 14.12$ Hz, 1H), 3.22 (m, 3H), 2.08 (d, $J = 16.6$ Hz, 1H), 1.92 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.3, 133.2, 129.4, 129.0, 128.7, 127.9, 127.5, 125.8, 78.3, 49.9, 41.3, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 606.2752 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{ClN}_3\text{O}_5$, 606.2734. HPLC purity: 96.3%.

12j yellow oil (42.8% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.75 (br s, 1H), 9.05 (br s, 1H), 7.90 (m, 2H), 7.48 (m, 7H), 6.43 (d, $J = 16.6$ Hz, 1H), 6.08 (d, $J = 16.2$ Hz, 1H), 5.80 (s, 1H), 5.64 (s, 1H), 5.15 (s, 1H), 3.82 (d, $J = 14.12$ Hz, 1H), 3.45 (d, $J = 14.12$ Hz, 1H), 3.25 (s, 2H), 3.10 (m, 2H), 2.98 (m, 2H), 2.07 (d, $J = 16.6$ Hz, 1H), 1.92 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.5, 143.8, 135.3, 129.1, 128.4, 128.0, 125.8, 78.4, 49.4, 41.3, 28.2, 24.1, 23.1, 19.0. HR-ESIMS: m/z 650.2252 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{BrN}_3\text{O}_5$, 650.2229. HPLC purity: 96.7%.

12k yellow oil (45.1% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.75 (br s, 1H),

9.02 (br s, 1H), 8.16 (d, $J = 8.44$ Hz, 2H), 7.84 (d, $J = 16.6$ Hz, 1H), 7.66 (d, $J = 8.44$ Hz, 2H), 7.46 (m, 5H), 6.43 (d, $J = 16.6$ Hz, 1H), 6.05 (d, $J = 16.2$ Hz, 1H), 5.79 (s, 1H), 5.69 (s, 1H), 5.12 (s, 1H), 3.70 (s, 2H), 3.62 (s, 2H), 3.24 (m, 2H), 2.07 (d, $J = 16.6$ Hz, 1H), 1.92 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 165.3, 163.5, 147.9, 146.5, 143.7, 135.2, 133.6, 129.5, 129.1, 127.9, 127.4, 125.8, 123.2, 121.4, 78.3, 57.5, 56.9, 52.9, 49.4, 41.3, 36.2, 24.1, 23.1, 20.8, 18.9. HR-ESIMS: m/z 617.2995 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{34}\text{H}_{41}\text{N}_4\text{O}_7$, 617.2975. HPLC purity: 95.4%.

12l yellow oil (42.0% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.91 (br s, 1H), 8.39 (br s, 1H), 7.81 (m, 2H), 7.53 (m, 6H), 7.00 (d, $J = 8.16$ Hz, 2H), 6.53 (d, $J = 15.84$ Hz, 1H), 6.12 (d, $J = 16.12$ Hz, 1H), 5.82 (s, 1H), 5.63 (s, 1H), 5.20 (br s, 1H), 3.78 (s, 3H), 3.55 (m, 2H), 3.00 (m, 2H), 2.09 (d, $J = 16.6$ Hz, 1H), 1.92 (s, 3H), 1.83 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 197.2, 166.2, 163.4, 160.1, 145.4, 137.5, 136.1, 135.4, 133.0, 132.0, 130.6, 129.4, 128.0, 127.8, 125.9, 121.2, 120.2, 114.8, 78.4, 56.1, 55.1, 50.9, 49.3, 41.3, 33.6, 24.1, 23.1, 20.8, 19.0. HR-ESIMS: m/z 602.3242 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_6$, 602.3230. HPLC purity: 95.6%.

12m yellow oil (35.6% yield). ^1H NMR (400 MHz, DMSO- d_6): δ 10.93 (br s, 1H), 8.42 (br s, 1H), 7.82 (m, 2H), 7.65 (m, 2H), 7.40 (m, 4H), 7.18 (d, $J = 7.36$ Hz, 1H), 6.99 (d, $J = 7.68$ Hz, 1H), 6.56 (d, $J = 15.84$ Hz, 1H), 6.12 (d, $J = 16.12$ Hz, 1H), 5.81 (s, 1H), 5.64 (s, 1H), 5.20 (br s, 1H), 4.38 (s, 2H), 4.35 (s, 2H), 3.79 (s, 3H), 3.00 (m, 2H), 2.08 (d, $J = 16.6$ Hz, 1H), 1.92 (s, 3H), 1.83 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (100 MHz,

DMSO-*d*6): δ 197.2, 166.0, 163.4, 162.4, 159.4, 145.2, 137.5, 136.0, 135.4, 132.2, 131.1, 130.8, 130.5, 129.8, 129.3, 128.1, 127.7, 125.9, 123.4, 120.2, 116.5, 115.4, 78.4, 56.6, 56.3, 55.2, 51.2, 49.3, 43.9, 41.2, 33.5, 24.1, 23.1, 20.8, 19.0. HR-ESIMS: m/z 602.3241 $[M+H]^+$, calcd for $C_{35}H_{44}N_3O_6$, 602.3230. HPLC purity: 96.7%.

12n yellow oil (25.8% yield). 1H NMR (400 MHz, DMSO-*d*6): δ 10.74 (br s, 1H), 9.04 (br s, 1H), 7.85 (m, 2H), 7.37 (m, 8H), 6.47 (d, $J = 15.76$ Hz, 1H), 6.05 (d, $J = 16.12$ Hz, 1H), 5.79 (s, 1H), 5.69 (s, 1H), 5.12 (s, 1H), 3.59 (s, 4H), 3.24 (m, 2H), 2.07 (d, $J = 16.64$ Hz, 1H), 1.90 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 165.3, 163.5, 143.6, 142.0, 139.9, 135.1, 134.8, 133.0, 130.0, 128.8, 128.1, 128.0, 127.1, 126.8, 125.8, 121.4, 78.3, 56.9, 52.7, 49.4, 41.3, 36.2, 24.1, 23.1, 20.7, 18.9. HR-ESIMS: m/z 606.2759 $[M+H]^+$, calcd for $C_{34}H_{41}ClN_3O_5$, 606.2734. HPLC purity: 96.9%.

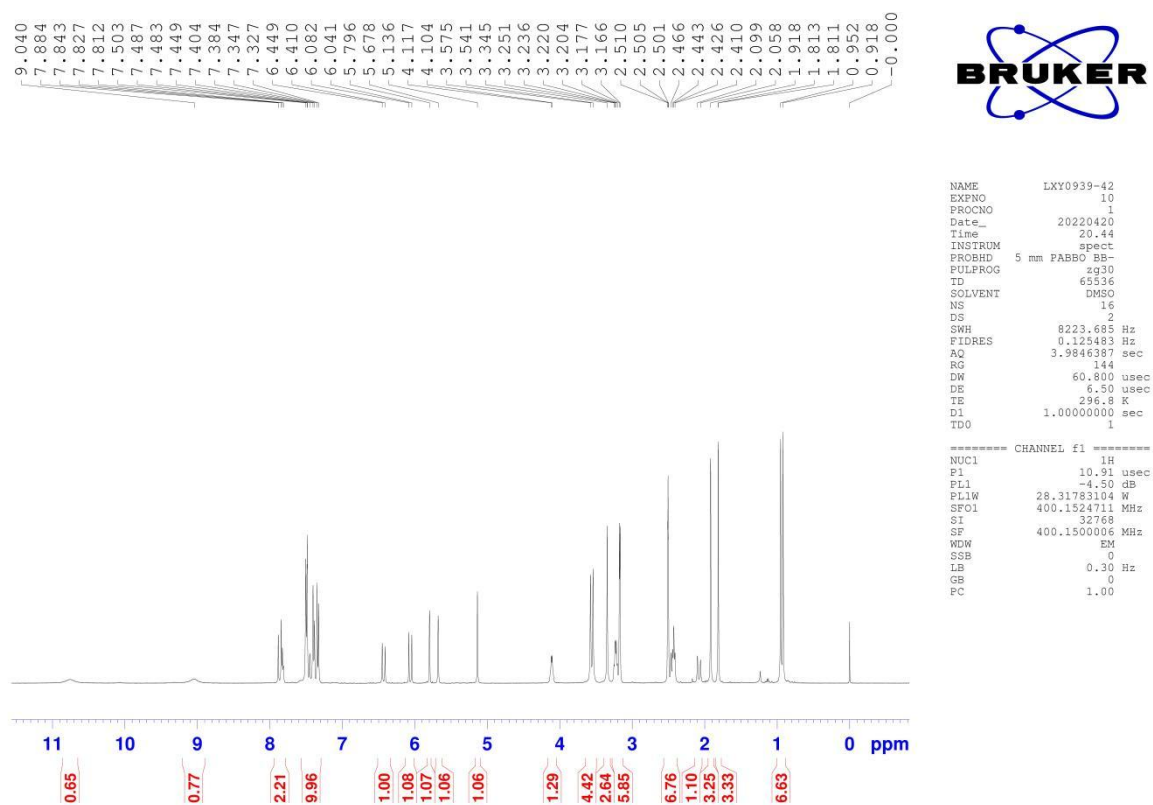
12o yellow oil (37.2% yield). 1H NMR (400 MHz, DMSO-*d*6): δ 10.75 (br s, 1H), 9.04 (br s, 1H), 7.84 (m, 2H), 7.67 (d, $J = 6.44$ Hz, 1H), 7.39 (m, 7H), 6.45 (d, $J = 15.8$ Hz, 1H), 6.05 (d, $J = 16.08$ Hz, 1H), 5.79 (s, 1H), 5.68 (s, 1H), 5.12 (s, 1H), 3.70 (s, 2H), 3.64 (s, 2H), 3.26 (m, 2H), 2.07 (d, $J = 16.64$ Hz, 1H), 1.90 (s, 3H), 1.80 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 165.3, 163.5, 143.7, 139.9, 136.6, 135.1, 134.7, 133.0, 130.6, 129.6, 129.1, 128.7, 128.4, 128.0, 127.7, 127.0, 125.9, 125.7, 121.4, 119.0, 78.3, 54.8, 53.0, 49.4, 41.3, 36.2, 24.1, 23.1, 18.9. HR-ESIMS: m/z 606.2764 $[M+H]^+$, calcd for $C_{34}H_{41}ClN_3O_5$, 606.2734. HPLC purity: 96.0%.

12p yellow oil (21.2% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.91 (br s, 1H), 9.08 (br s, 1H), 8.40 (s, 1H), 7.81 (m, 2H), 7.62 (d, J = 6.80 Hz, 2H), 7.48 (m, 4H), 7.25 (d, J = 7.00 Hz, 2H), 6.53 (d, J = 15.80 Hz, 1H), 6.11 (d, J = 16.08 Hz, 1H), 5.81 (s, 1H), 5.63 (s, 1H), 5.19 (s, 1H), 4.34 (s, 4H), 3.56 (br s, 2H), 3.01 (br s, 2H), 2.33 (s, 3H), 2.07 (d, J = 16.64 Hz, 1H), 1.92 (s, 3H), 1.83 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 166.2, 163.4, 139.1, 136.1, 135.4, 131.4, 130.6, 129.4, 127.8, 126.5, 125.9, 120.2, 78.4, 59.7, 56.1, 51.0, 49.3, 43.9, 41.30, 33.6, 24.1, 23.1, 20.8, 19.0. HR-ESIMS: m/z 586.3296 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_5$, 586.3281. HPLC purity: 97.1%.

12q yellow oil (36.1% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.76 (br s, 1H), 9.05 (br s, 1H), 7.84 (m, 2H), 7.42 (m, 4H), 7.21 (m, 3H), 7.03 (d, J = 6.84 Hz, 1H), 6.47 (d, J = 15.8 Hz, 1H), 6.05 (d, J = 16.08 Hz, 1H), 5.79 (s, 1H), 5.69 (s, 1H), 5.12 (s, 1H), 3.58 (s, 2H), 3.54 (s, 2H), 3.24 (m, 2H), 2.29 (s, 3H), 2.07 (d, J = 16.64 Hz, 1H), 1.90 (s, 3H), 1.81 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 197.2, 165.2, 143.6, 139.0, 129.2, 128.7, 128.0, 127.6, 125.8, 125.6, 121.4, 78.3, 49.4, 41.3, 24.1, 23.1, 20.7, 19.0. HR-ESIMS: m/z 586.3293 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_5$, 586.3281. HPLC purity: 96.7%.

12r yellow oil (27.9% yield). ^1H NMR (400 MHz, DMSO-*d*6): δ 10.77 (br s, 1H), 9.07 (br s, 1H), 7.81 (m, 2H), 7.39 (m, 5H), 7.21 (m, 3H), 7.13 (m, 3H), 6.45 (d, J = 15.8 Hz, 1H), 6.05 (d, J = 16.08 Hz, 1H), 5.79 (s, 1H), 5.65 (s, 1H), 5.12 (s, 1H), 3.58 (s, 2H), 3.57 (s, 2H), 3.25 (m, 2H), 2.28 (s, 3H), 2.07 (d, J = 16.64 Hz, 1H), 1.89 (s, 3H), 1.81 (s, 3H), 0.95 (s,

3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*₆): δ 197.2, 165.2, 143.6, 137.1, 130.1, 128.7, 126.8, 125.8, 121.4, 78.3, 48.6, 41.3, 24.1, 23.1. 20.7, 18.9. HR-ESIMS: m/z 586.3288 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{35}\text{H}_{44}\text{N}_3\text{O}_5$, 586.3281. HPLC purity: 97.3%.



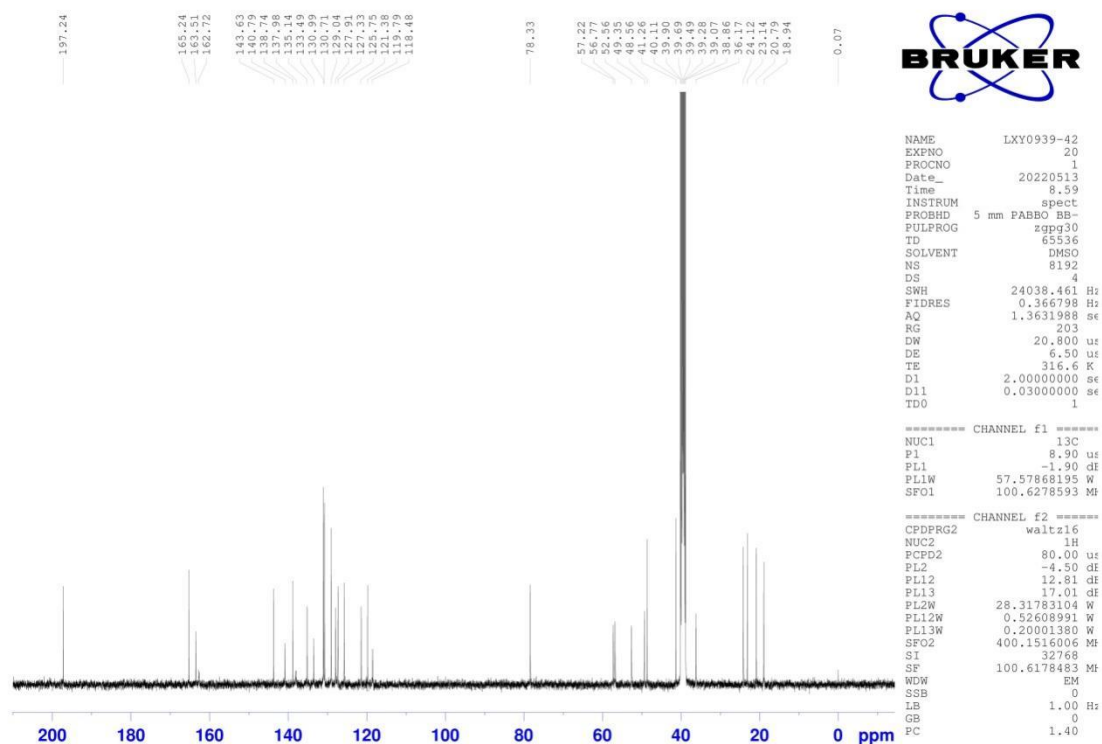
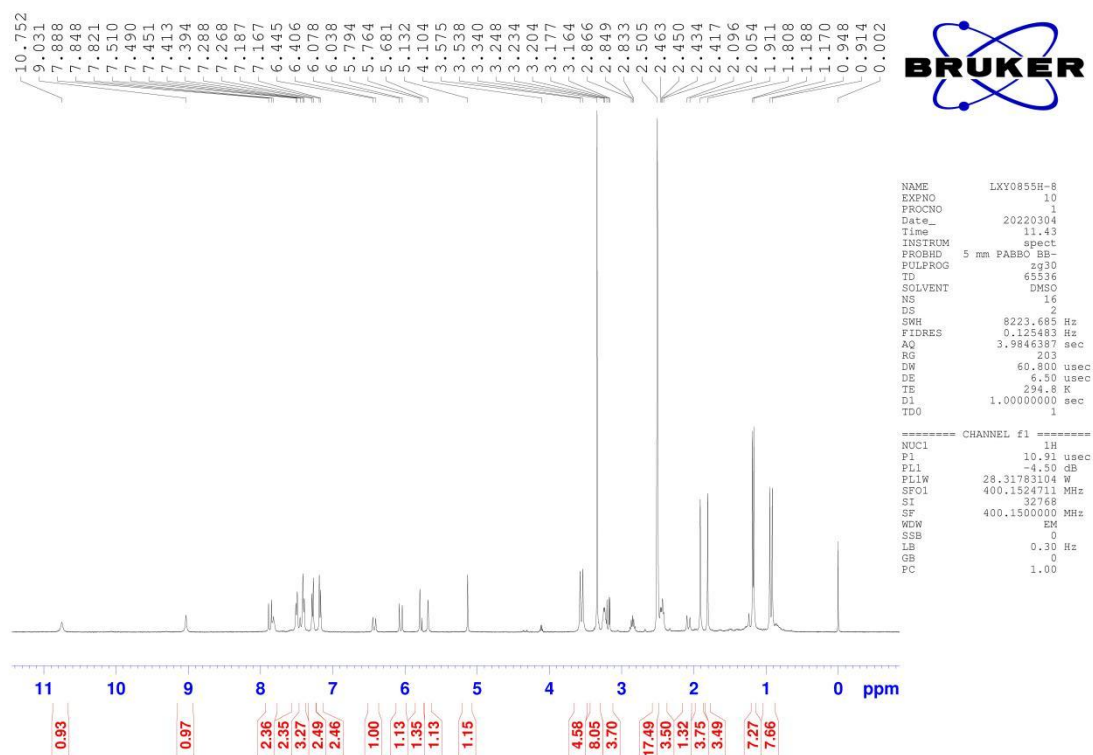


Figure S1. ^1H , ^{13}C NMR spectrum of compound 12a.



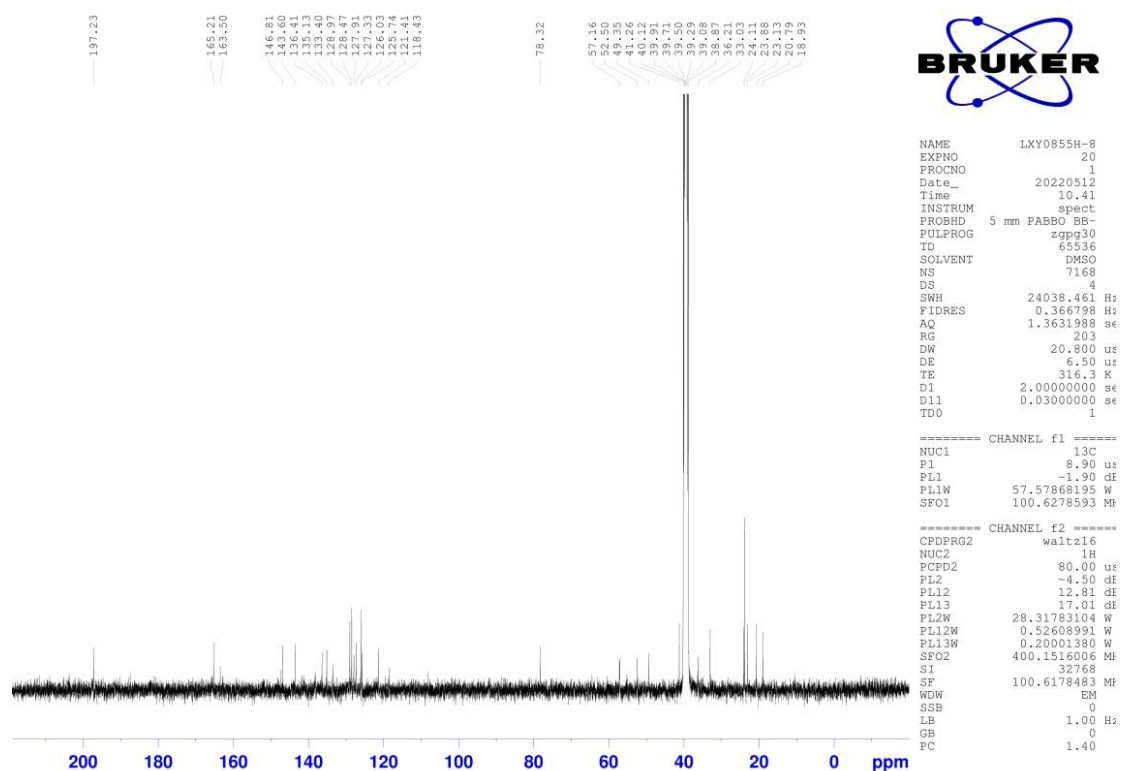
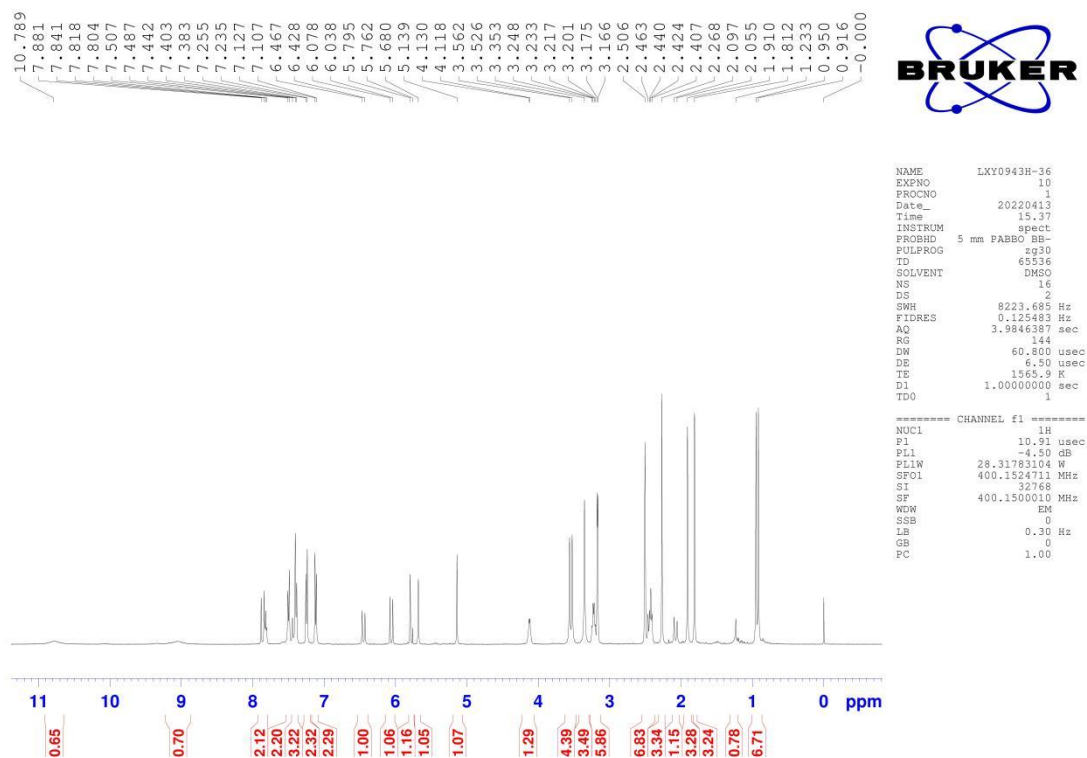


Figure S2. ^1H , ^{13}C NMR spectrum of compound 12b.



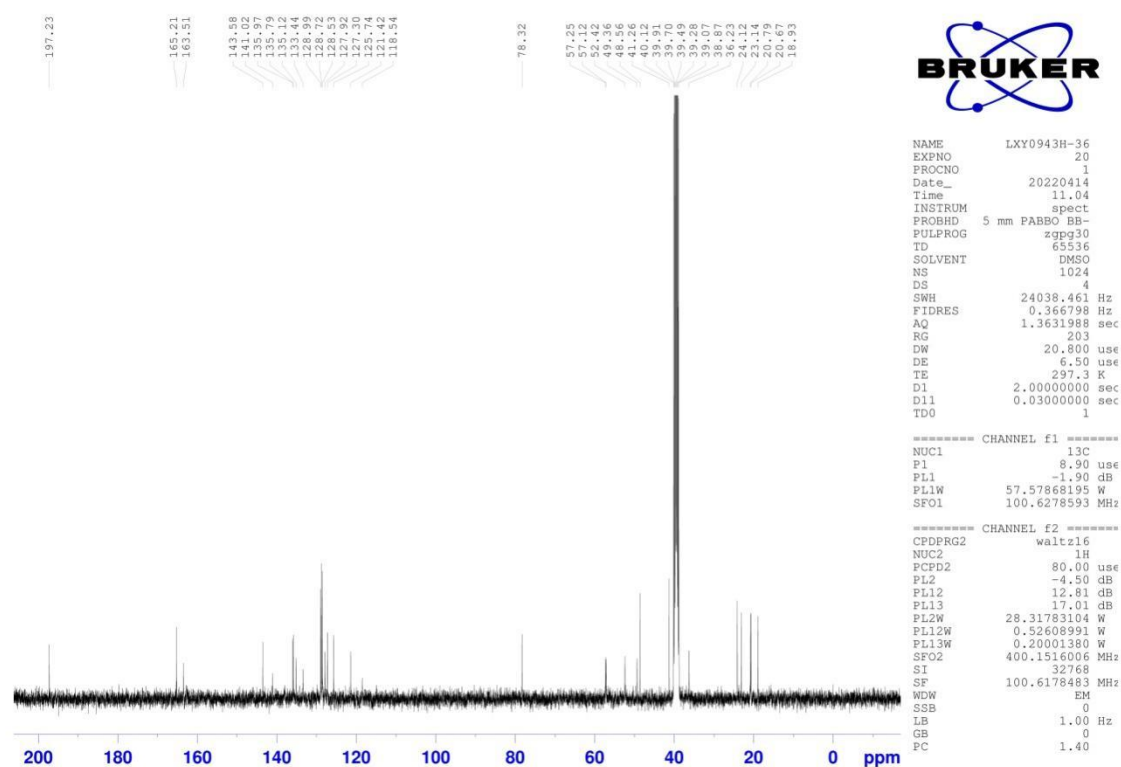


Figure S3. ^1H , ^{13}C NMR spectrum of compound 12c.

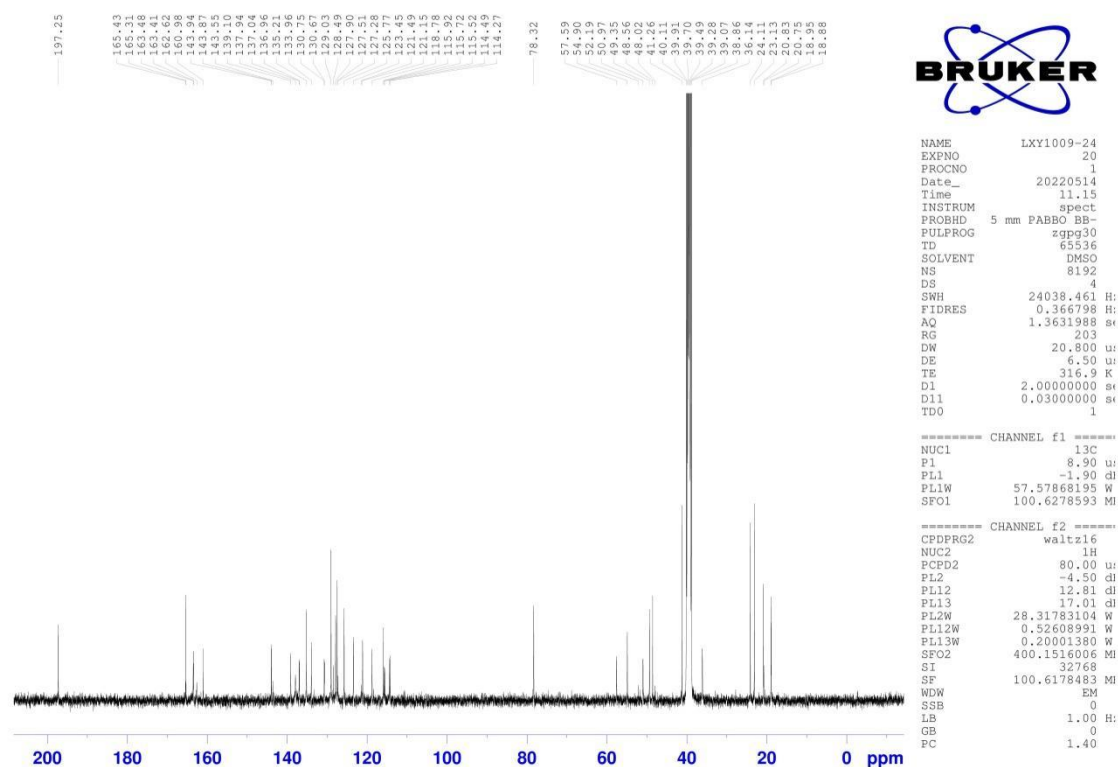
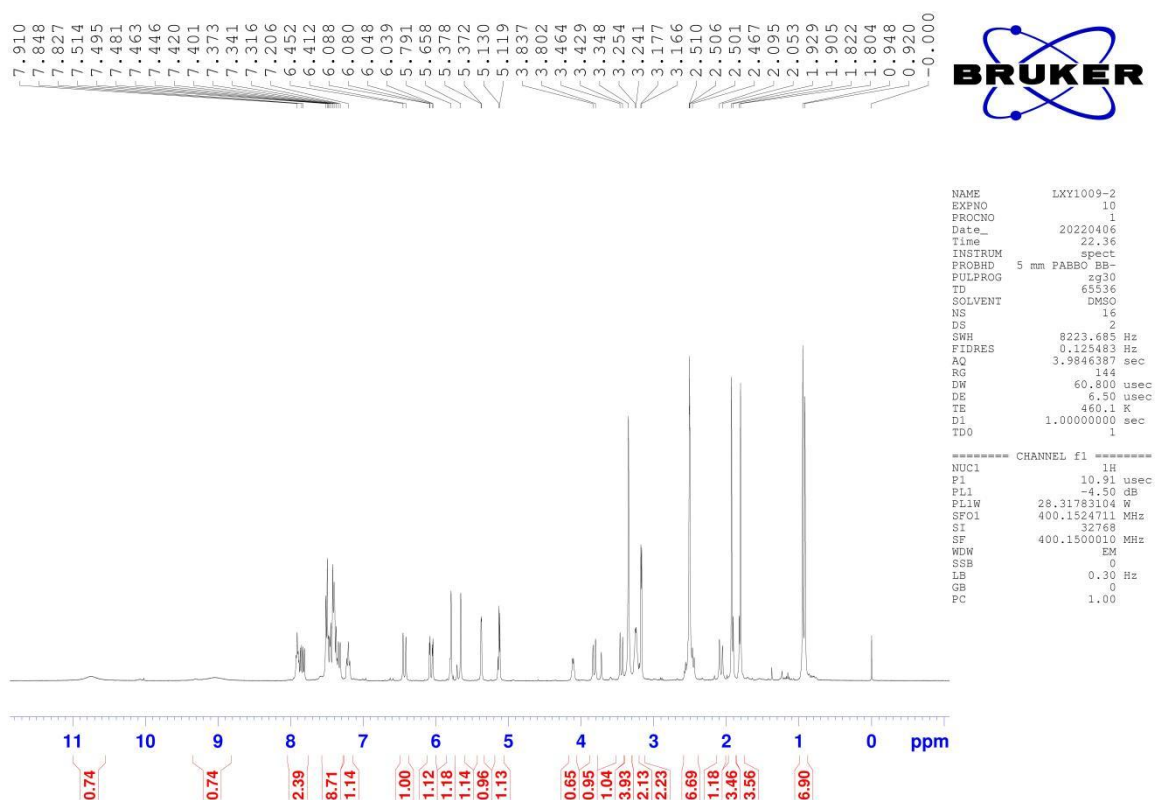


Figure S4. ¹H, ¹³C NMR spectrum of compound 12d.

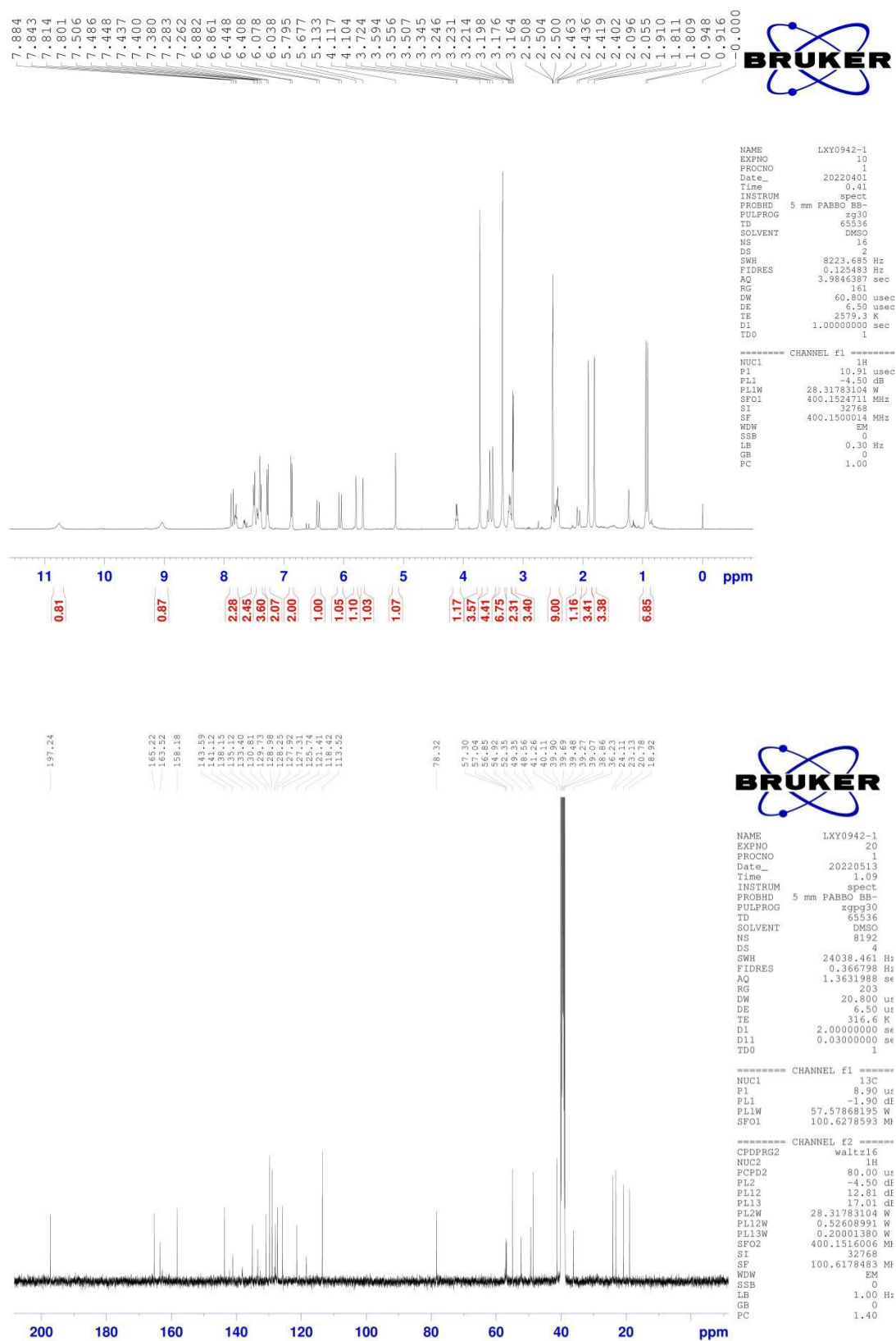


Figure S5. ¹H, ¹³C NMR spectrum of compound 12e.

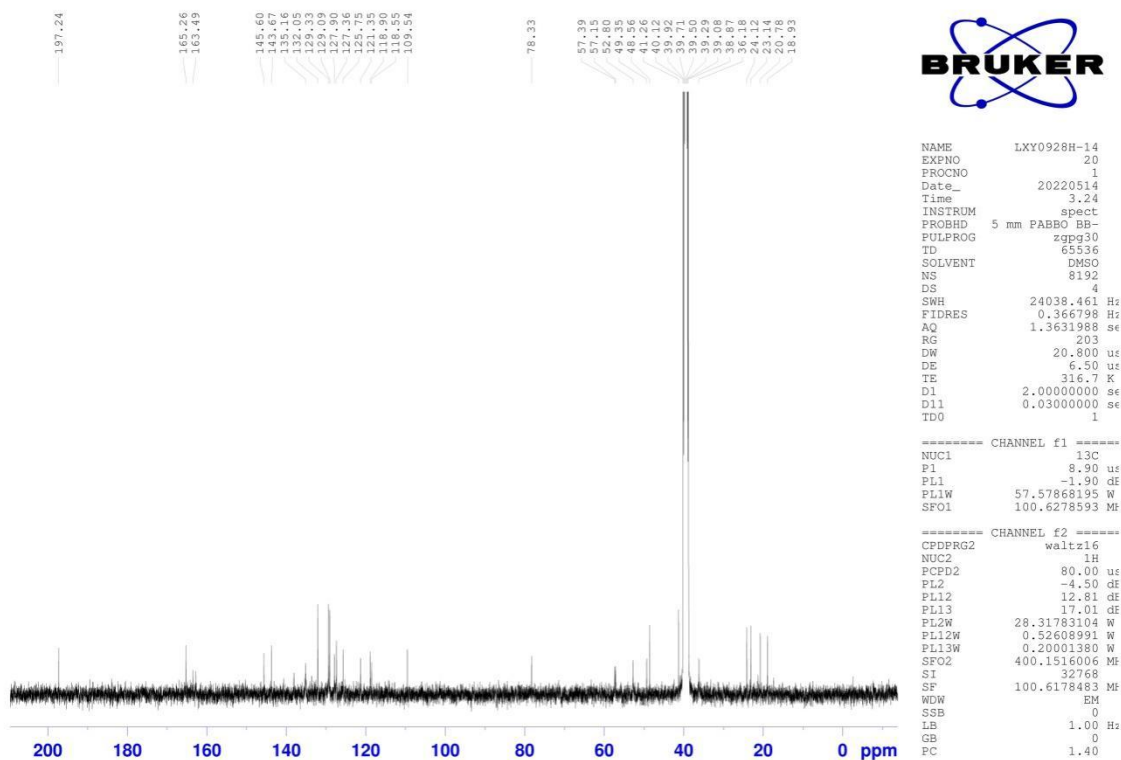
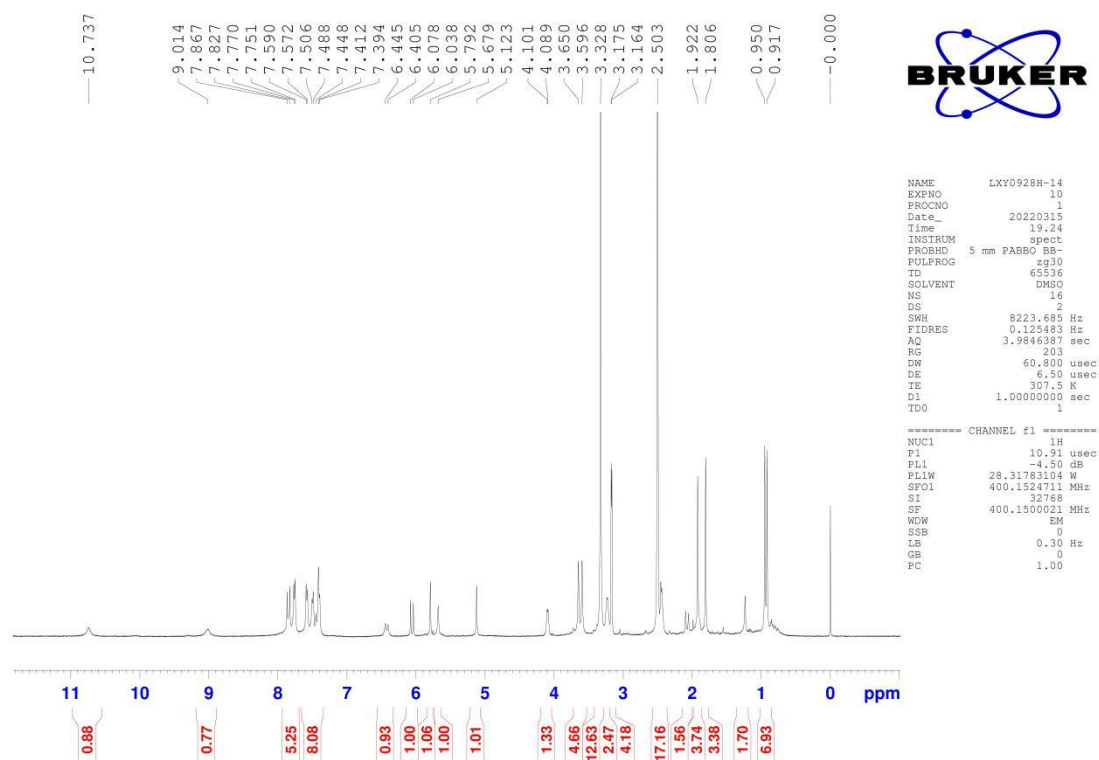


Figure S6. ^1H , ^{13}C NMR spectrum of compound 12f.

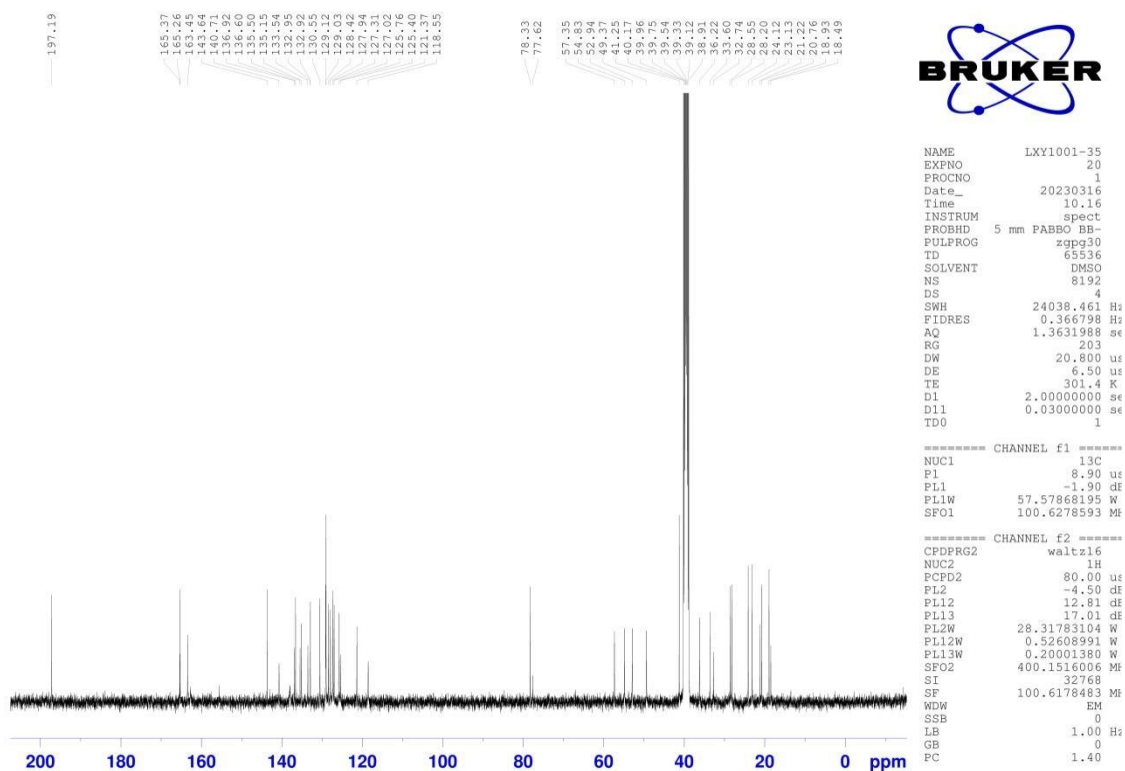
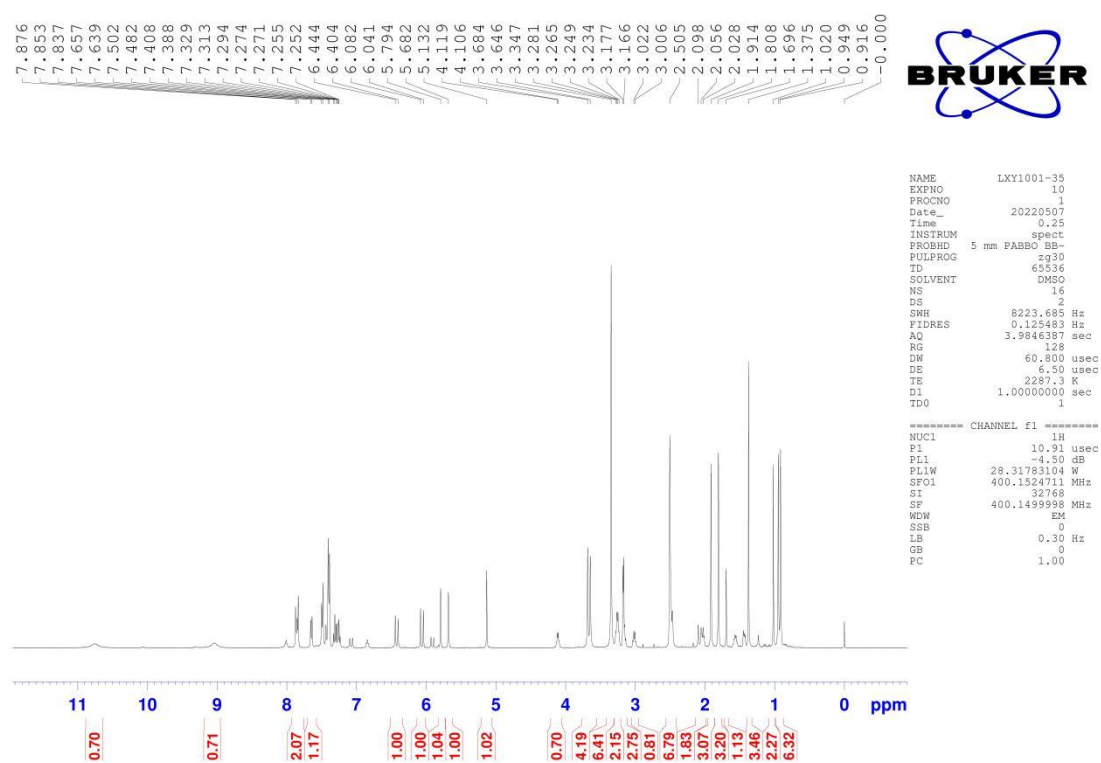


Figure S7. ¹H, ¹³C NMR spectrum of compound 12g.

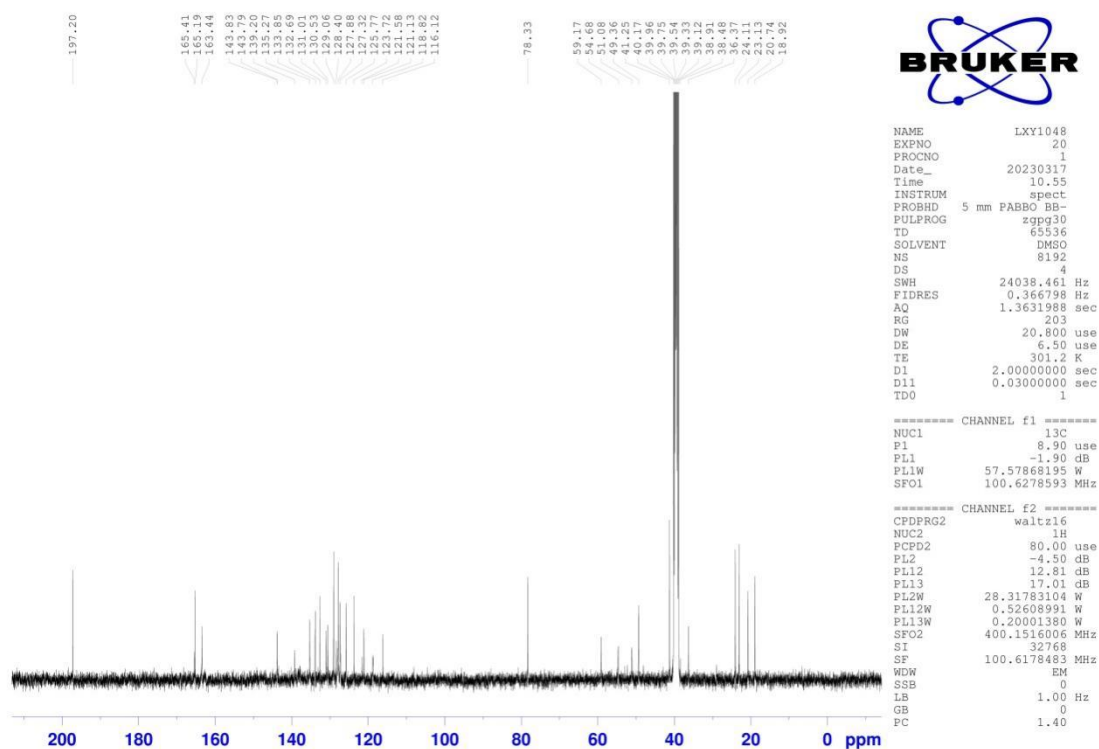
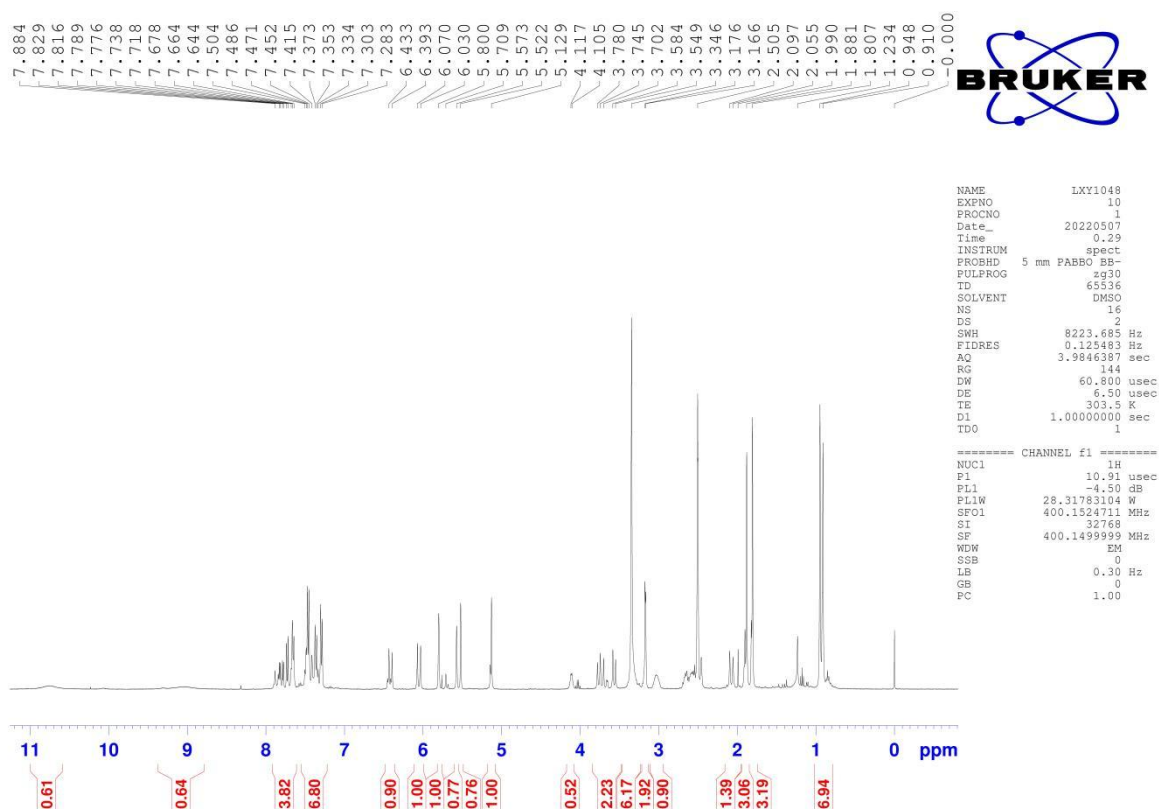


Figure S8. ^1H , ^{13}C NMR spectrum of compound 12h.

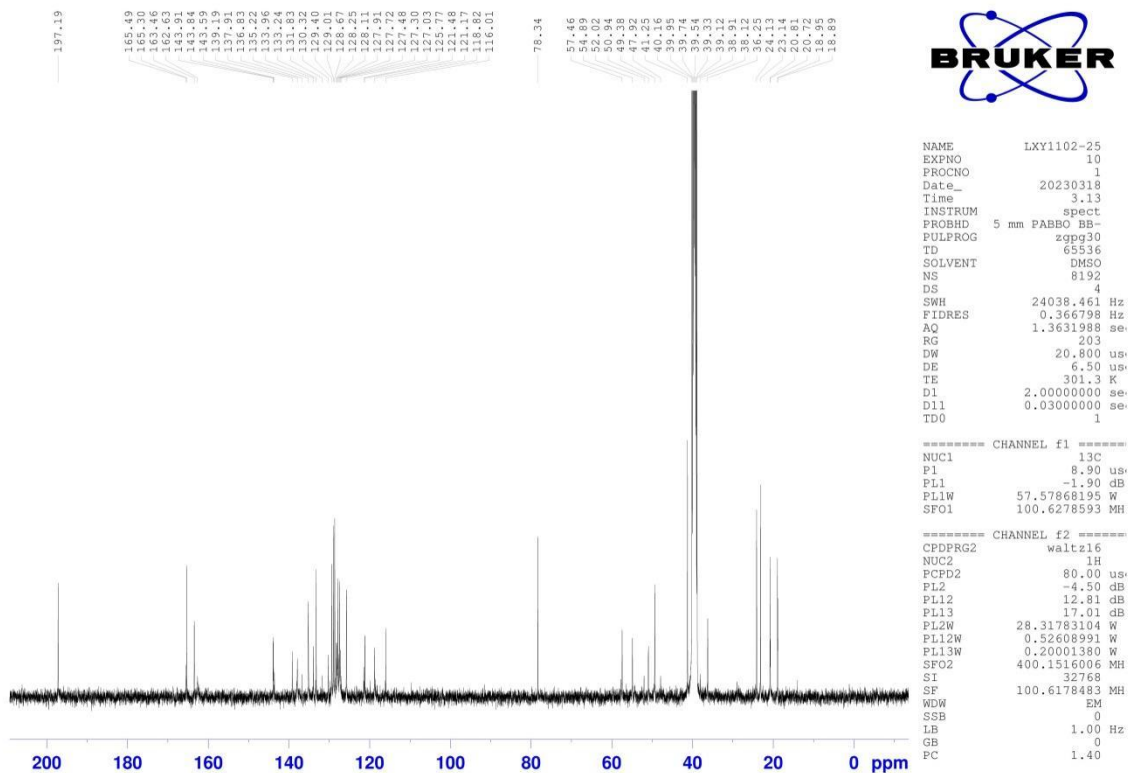
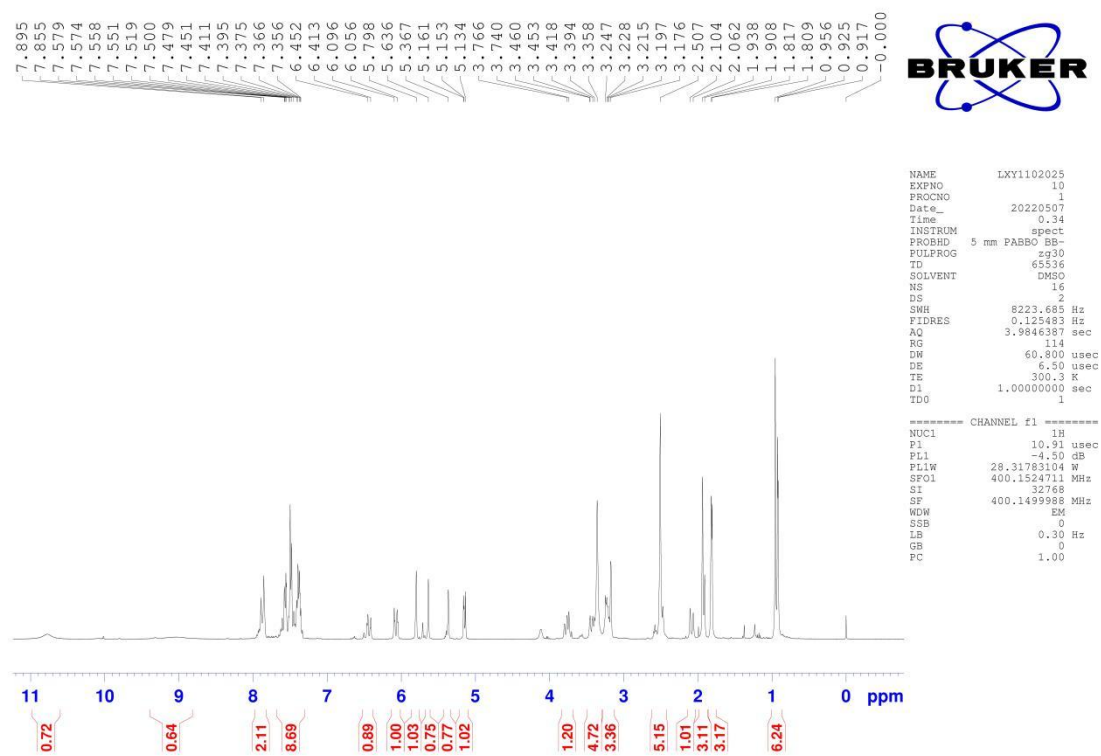


Figure S9. ^1H , ^{13}C NMR spectrum of compound 12i.

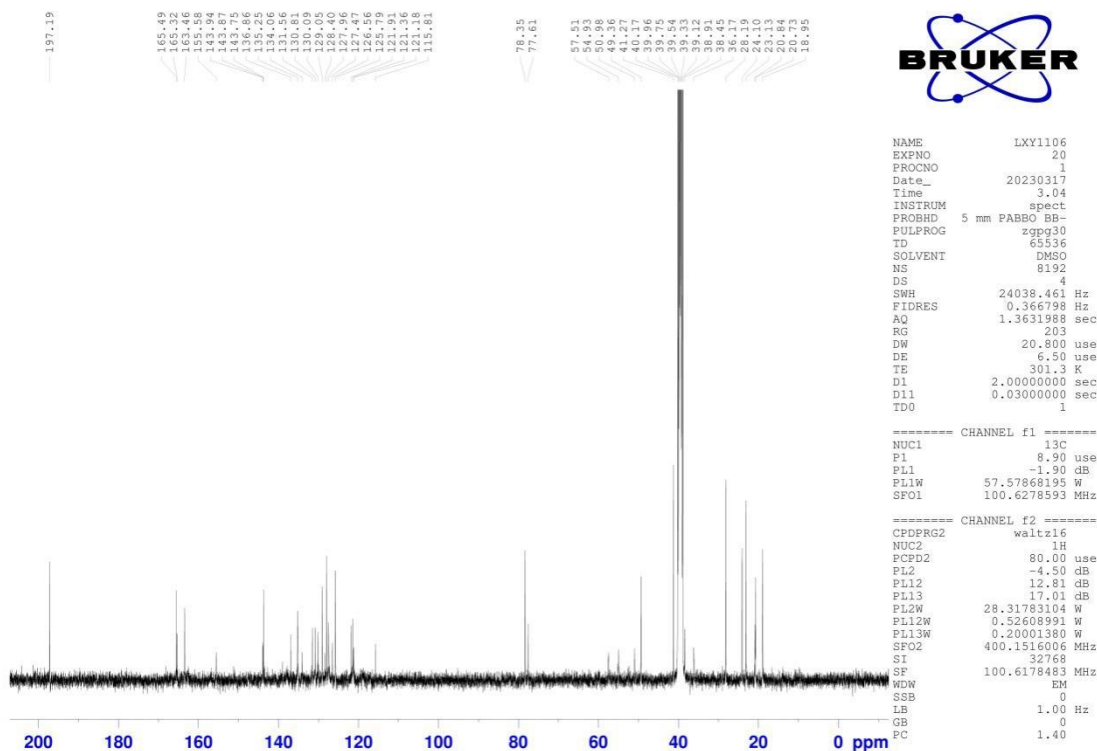
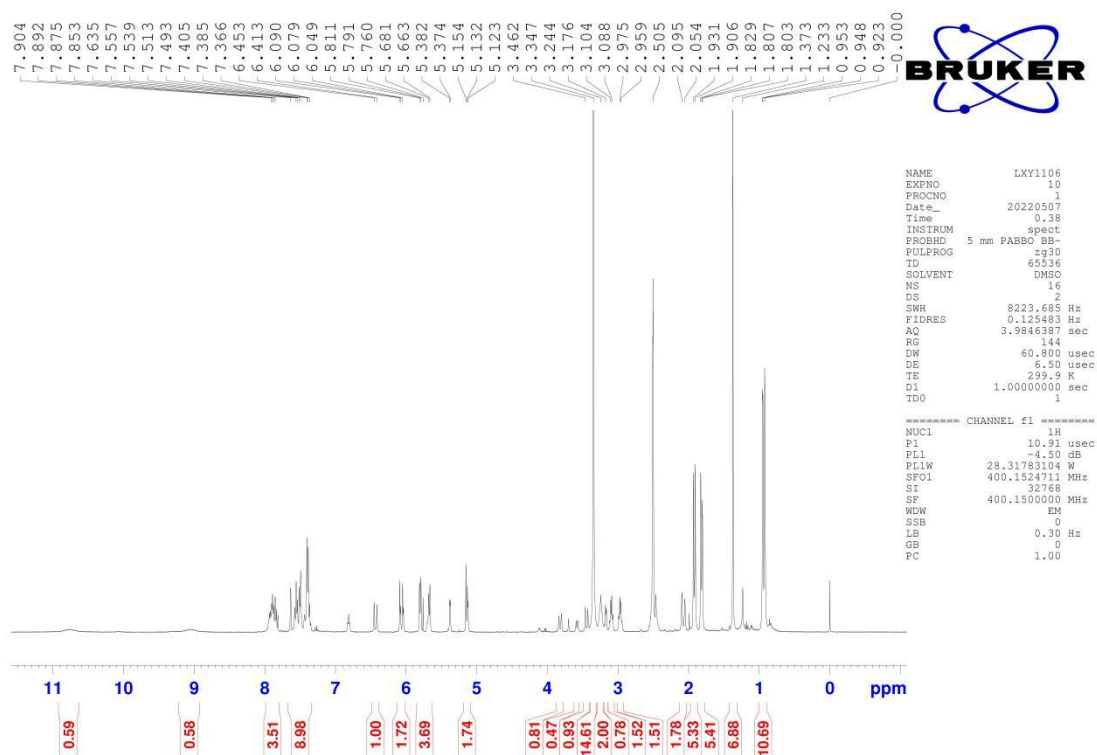


Figure S10. ¹H, ¹³C NMR spectrum of compound 12j.

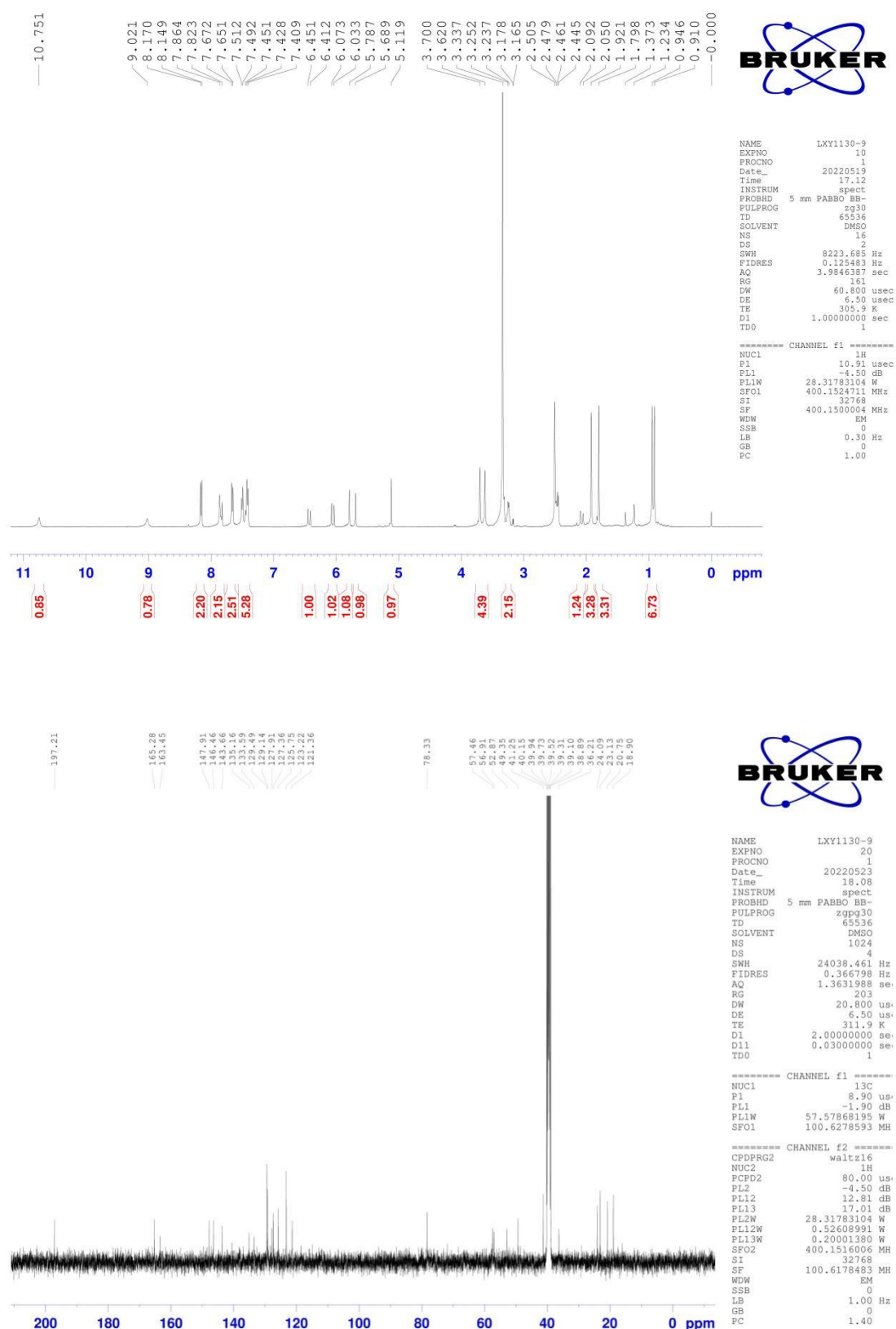


Figure S11. ¹H, ¹³C NMR spectrum of compound 12k.

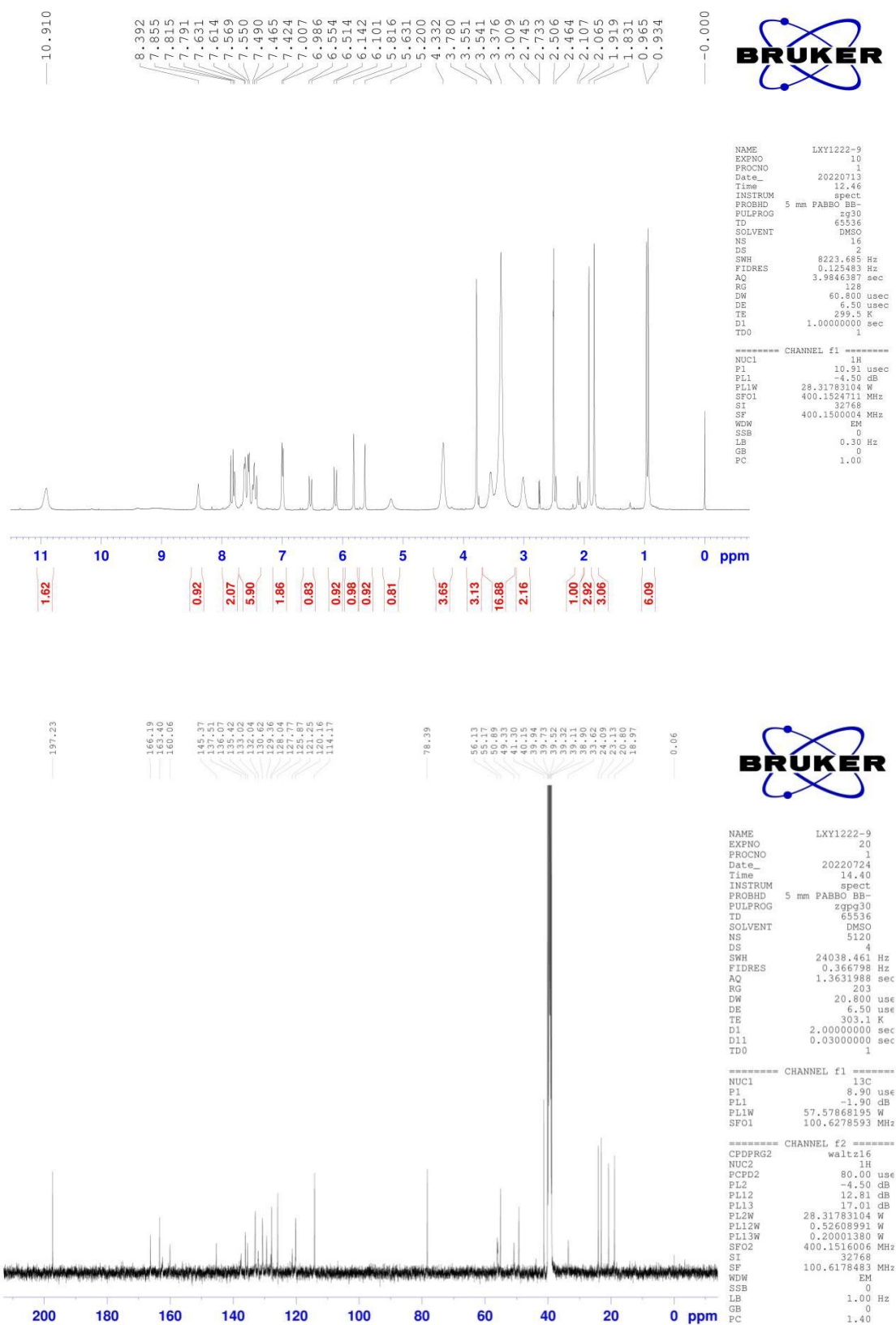


Figure S12. ¹H, ¹³C NMR spectrum of compound 12l.

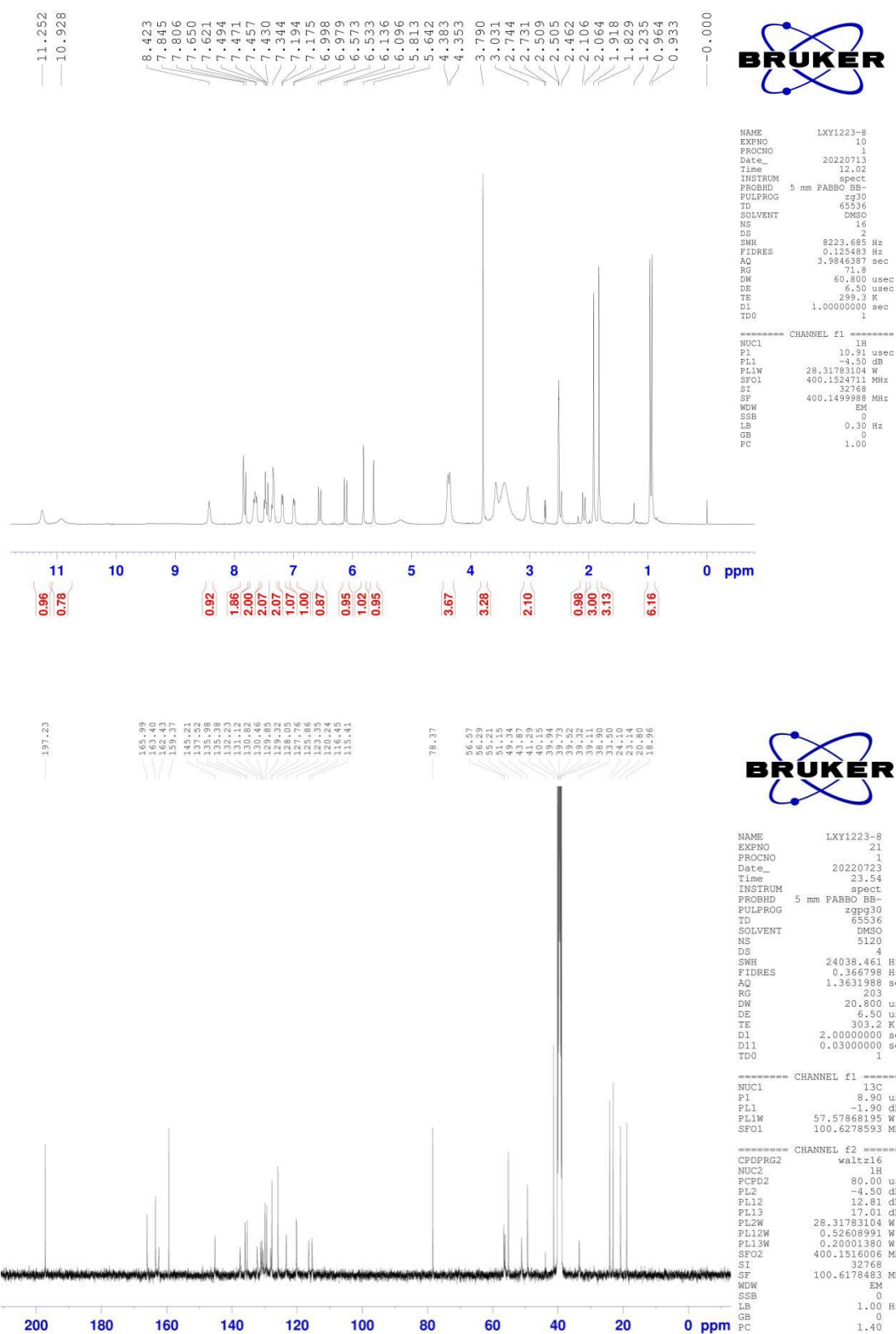


Figure S13. ¹H, ¹³C NMR spectrum of compound 12m.

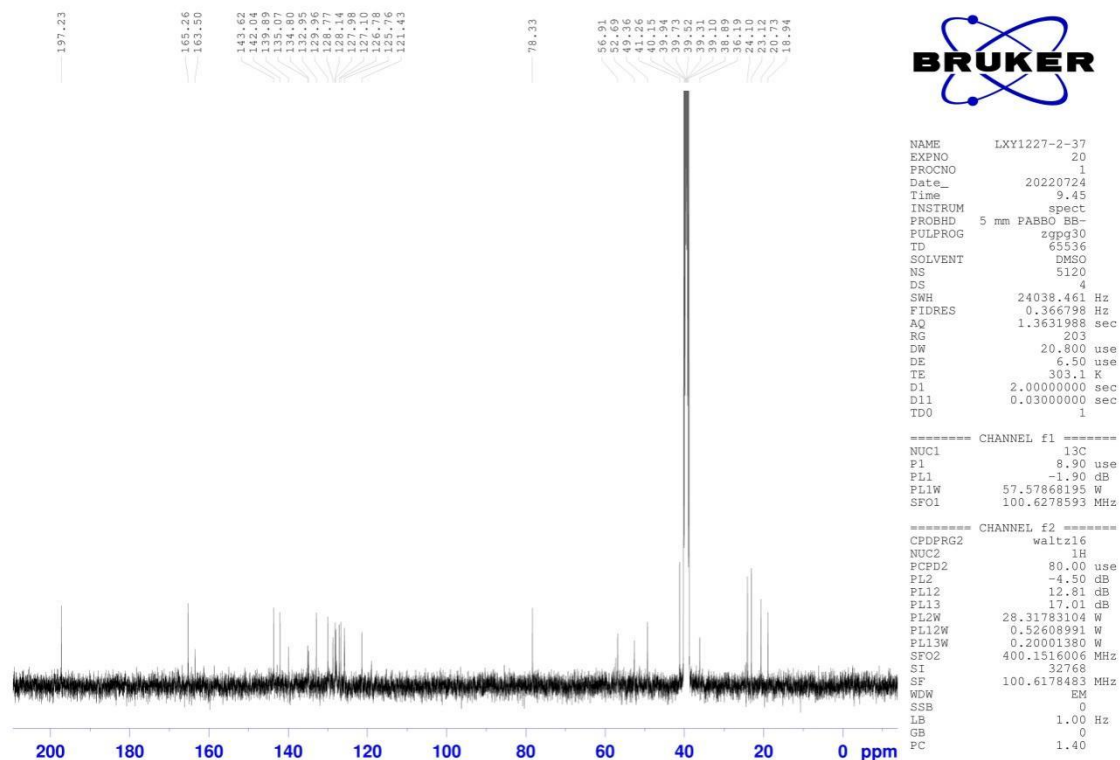
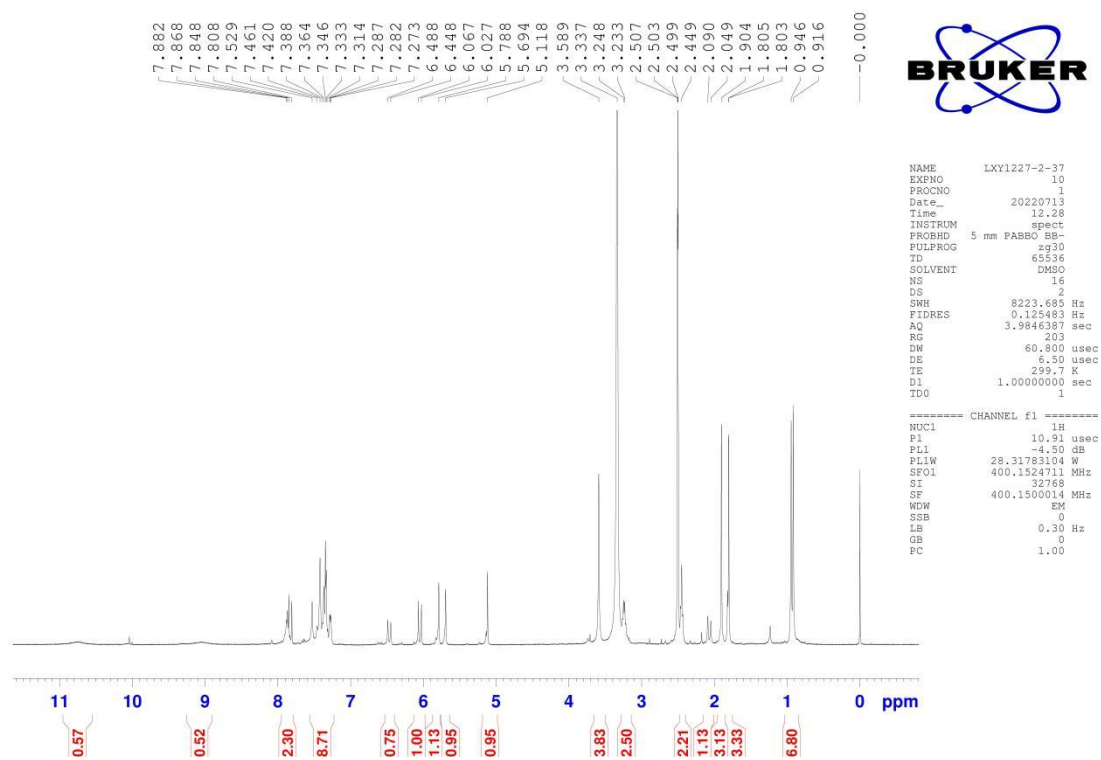


Figure S14. ^1H , ^{13}C NMR spectrum of compound 12n.

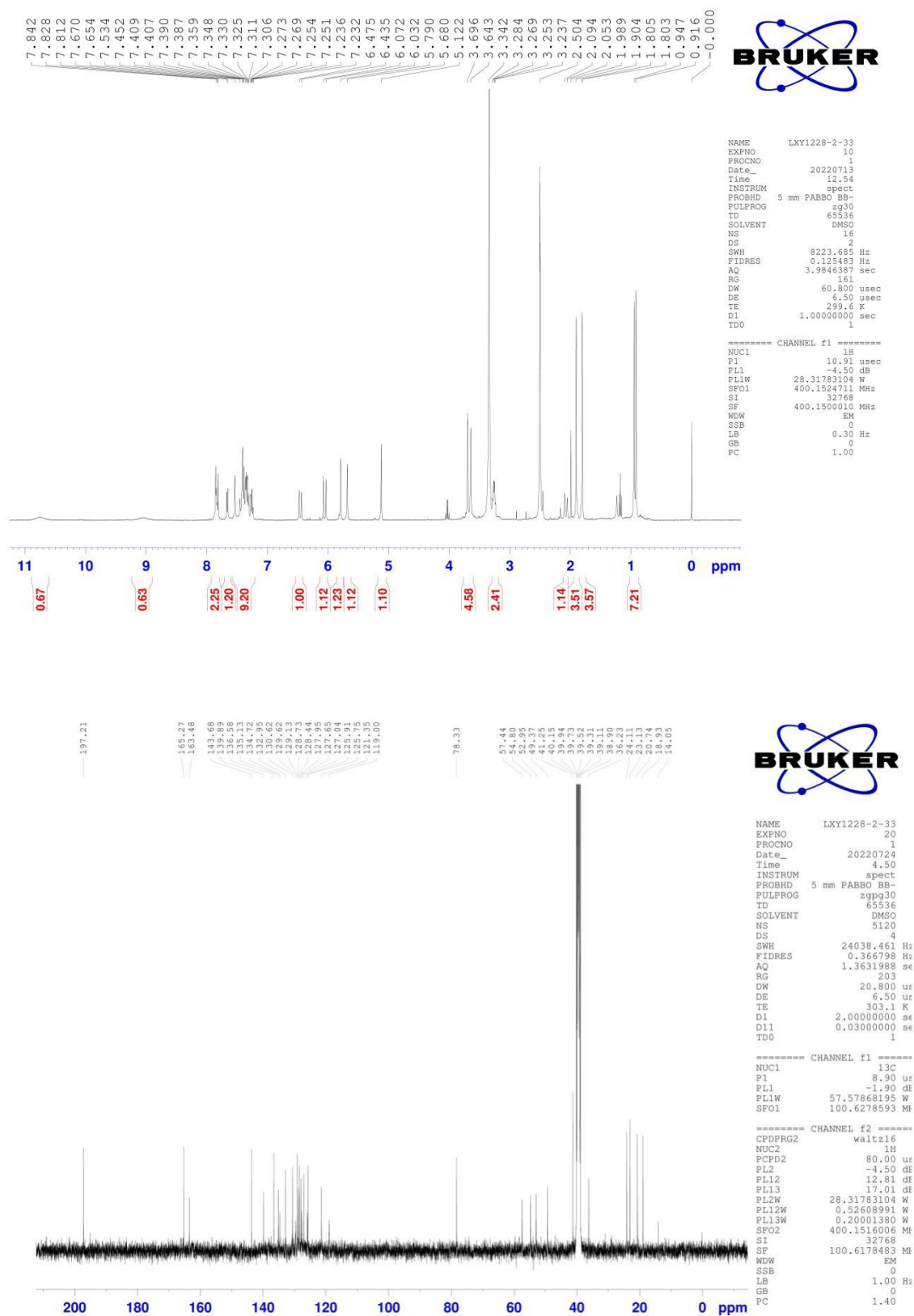


Figure S15. ¹H, ¹³C NMR spectrum of compound 12o.

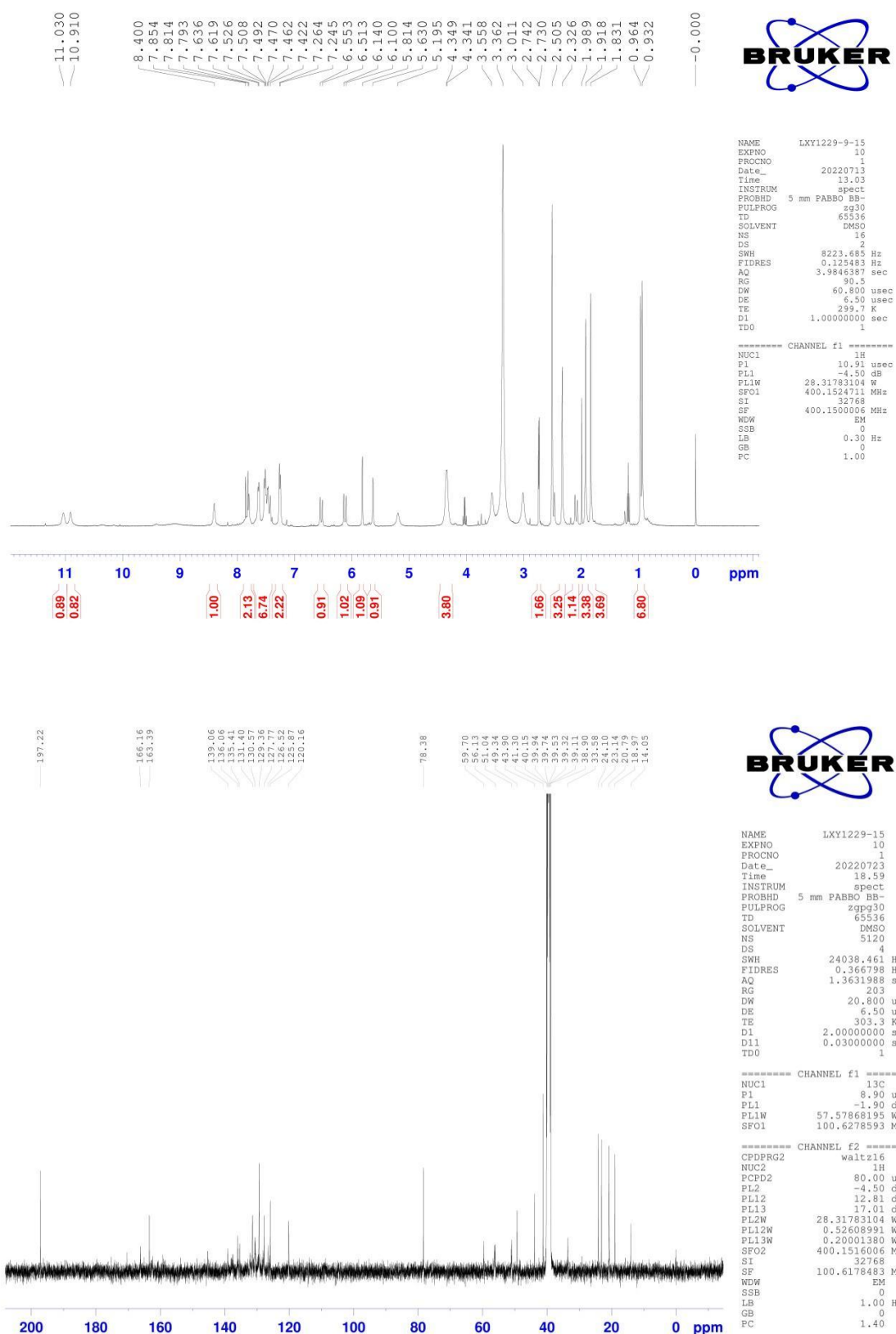


Figure S16. ¹H, ¹³C NMR spectrum of compound 12p.

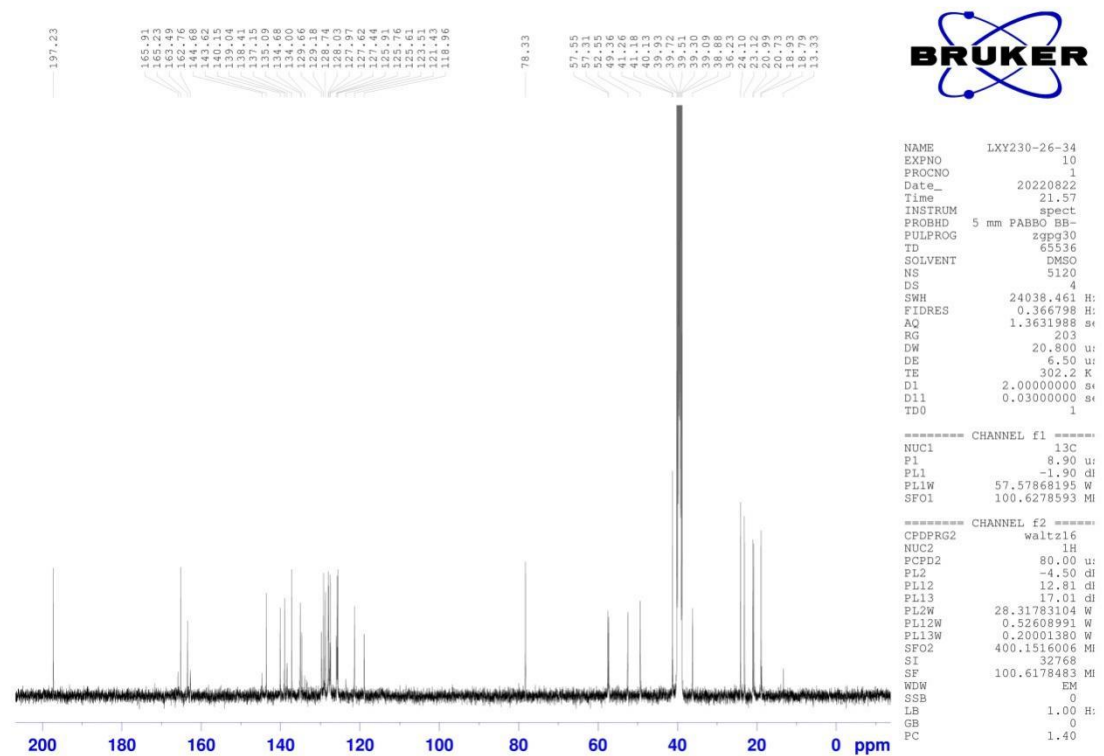
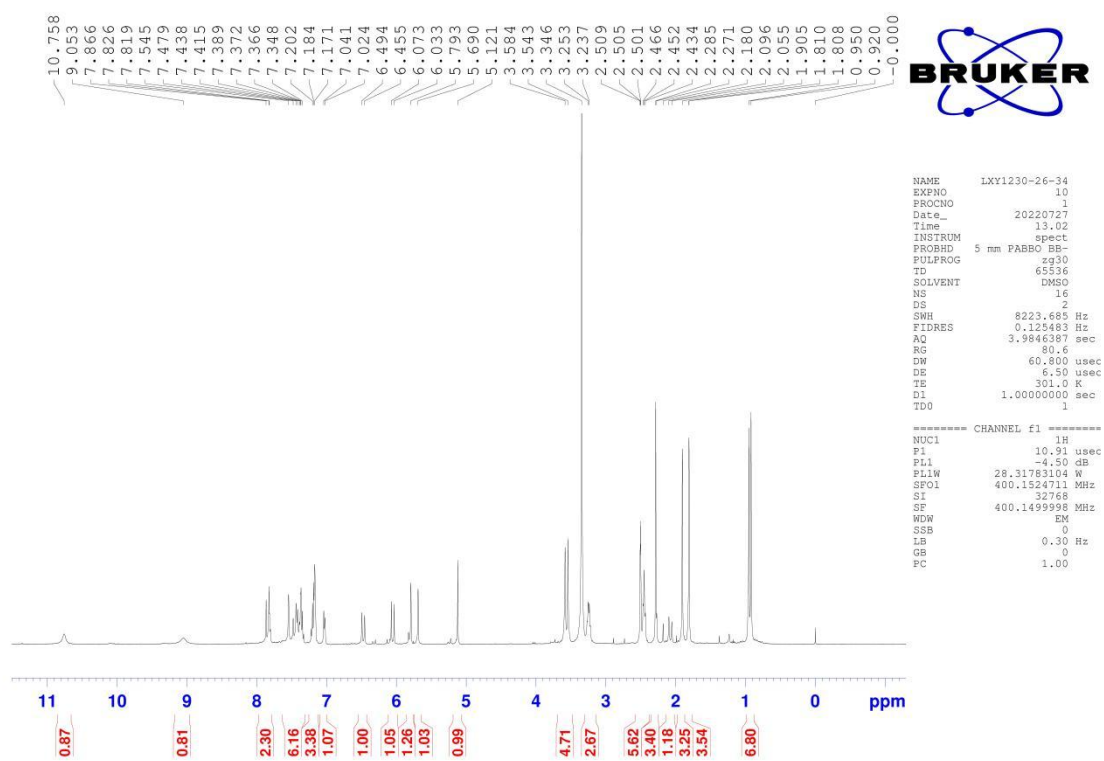


Figure S17. ¹H, ¹³C NMR spectrum of compound 12q.

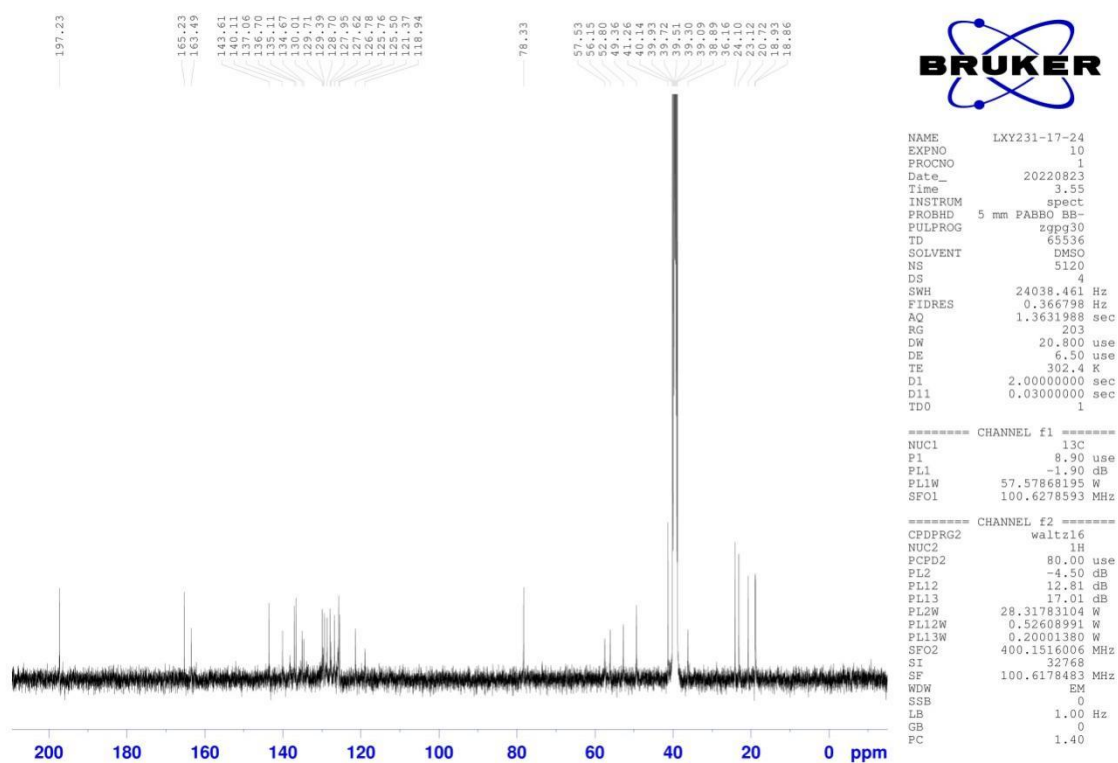
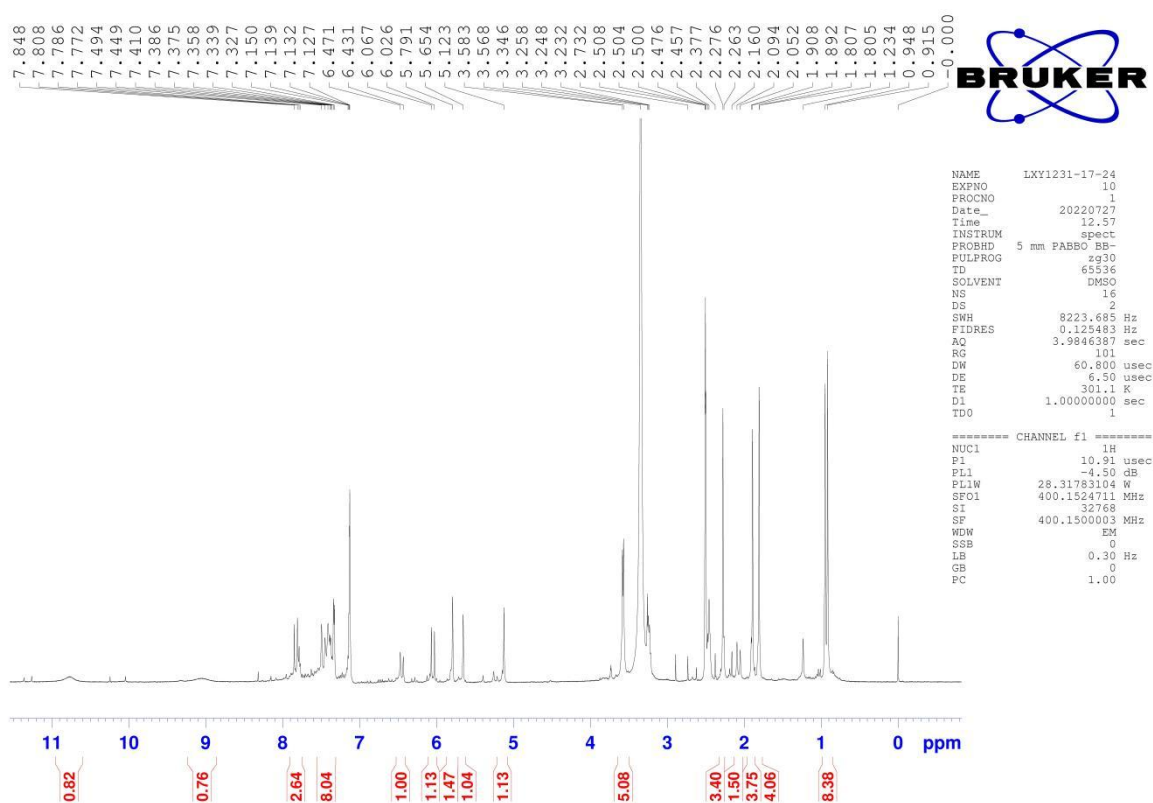


Figure S18. ^1H , ^{13}C NMR spectrum of compound 12r.

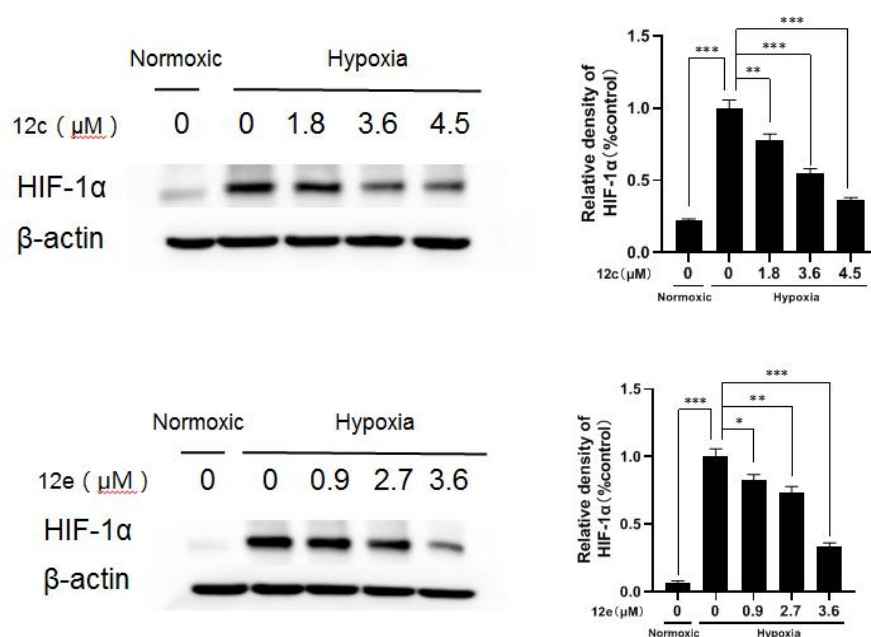


Figure S19. Dose-dependent effects of derivatives 12c and 12e on HIF-1α expression in MDA-MB-231 cells.

Representative Western blot bands (left) and quantitative analysis (right) of HIF-1α protein levels under normoxic and hypoxic conditions. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. ns not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

Note: As the western blot analysis method is a semi-quantitative analysis, it is impossible to calculate the IC50 for a quantitative half-maximal inhibitory concentration. Therefore, in this study, two preferred derivatives with better activity were placed on a single microplate. Using the same batch of cells and the same blank control, the strength of the activity of the two derivatives could be precisely compared.

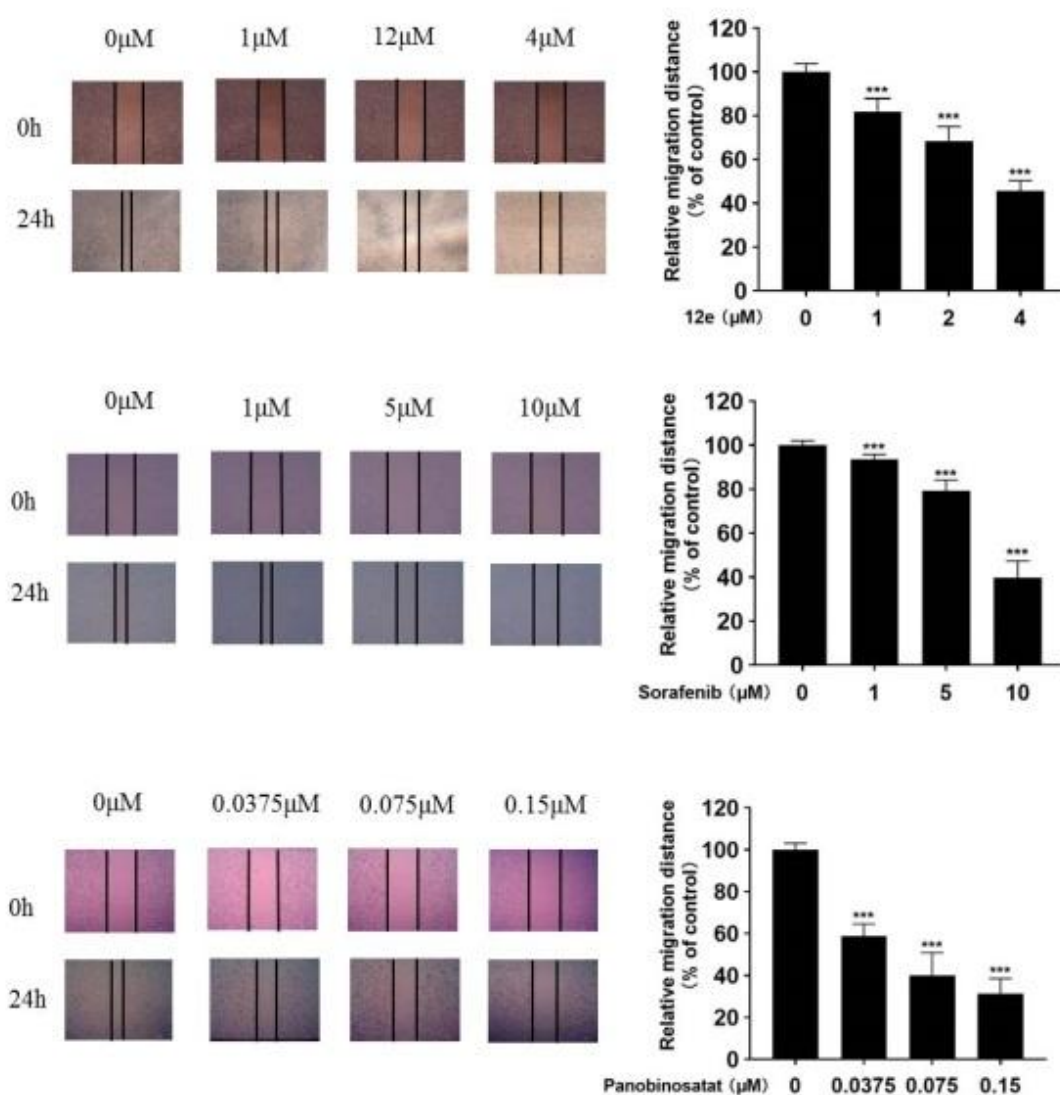


Figure S20. Effects of derivatives 12e, Sorafenib and Panobinostat on the migration of MDA-MB-231 cells.

Representative images of wound healing assays (left) and quantitative analysis of relative migration distance (right) for MDA-MB-231 cells treated with **12e**, Sorafenib, and Panobinostat. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

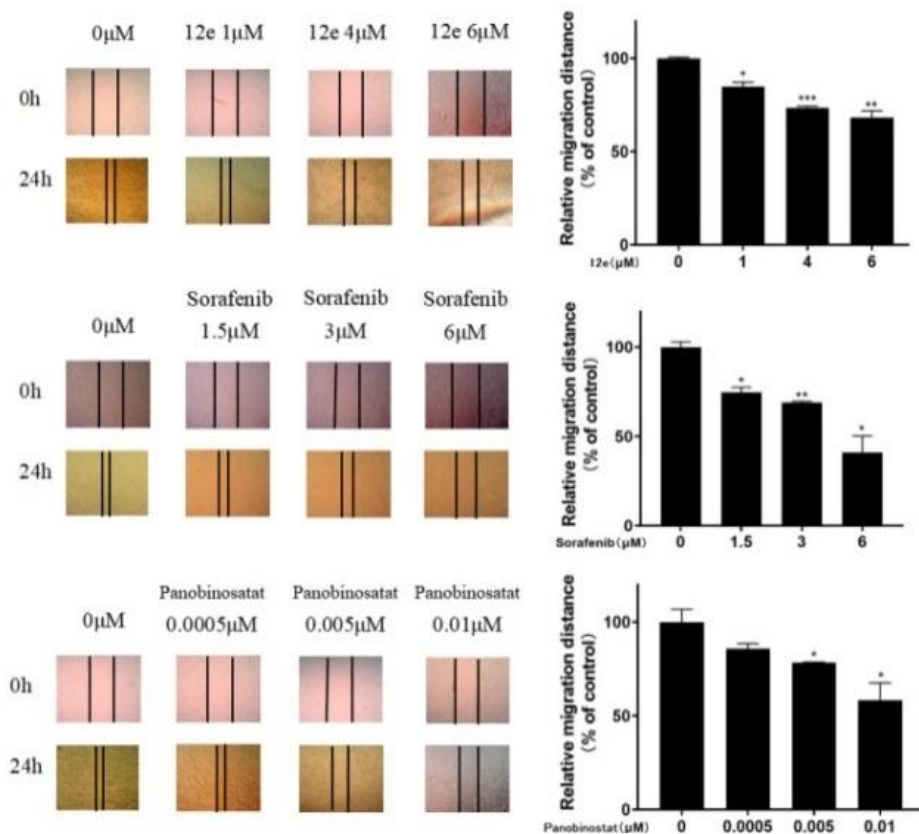


Figure S21. Effects of derivatives 12e, Sorafenib and Panobinostat on the migration of 4T1 cells.

Representative images of wound healing assays (left) and quantitative analysis of relative migration distance (right) for 4T1 cells treated with 12e, Sorafenib, and Panobinostat. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

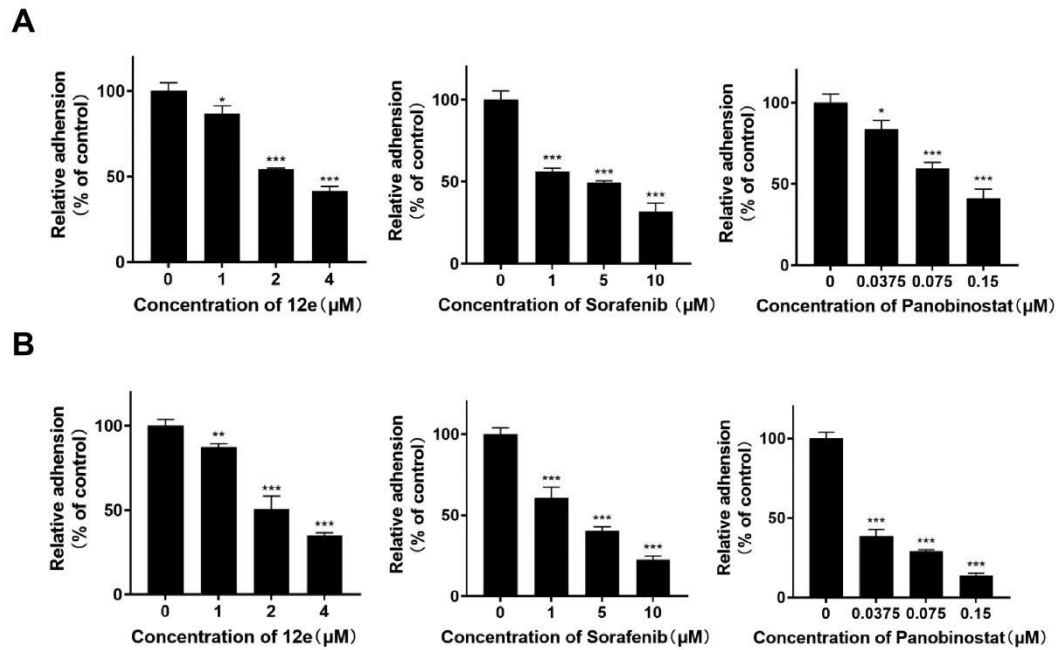


Figure S22. Effects of derivatives 12e, Sorafenib and Panobinostat on cell adhesion.

(A) Quantitative analysis of the adhesion ability of MDA-MB-231 cells (top row). (B) Quantitative analysis of the adhesion ability of 4T1 cells (bottom row). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

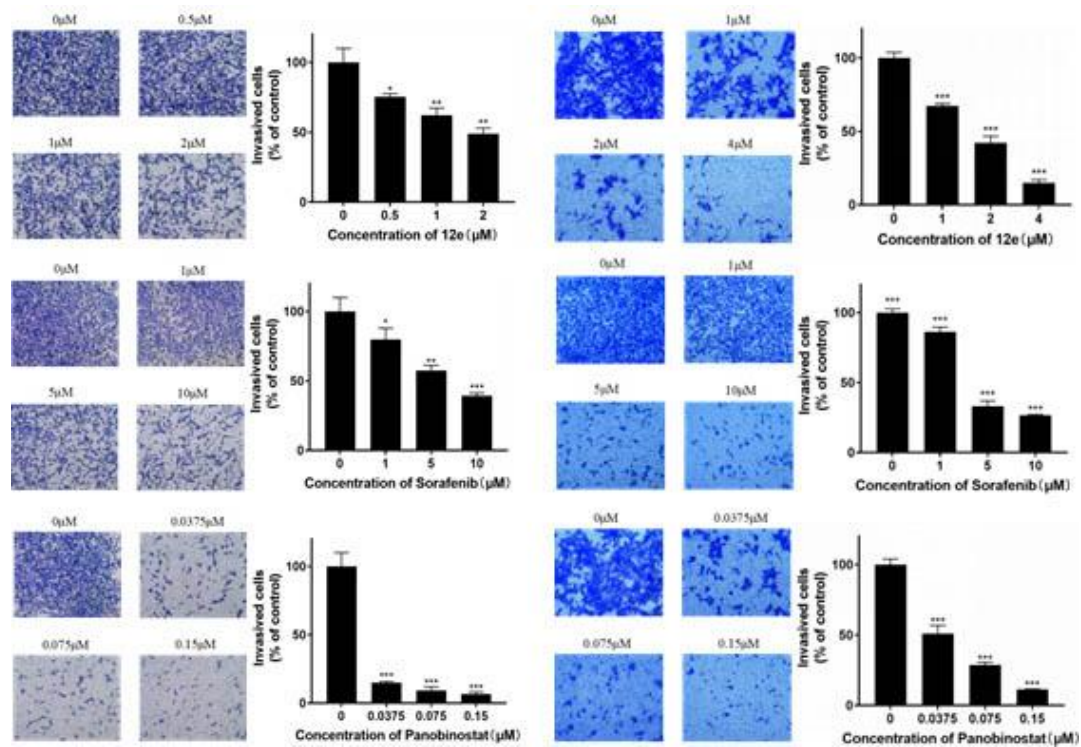
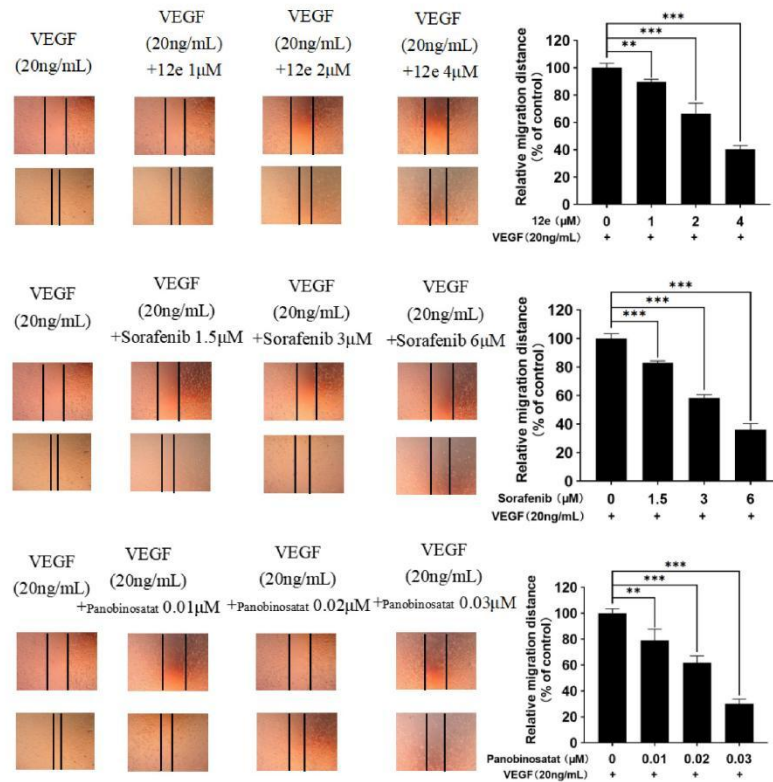


Figure S23. Effects of derivatives 12e, Sorafenib and Panobinostat on cell invasion.

Representative images of Transwell invasion assays (left) and quantitative analysis of invaded cells (right) for MDA-MB-231 cells (top panels) and 4T1 cells (bottom panels). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

A



B

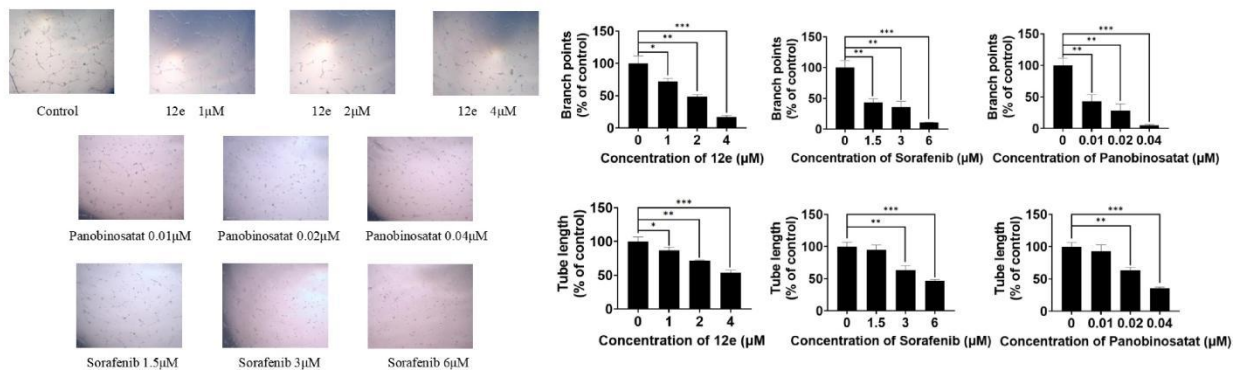


Figure S24 Effects of derivatives **12e**, sorafenib, and Panobinostat on migration and tube formation in HUVECs.

(A) Representative images of tube formation assays for cells treated with 12e (top), Panobinostat (middle), and Sorafenib (bottom). (B) Quantitative analysis of branch points (left column) and tube length (right column). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

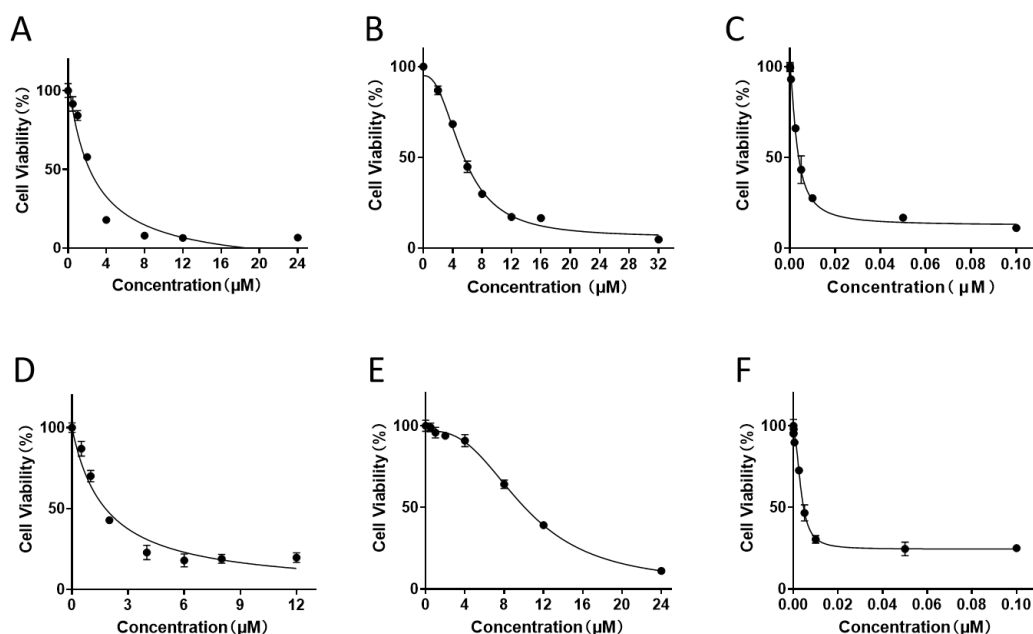


Figure S25. The growth inhibitory effects of derivative 12e, Sorafenib, and Panobinostat on breast cancer cells.

(A–C) Cell viability curves of 4T1 cells treated with 12e (A), Sorafenib (B), and Panobinostat (C).

(D–F) Cell viability curves of MDA-MB-231 cells treated with 12e (D), Sorafenib (E), and Panobinostat (F). The IC₅₀ value showing as follows: 12e, 2.51 ± 0.01 μM (4T1) and 1.85 ± 0.08 μM (MDA-MB-231); Sorafenib, 5.54 ± 0.17 μM (4T1) and 10.08 ± 0.18 μM (MDA-MB-231); Panobinostat, 0.0045 ± 0.0006 μM (4T1) and 0.0035 ± 0.0001 μM (MDA-MB-231). Data are presented as mean ± SD from three biological replicates (n = 6) and were analyzed using two-sample, two-tailed unpaired Student's *t*-test. ns not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

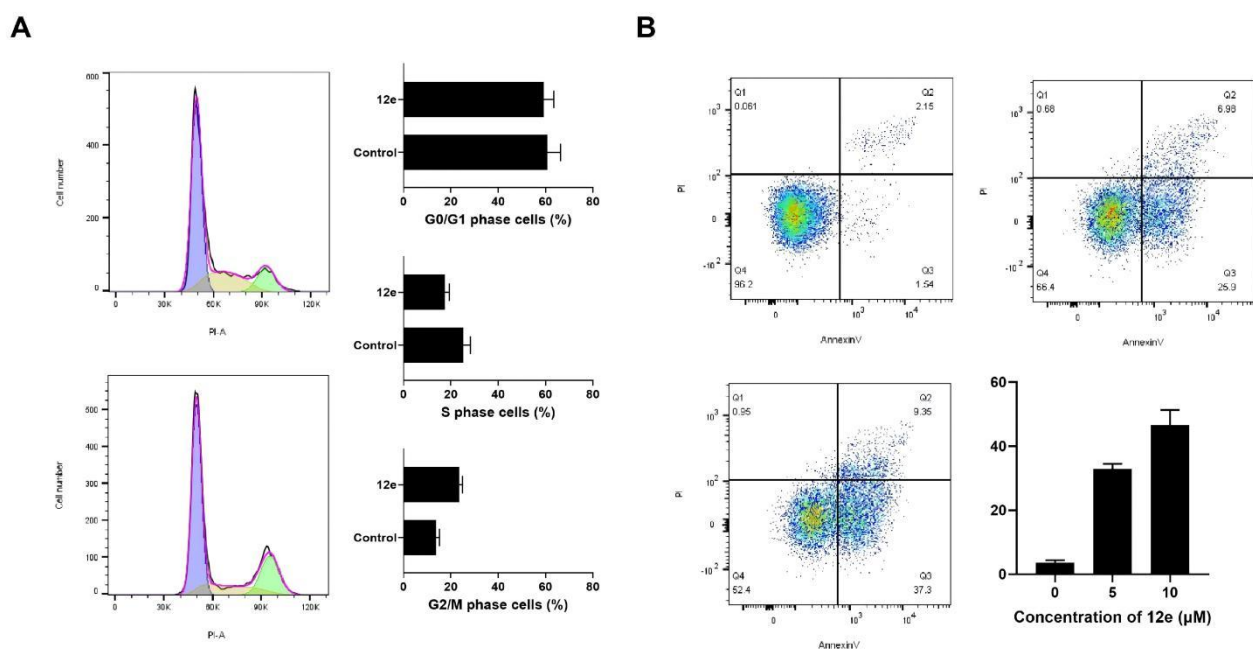


Figure S26. Effects of derivative 12e on cell cycle and apoptosis in MDA-MB-231 cells.

(A) Representative flow cytometry plots (left) and quantitative analysis (right) of cell cycle distribution (G0/G1, S, and G2/M phases). (B) Representative flow cytometry plots (left) and quantitative analysis (right) of cell apoptosis visualized by Annexin V-FITC/PI staining. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. ns not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

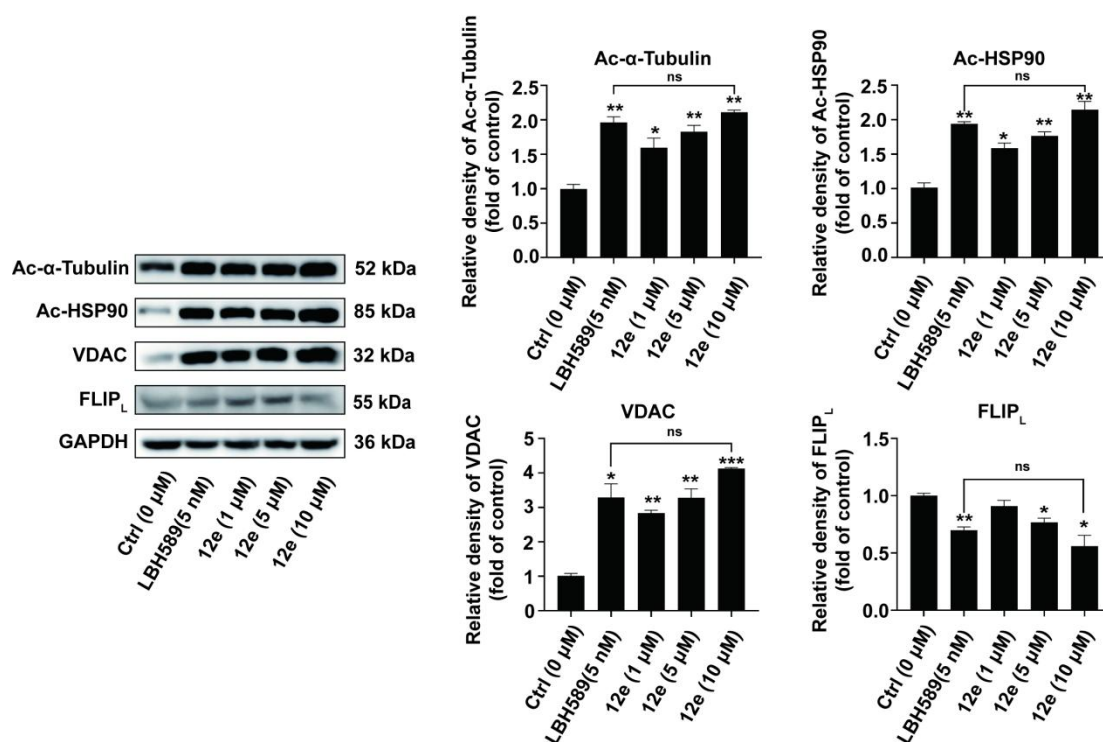


Figure S27. Effects of derivative 12e on the protein expression of Ac- α -tubulin, AC-HSP90, VDAC, and FLIP_L in MDA-MB-231 cells.

Representative western blot bands (left) and quantitative analysis (right) of the protein levels of Ac- α -tubulin, Ac-HSP90, VDAC, and FLIP_L. Data are presented as the means $\pm$ SDs from three biological replicates and were analysed using a two-sample, two-tailed, unpaired Student's *t* test. Compared with the control group, ns indicates not significant, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; compared with the LBH589 group, ns indicates not significant, #*P* < 0.05, ##*P* < 0.01 and ###*P* < 0.001.

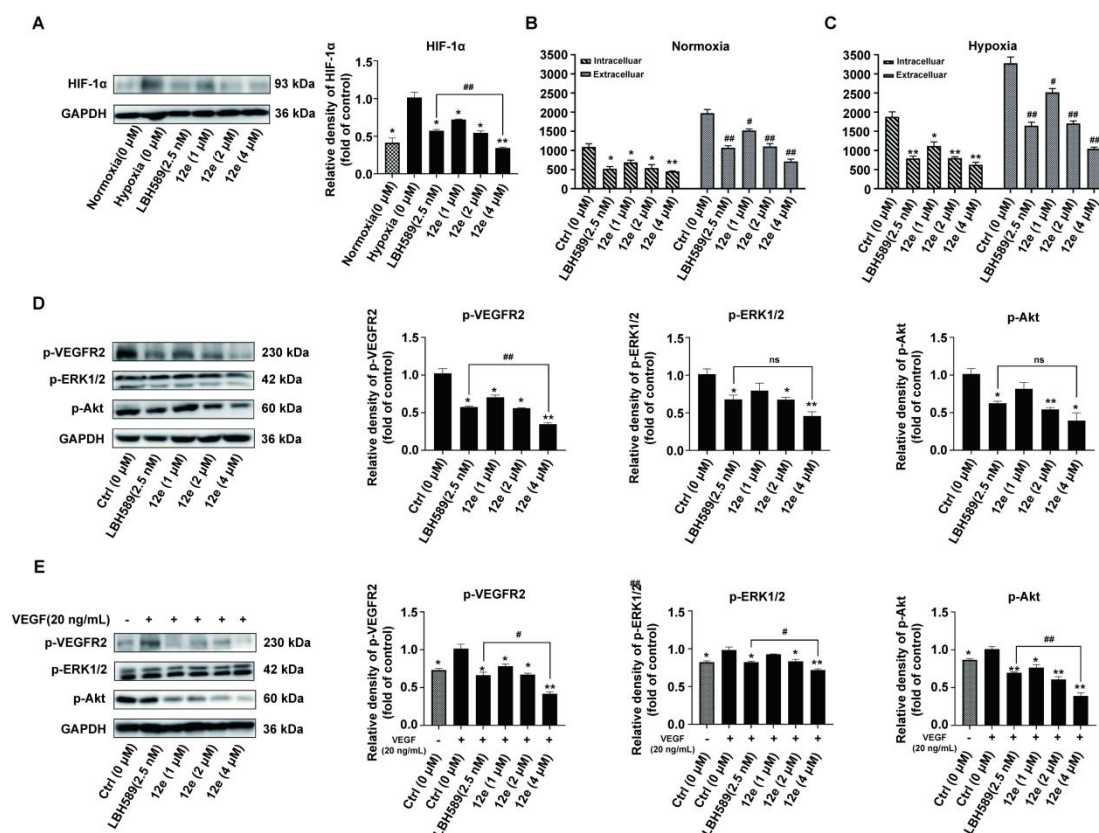


Figure S28. Effects of derivative 12e on the HIF-1α/VEGF/VEGFR2 signalling pathway in MDA-MB-231 cells and VEGF-induced HUVECs.

(A) Western blot analysis of HIF-1α protein levels. (B, C) ELISA analysis of intracellular and extracellular VEGF concentrations under normoxia (B) and hypoxia (C). (D, E) Representative western blot bands (left) and quantitative analysis (right) of p-VEGFR2, p-ERK1/2, and p-Akt protein levels in MDA-MB-231 cells (D) and VEGF-induced HUVECs (E). Data are presented as the means $\pm$ SDs from three biological replicates and were analysed using a two-sample, two-tailed, unpaired Student's *t* test. Compared with the Hypoxia, Intracellular Ctrl, Ctrl or Ctrl with VEGF groups, ns indicates not significant, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; Compared with the LBH589, Extracellular Ctrl or LBH589

with VEGF groups, ns indicates not significant, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

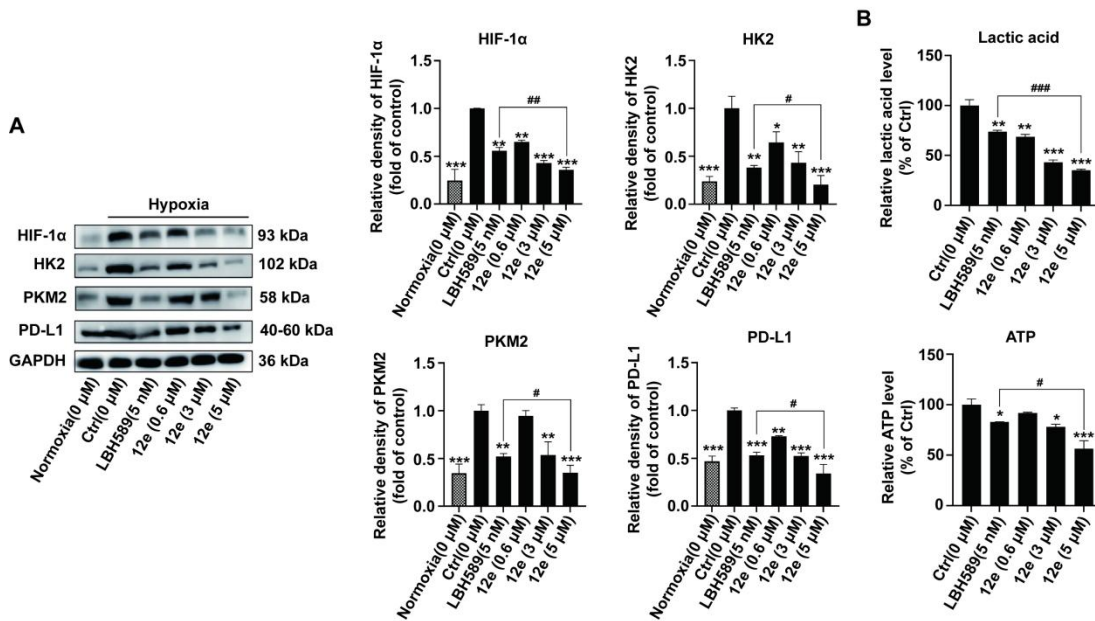


Figure S29. Effects of derivative 12e on the expression of glycolysis-related proteins and metabolites in MDA-MB-231 cells under hypoxia.

(A) Representative western blot bands (left) and quantitative analysis (right) of HIF-1 α , HK2, PKM2, and PD-L1 protein levels. (B) Intracellular lactic acid and ATP levels under hypoxia. Data are presented as the means $\pm$ SDs from three biological replicates and were analysed using a two-sample, two-tailed, unpaired Student's t test. Compared with the Hypoxia Ctrl group, ns indicates not significant, $^{\ast}P < 0.05$, $^{\ast\ast}P < 0.01$ and $^{\ast\ast\ast}P < 0.001$; compared with the Hypoxia LBH589 group, ns indicates not significant, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$.

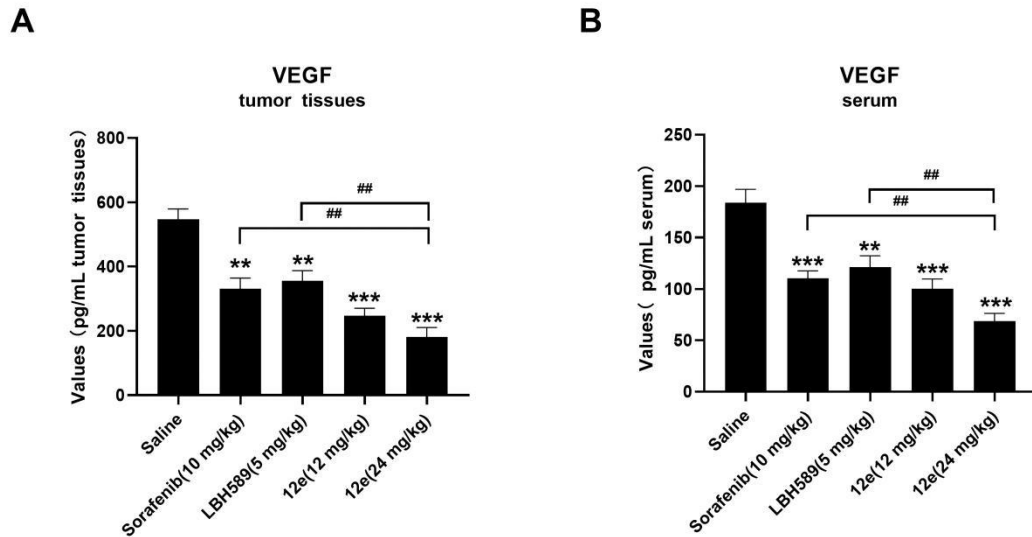
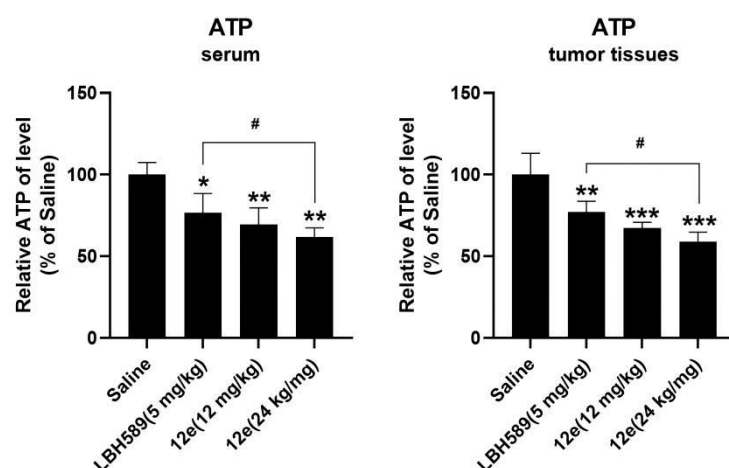


Figure S30. Analysis of VEGF levels in tumor tissues and serum.

(A) Quantification of VEGF protein levels in tumor tissues. (B) Quantification of VEGF protein levels in serum. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using two-sample, two-tailed unpaired Student's *t*-test. Compared with the Saline group, ns not significant, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; Compared with the Sorafenib group, ns not significant, #*P* < 0.05, ##*P* < 0.01 and ###*P* < 0.001.

A



B

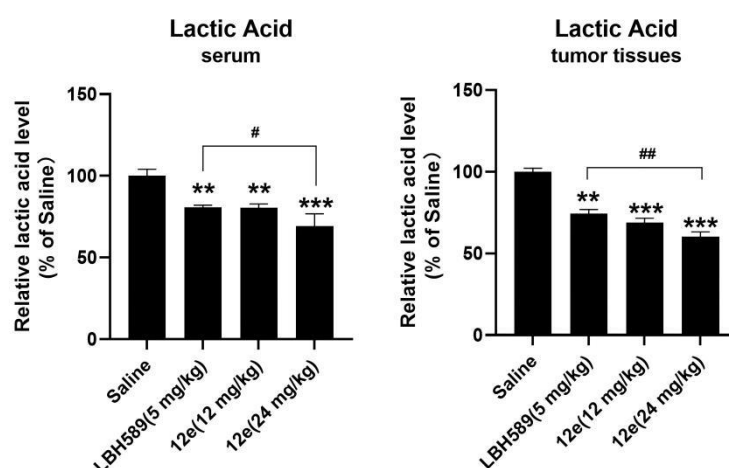
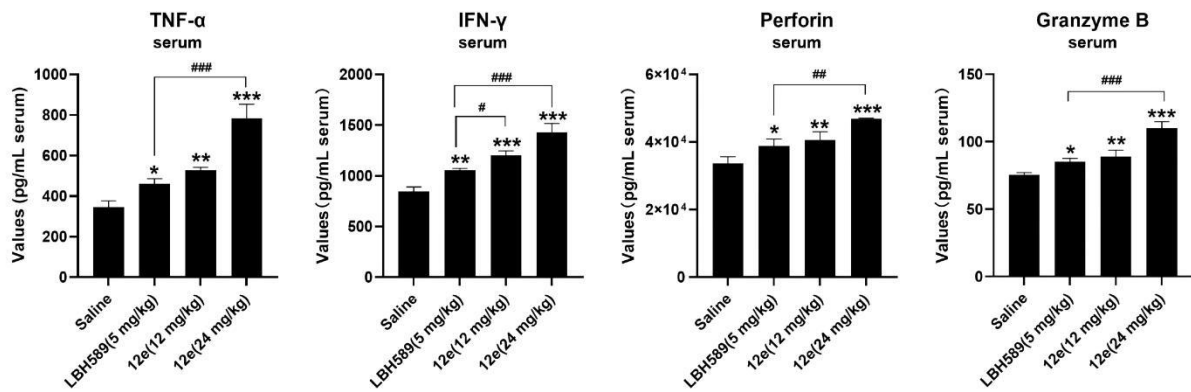


Figure S31. Effects of derivative 12e on metabolic markers in serum and tumor tissues.

(A) Analysis of ATP levels in serum (left) and tumor tissues (right). (B) Analysis of lactic acid levels in serum (left) and tumor tissues (right). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. Compared with the Saline group, ns not significant, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; compared with the LBH589 group, ns not significant, #*P* < 0.05, ##*P* < 0.01 and ###*P* < 0.001.

A



B

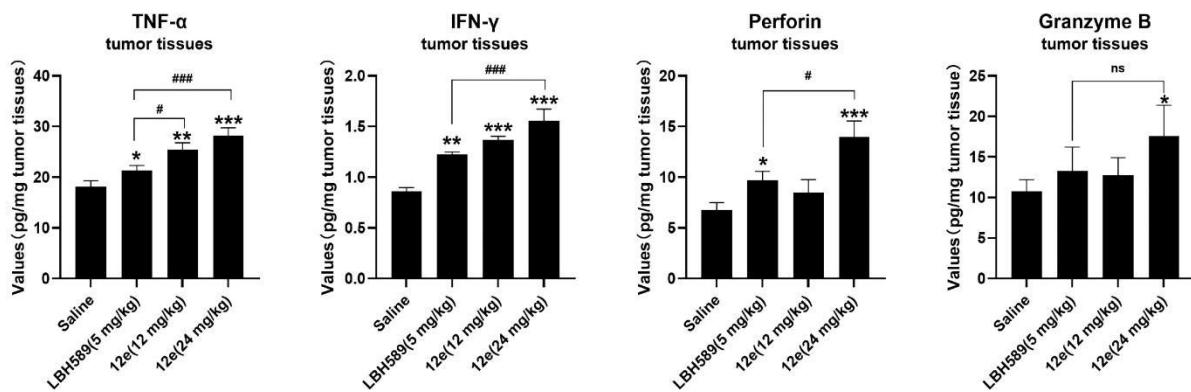


Figure S32. Analysis of cytokine and cytotoxic granule levels in serum and tumor tissues.

(A, B) Quantification of TNF- α , IFN- γ , Perforin, and Granzyme B levels in serum (A) and tumor tissues (B). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. Compared with the Saline group, ns not significant, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; compared with the LBH589 group, ns not significant, #*P* < 0.05, ##*P* < 0.01 and ###*P* < 0.001.

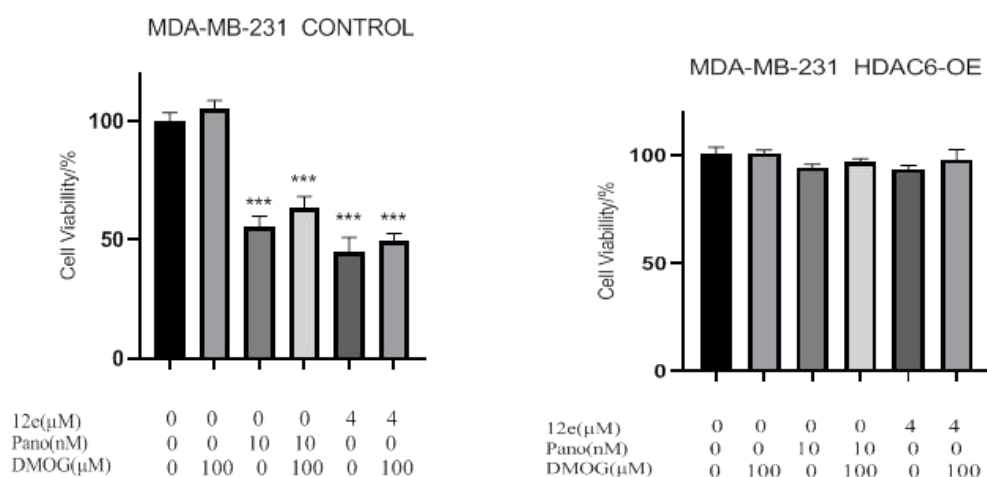
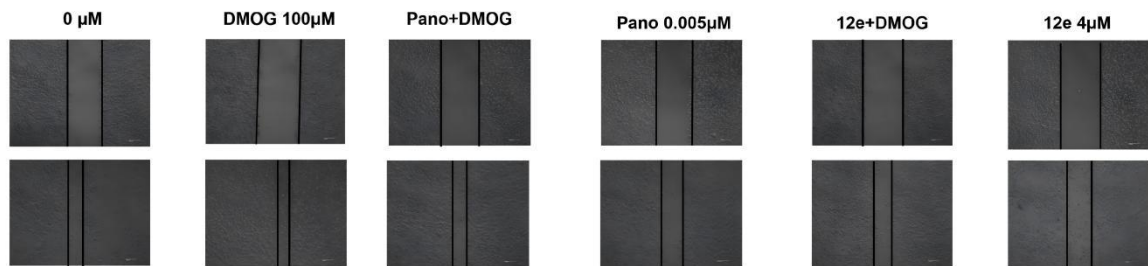


Figure S33. Effects of 12e on the viability of MDA-MB-231 cells under DMOG intervention or HDAC6 overexpression.

(A) Cell viability of MDA-MB-231 Control cells treated with 12e, Panobinostat, or DMOG. (B) Cell viability of HDAC6-overexpressing (HDAC6-OE) MDA-MB-231 cells under the same treatment conditions. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

A



B

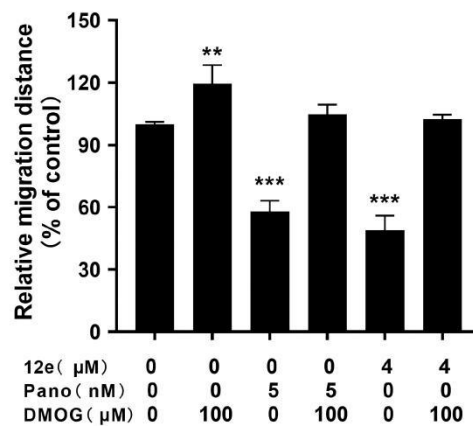
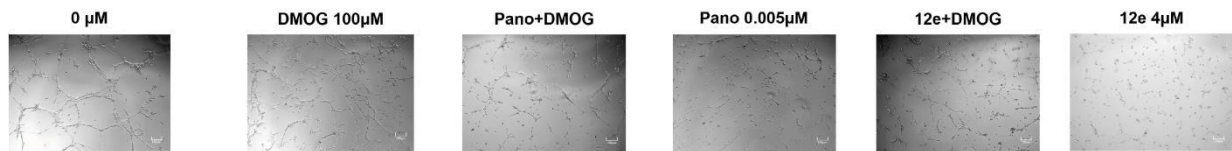


Figure S34. Effects of 12e on the migration of MDA-MB-231 cells under DMOG intervention. (A) Representative images of wound healing assays. (B) Quantitative analysis of relative migration distance. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001.

A



B

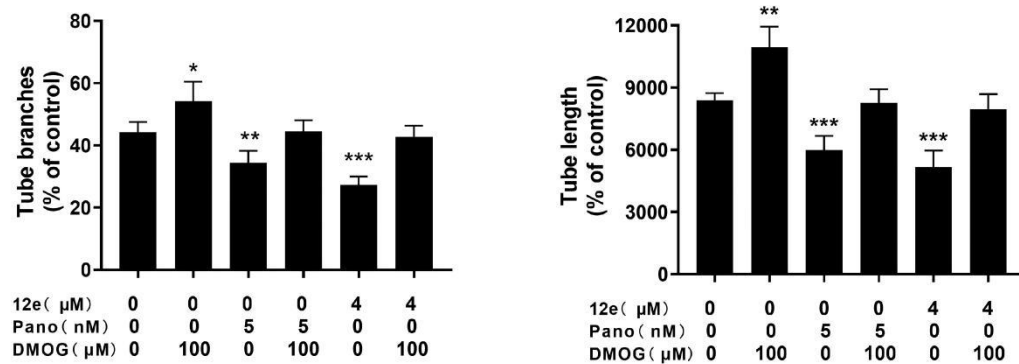


Figure S35. Effects of 12e on tube formation of HUVECs under DMOG intervention.

(A) Representative images of tube formation assays. (B) Quantitative analysis of tube branches (left) and tube length (right). Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

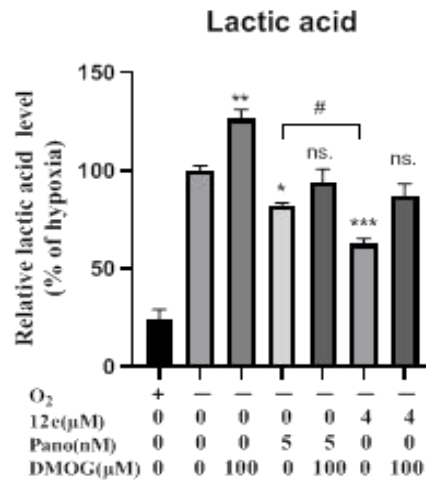


Figure S36. Effects of 12e on lactic acid production in MDA-MB-231 cells under hypoxia and DMOG intervention.

Quantitative analysis of relative lactic acid levels. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. Compared with the Ctrl group, ns not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001; compared with the Panobinostat group, ns not significant, #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 and ####*P* < 0.0001.

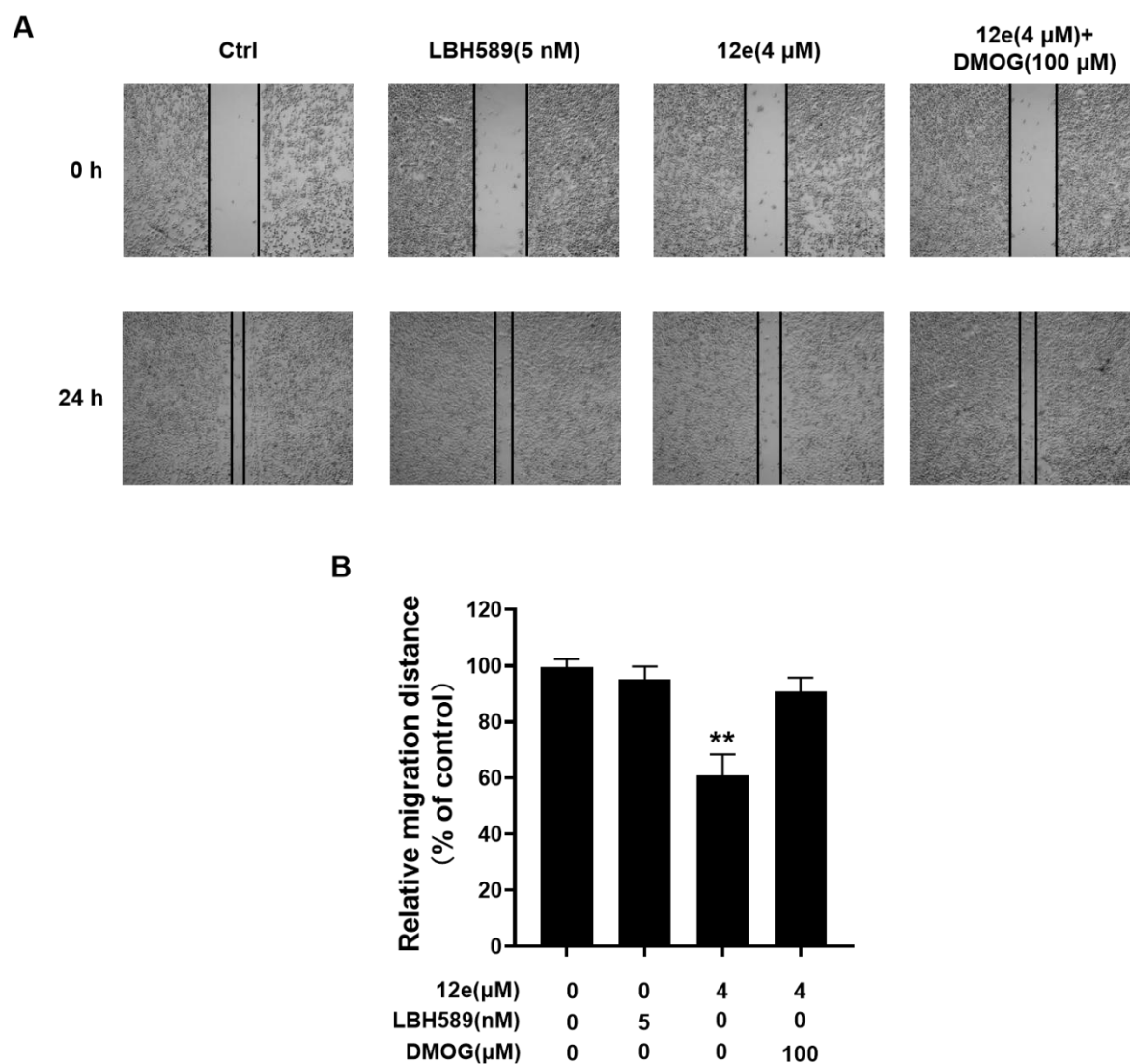


Figure S37. Effects of LBH589 and 12e on the migration of HDAC6-overexpressing (HDAC6-OE) MDA-MB-231 cells under DMOG intervention. (A) Representative images of wound healing assays. (B) Quantitative analysis of relative migration distance. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using two-sample, two-tailed unpaired Student's t-test. * P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

Table S1. Inhibitory effects of derivatives on the proliferation of MDA-MB-231 cancer cells and MCF10A normal cells (n = 3).

Compounds	IC ₅₀ (μM)	
	MDA-MB-231	MCF-10A
12a	3.86 ± 0.23	12.34 ± 0.55
12b	6.14 ± 0.41	13.12 ± 0.44
12c	2.94 ± 0.22	13.77 ± 0.79
12d	> 20.00	> 20.00
12e	1.85 ± 0.11	16.79 ± 1.01
12f	17.75 ± 0.59	> 20.00
12g	2.97 ± 0.14	14.39 ± 0.277
12h	> 20.00	> 20.00
12i	> 20.00	> 20.00
12j	19.24 ± 1.03	> 20.00
12k	13.14 ± 0.87	> 20.00
12l	13.87 ± 0.93	> 20.00
12m	17.33 ± 0.99	> 20.00.
12n	6.32 ± 0.41	19.24 ± 1.30
12o	10.22 ± 0.67	> 20.00
12p	> 20.00	> 20.00
12q	17.9 ± 1.33	> 20.00
12r	> 20.00	> 20.00

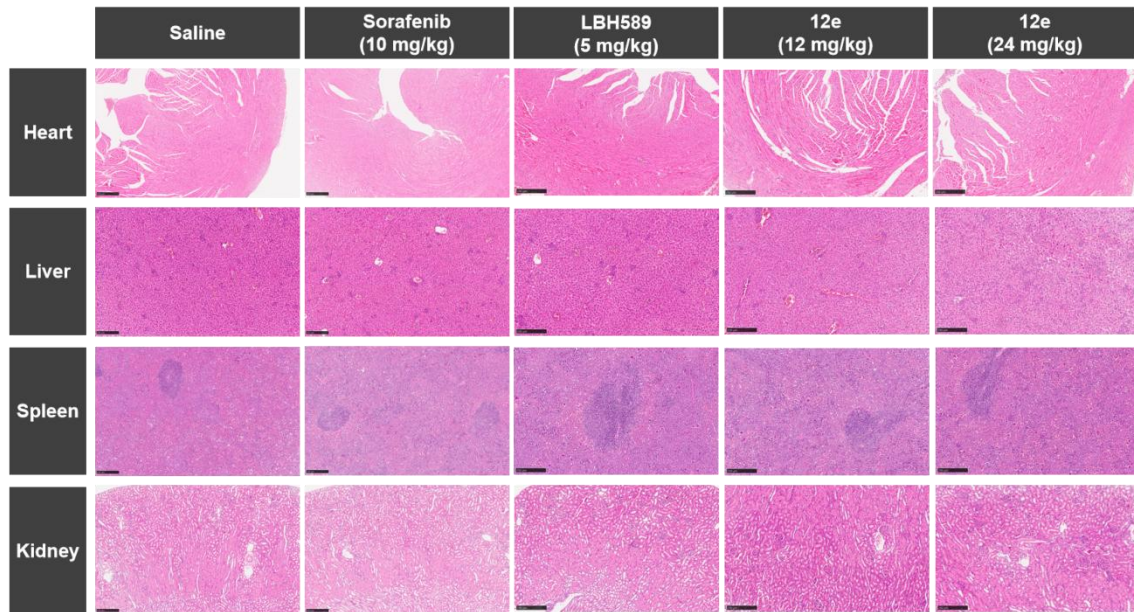


Figure S38. H&E staining of heart, liver, spleen and kidney tissues in each group of mice (100 X).

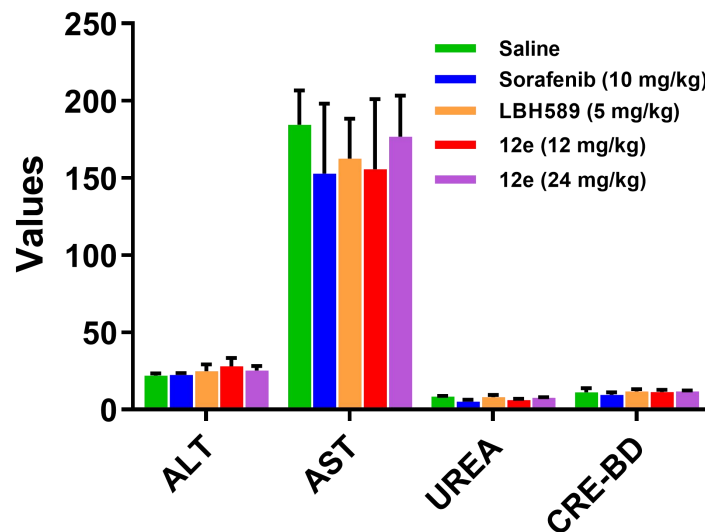


Figure S39. The results of blood biochemical tests for liver and kidney functions in each group of mice.

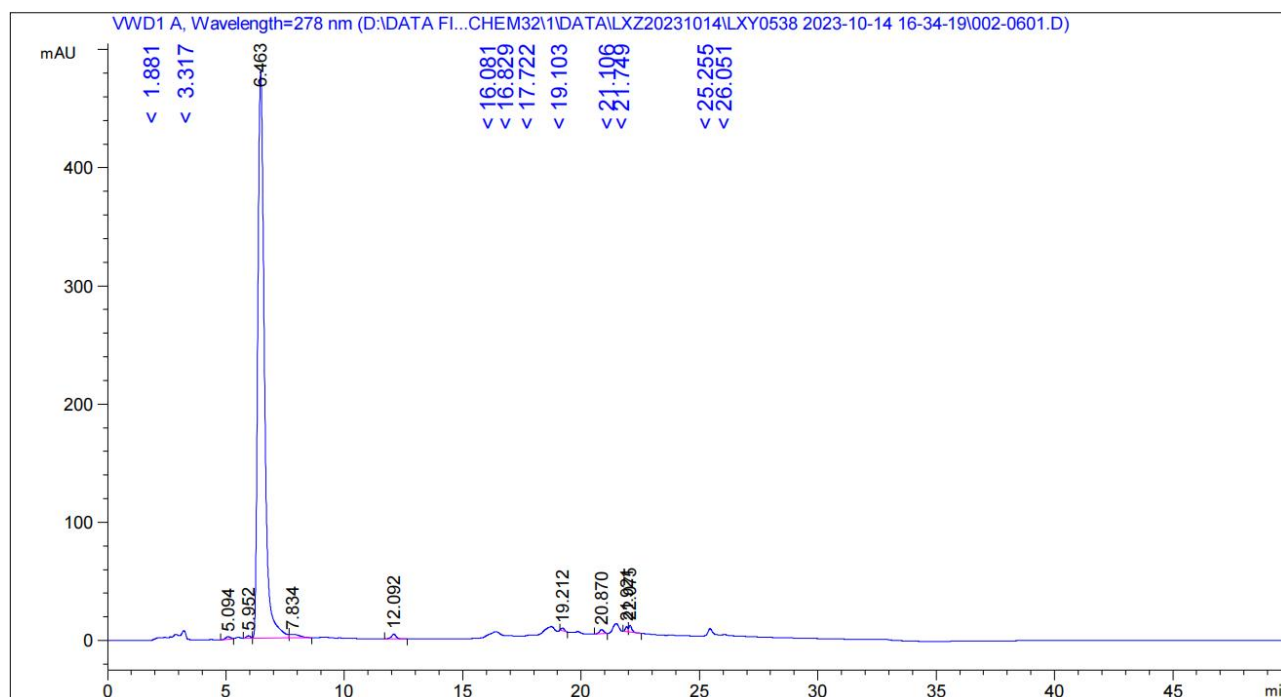


Figure S40. HPLC spectrum of 12e used for *in vivo* study (96.35%).

The purity of 12e was analyzed by HPLC and calculated by peak area normalization method. Column: Agilent Eclipse XDB-C18 250×4.6 mm 5 μm. Wave length: 278 nm. Temperature: 35 °C. Flow rate: 1.0 mL/min. Concentration of compound: 100 μg/mL. Injection volume: 20 μL.

Table S2. The elution timetable of 12e.

Time (min)	Acetonitrile–water (0.5% phosphoric acid, 0.2% sodium dodecyl sulfonate) (V/V)
0.00	30:70
30.00	65:35
30.01	30:70
45.00	30:70

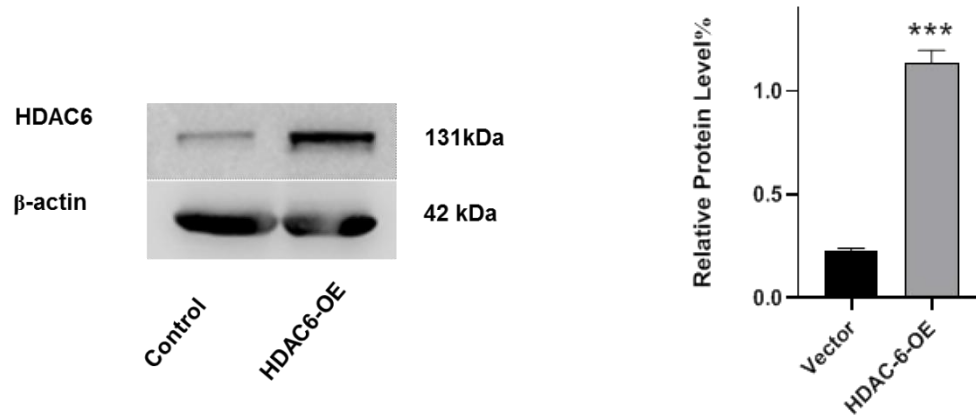


Figure S41. Verification of HDAC6 overexpression in MDA-MB-231 cells.

Representative Western blot bands (left) and quantitative analysis (right) of HDAC6 protein levels in Vector control and HDAC6-OE cells. Data are presented as mean $\pm$ SD from three biological replicates and were analyzed using a two-sample, two-tailed unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001.