Supporting information

Targeting EGFR/PI3K/AKT/mTOR Signaling in Lung and Colon Cancers: Synthesis, Antitumor Evaluation of New 1,2,4-oxdiazoles Tethered 1,2,3-Triazoles

Mohammed Salah Ayoup*[1,2], Islam Shawky [2], Hamida Abdel-Hamid[2], Doaa A. Ghareeb[3], Aliaa Masoud[3], Marwa F Harras[4], Mohamed El-Atawy[2,5]*, Nuha Salamah Alharbi[6] and Magda M. F. Ismail[4]

[1] Department of Chemistry, College of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[2] Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt.
[3] Bio-screening and preclinical trial lab, Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
[4] *Department of Pharmaceutical Medicinal Chemistry and Drug Design, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo 11754, Egypt*
[5]*Chemistry Department,* College *of Science at Yanbu, Taibah University, Yanbu 46423, Saudi Arabia;*
[6] Chemistry Department, College of Sciences, Taibah University, Al-Madina 30002, Saudi Arabia

*Corresponding authors: Email addresses: mayoup@kfu.edu.sa & mohammedsalahayoup@gmail.com, mohamed.elatawi@alexu.edu.eg

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1. ^{1}H, ^{13}C NMR and IR spectra of the synthesized compounds (Figure S 1-48)

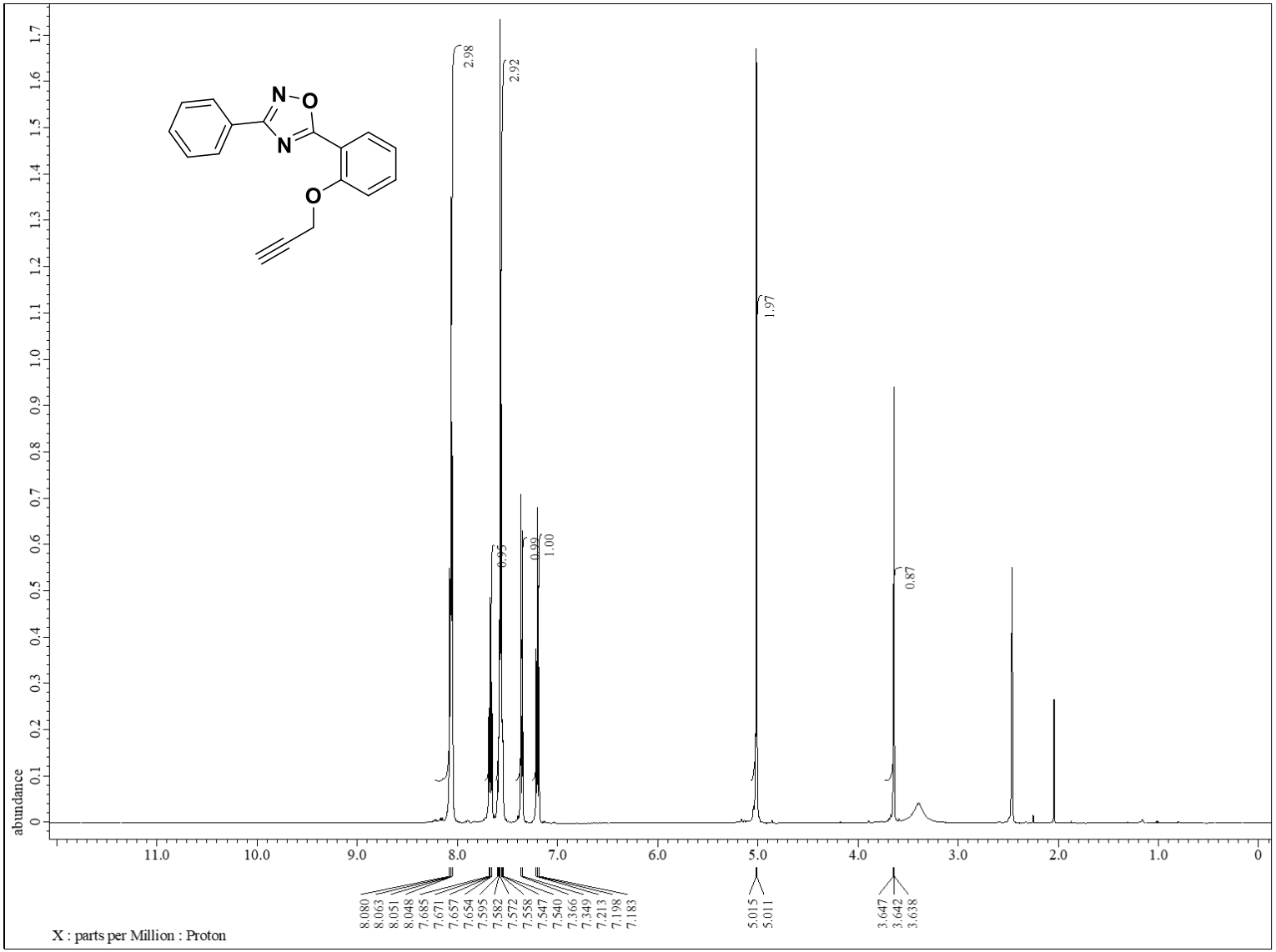

Figure S1 ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **3a**

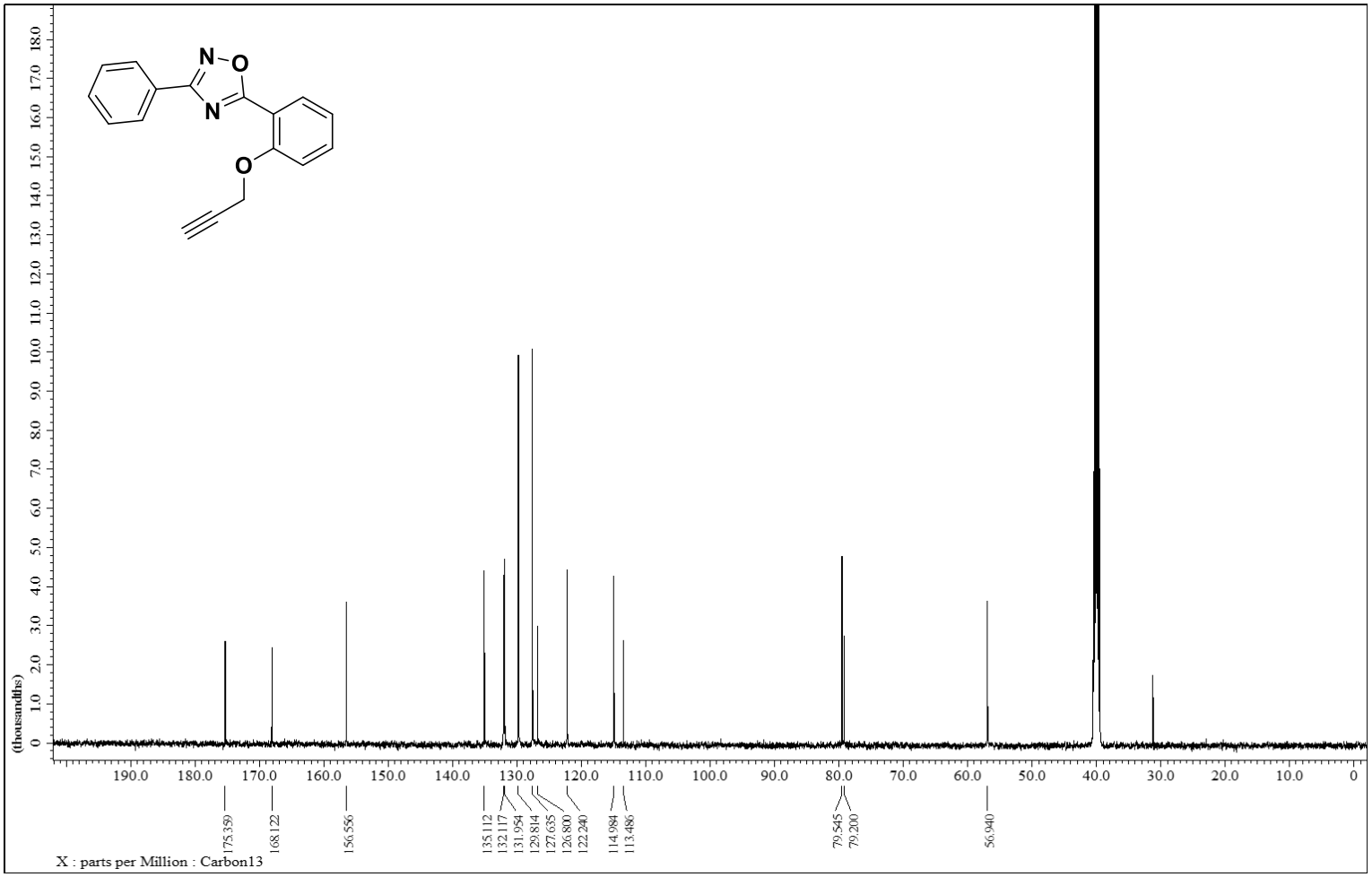

Figure S2 ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **3a.**

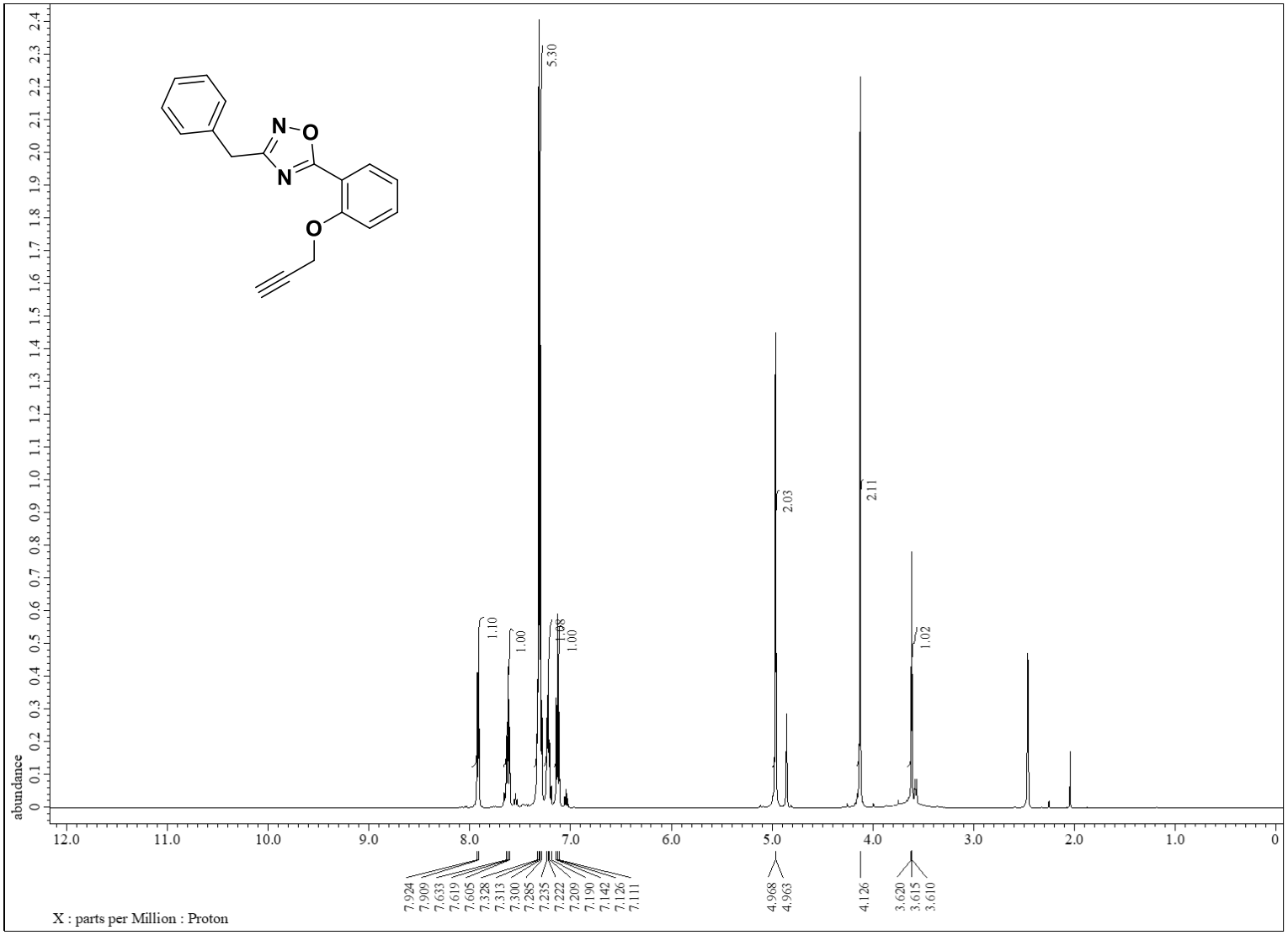

Figure S3. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **3b**

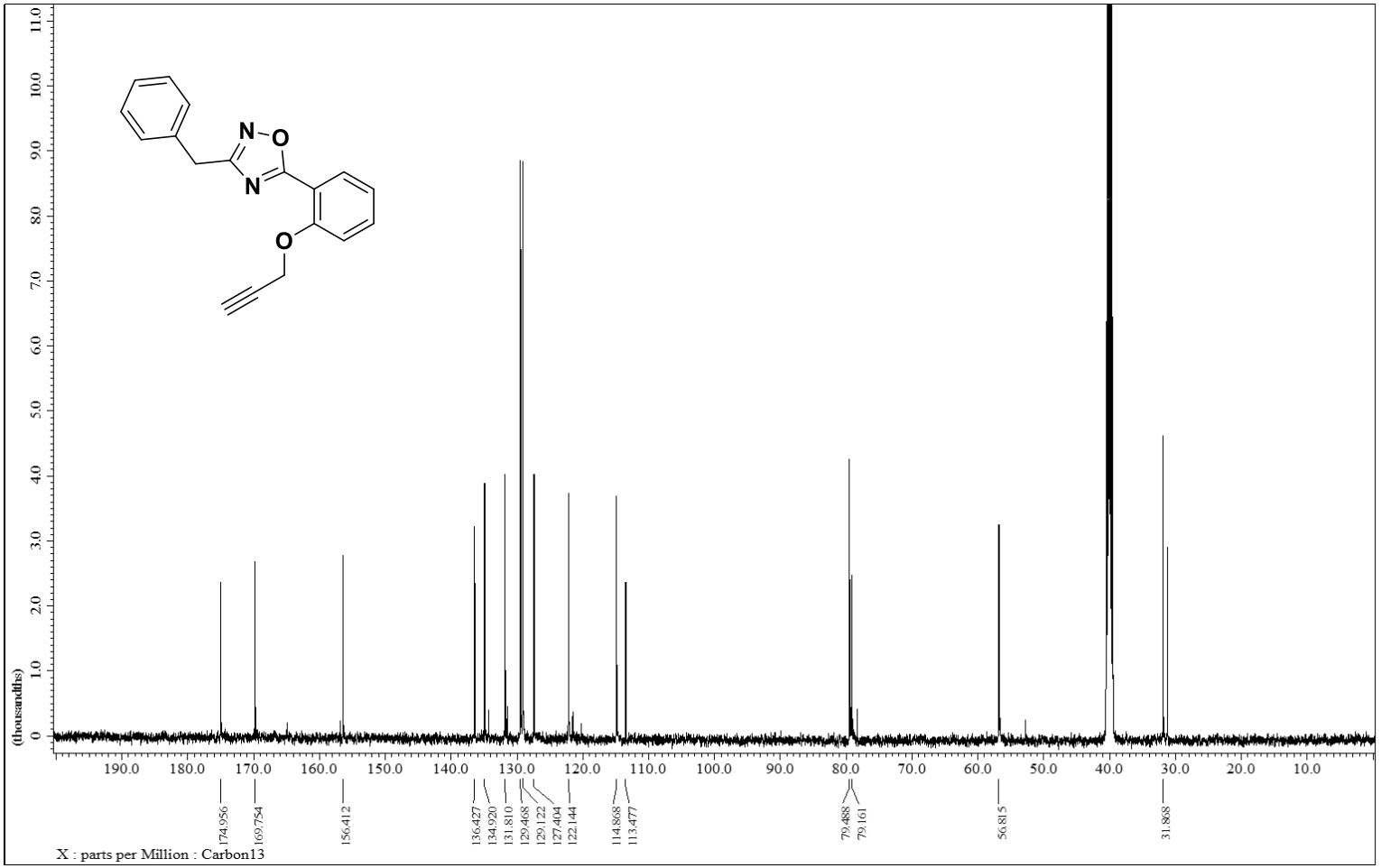

Figure S4. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **3b.**

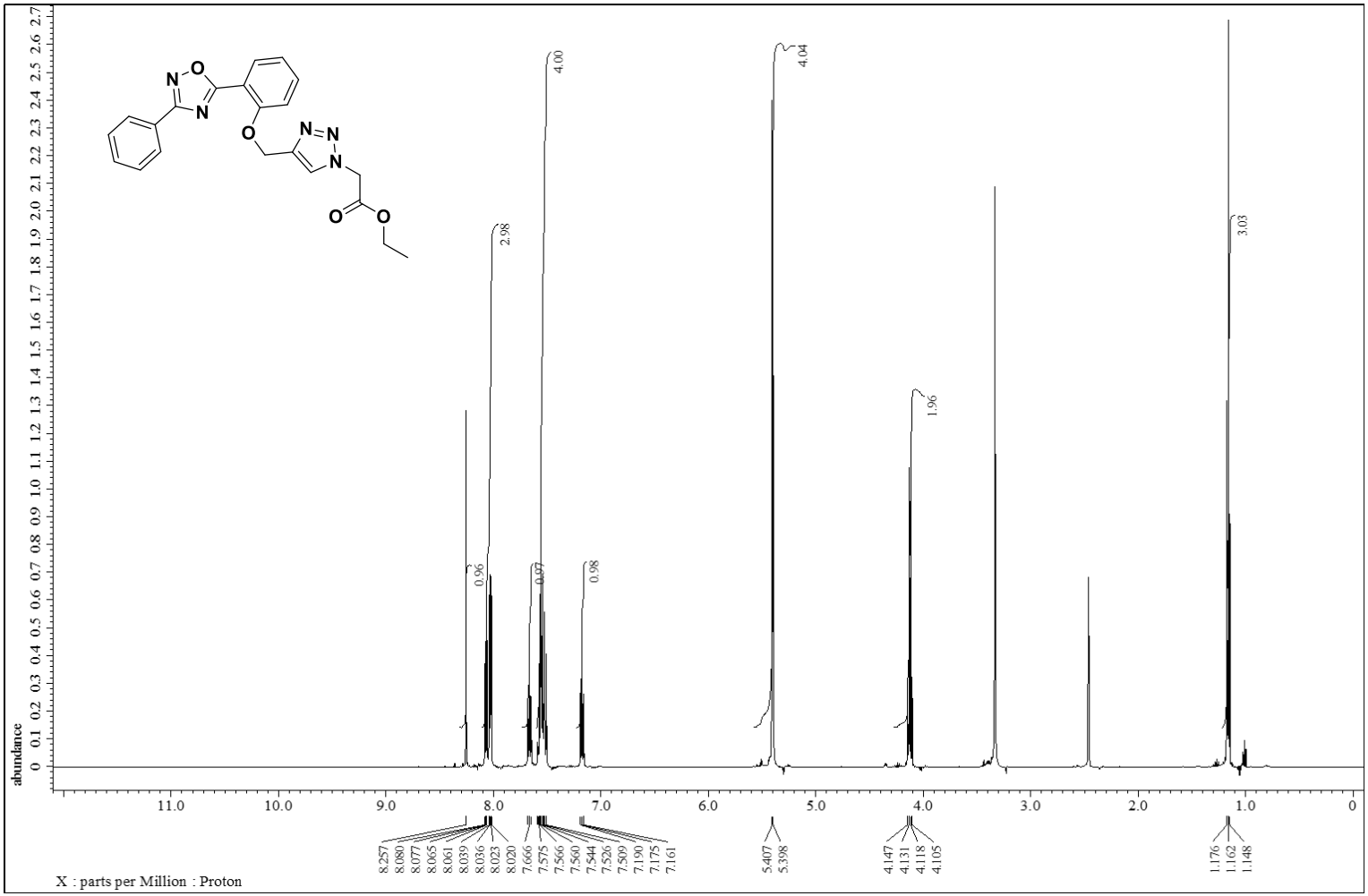

Figure S5. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **5a**

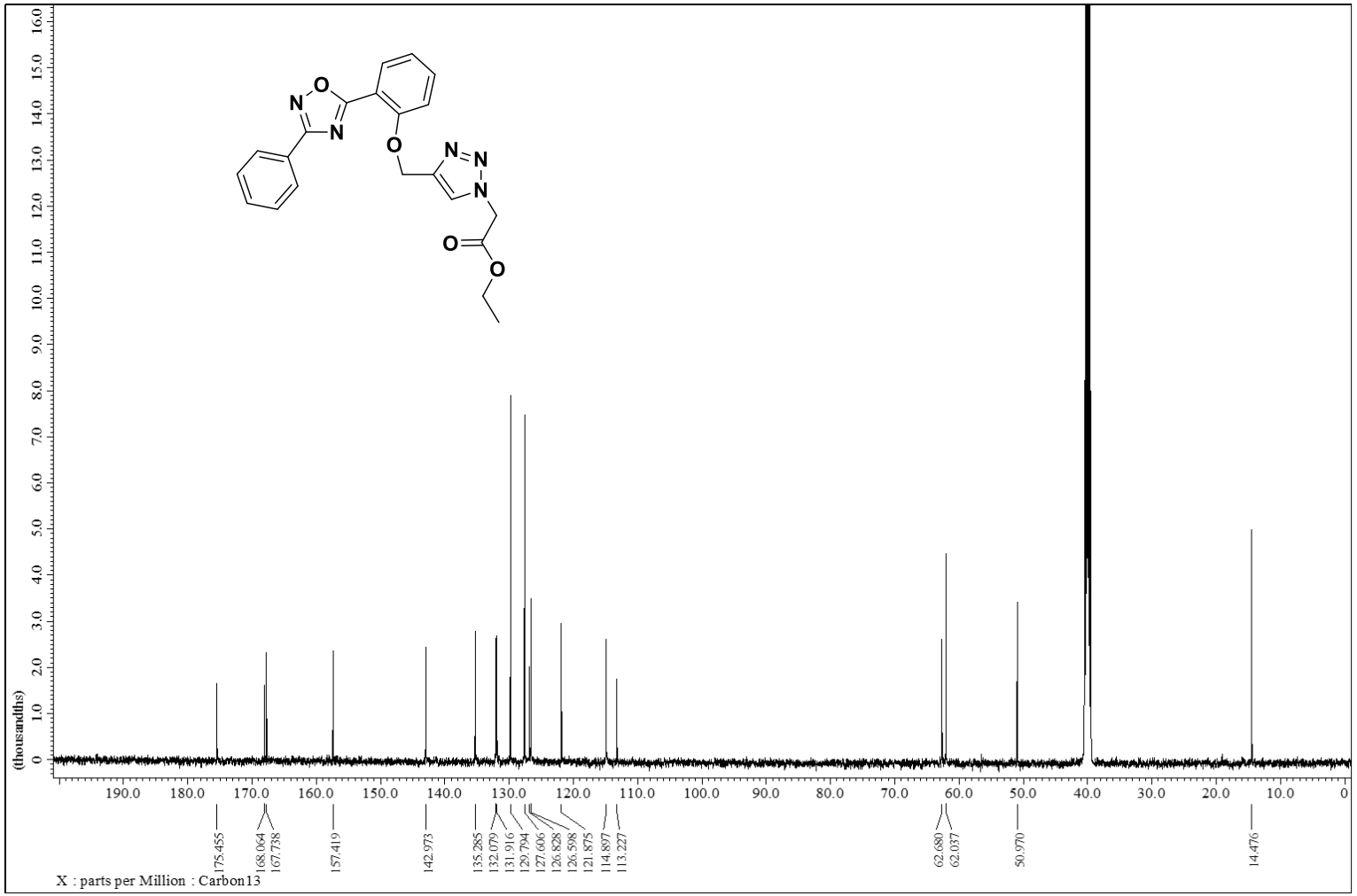

Figure S6. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **5a.**

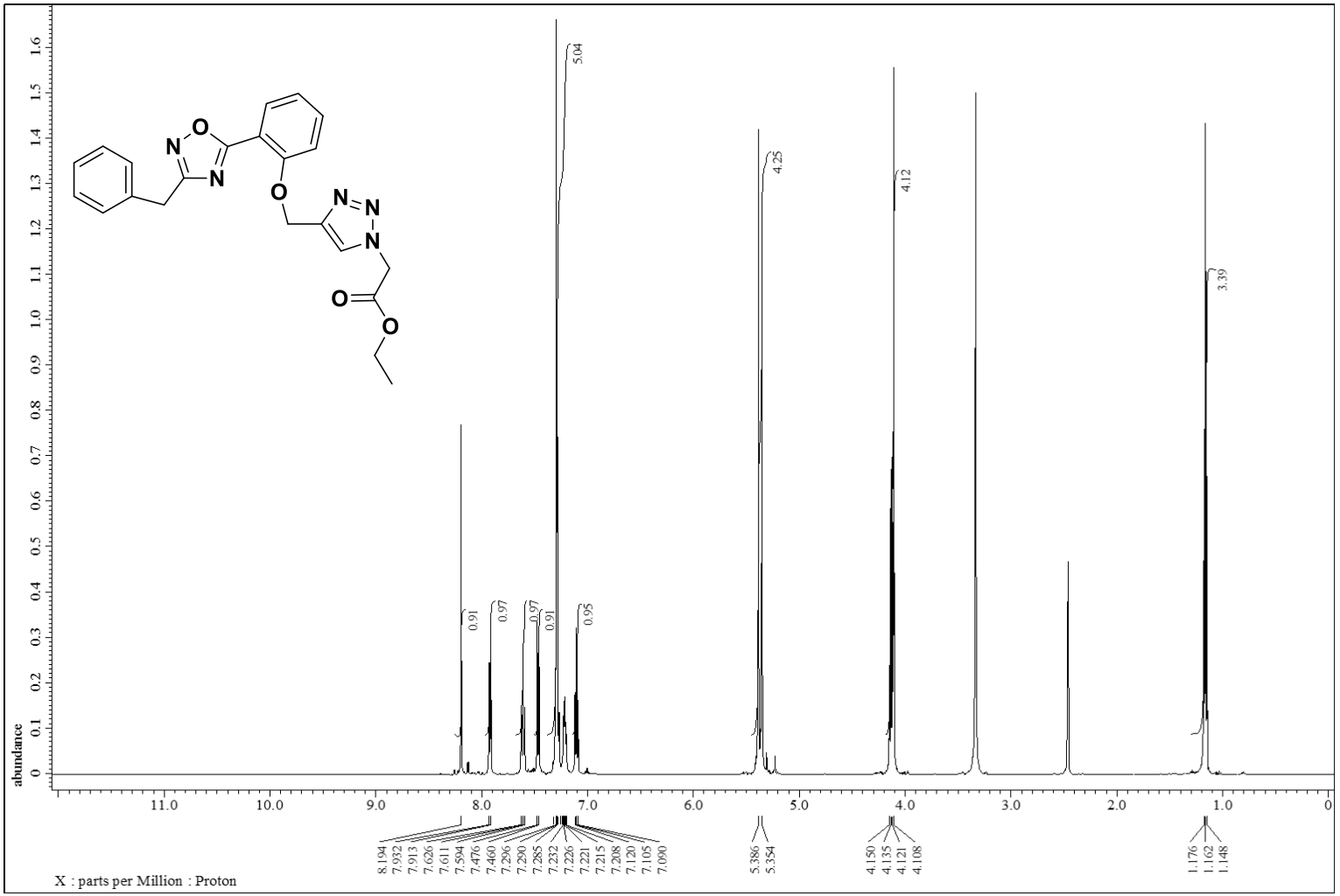

Figure S7. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **5b**

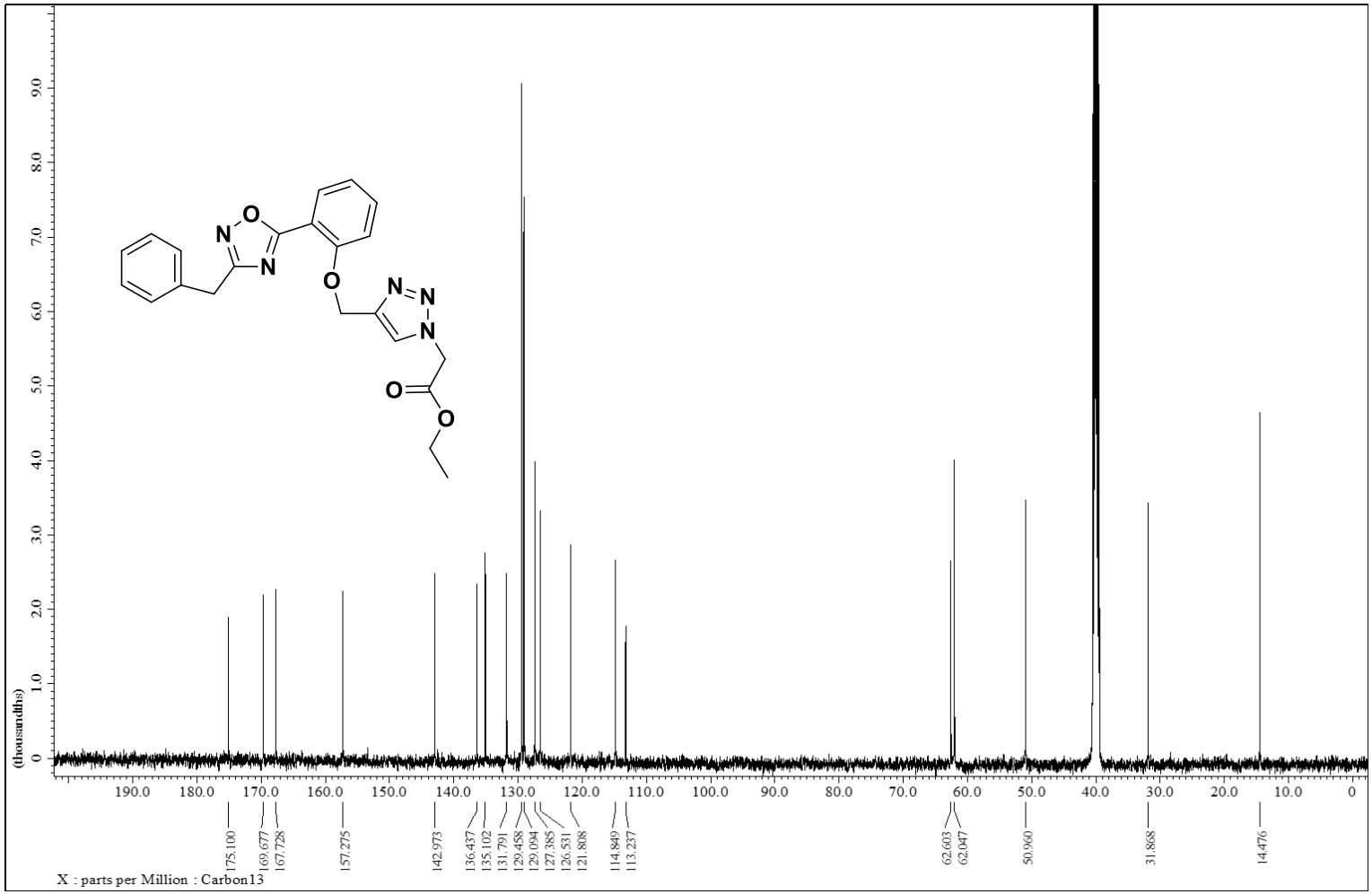

Figure S8. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **5b.**

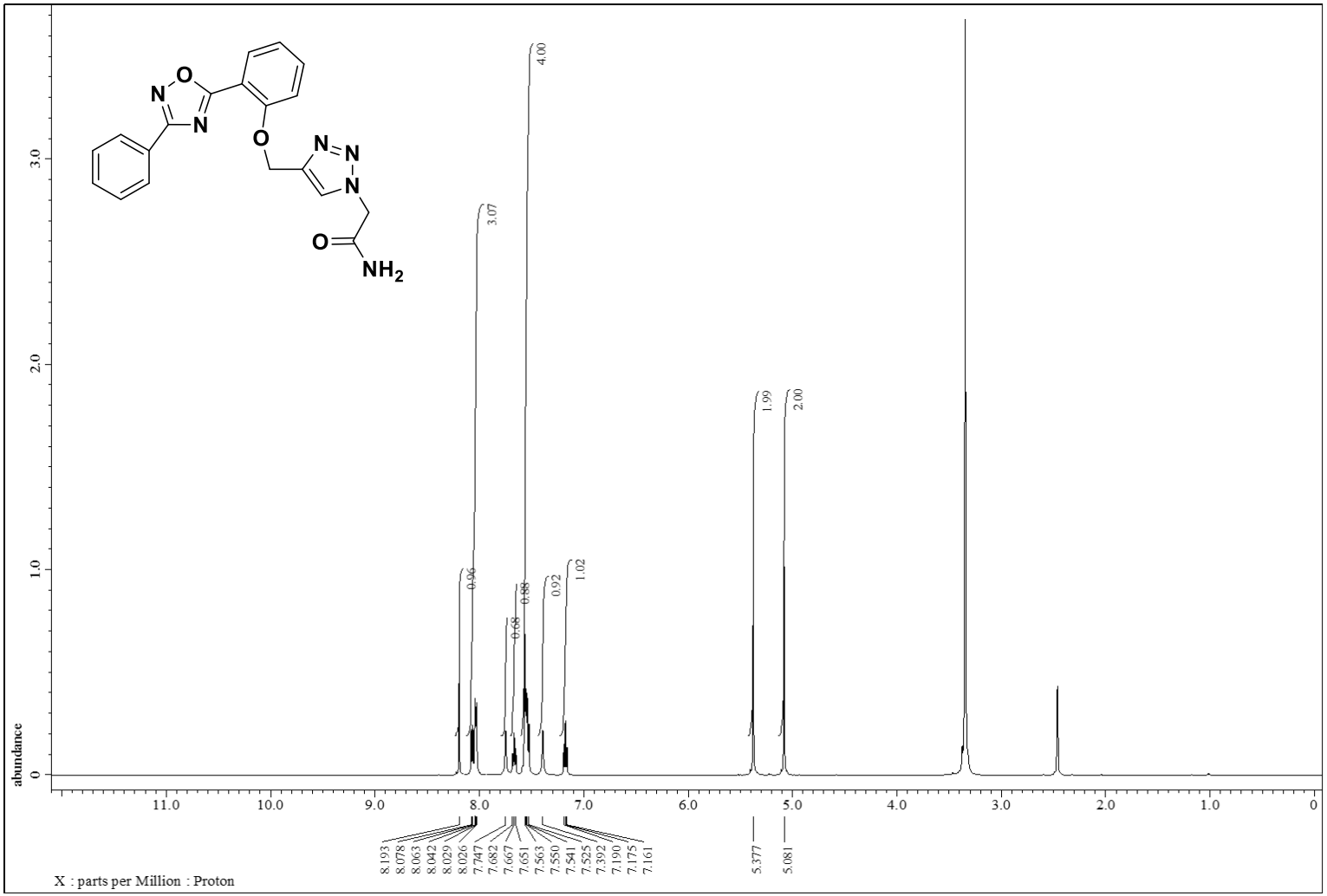

Figure S9. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **6a**

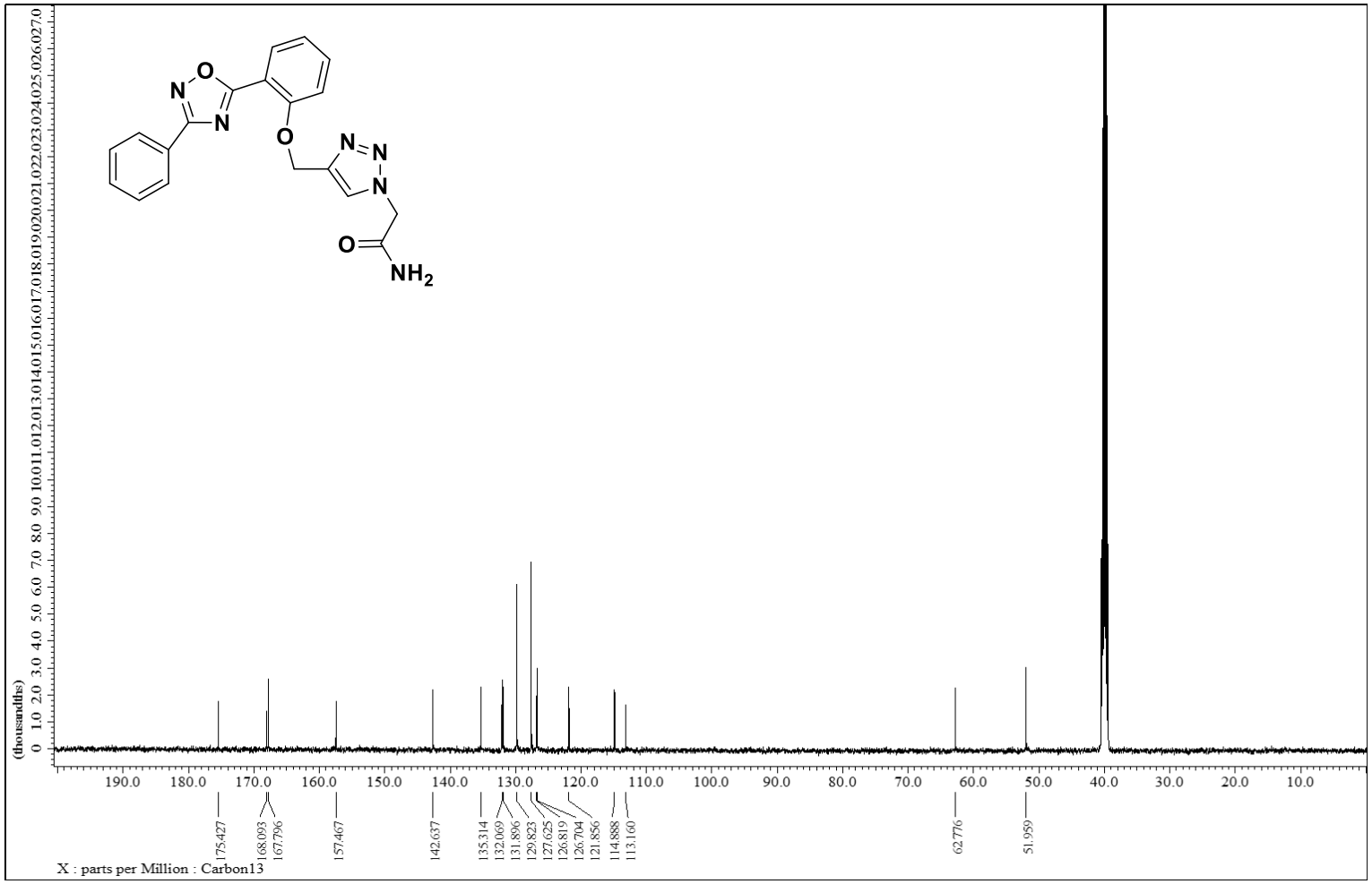

Figure S10. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **6a.**

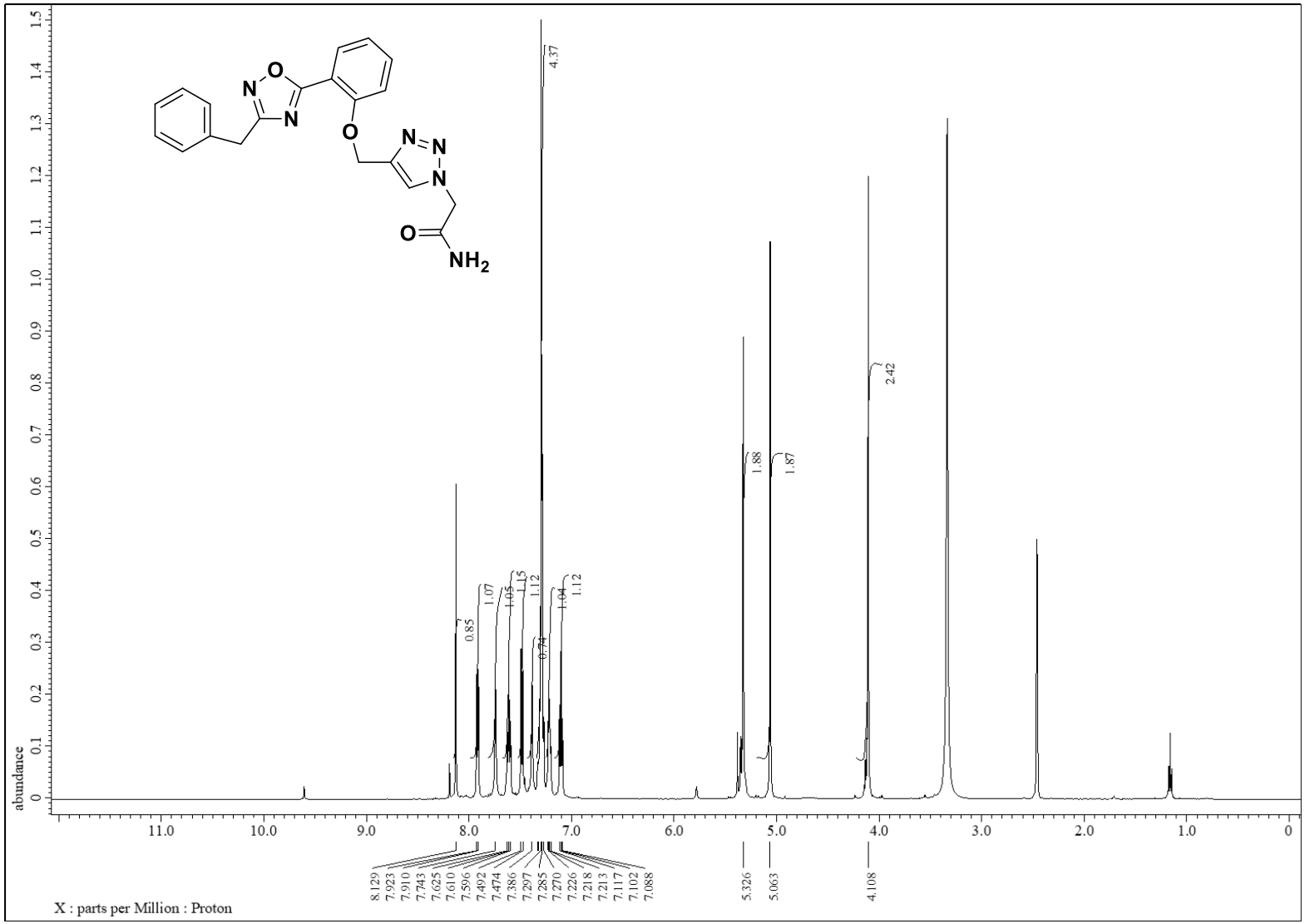

Figure S11. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **6b**

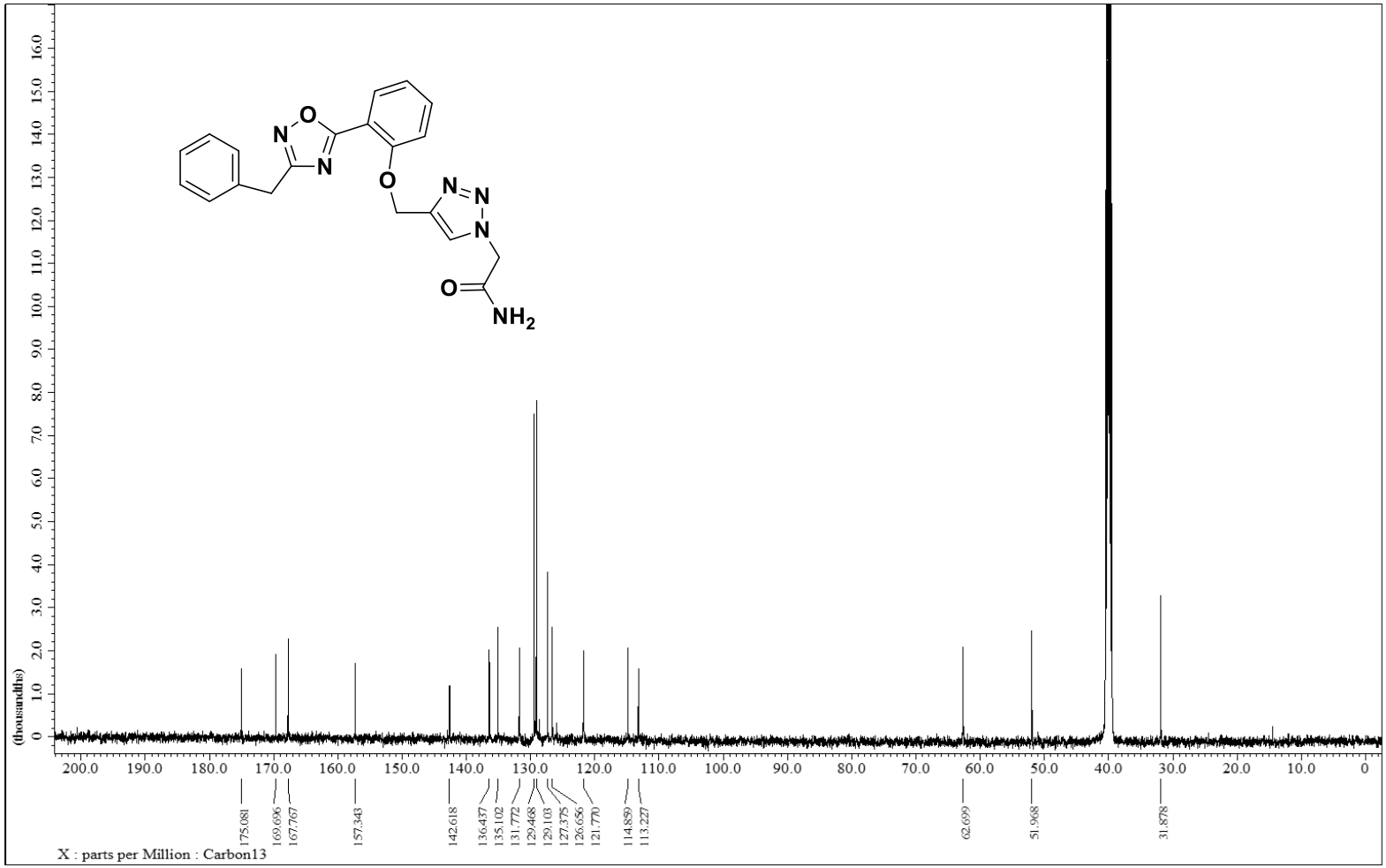

Figure S12. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **6b.**

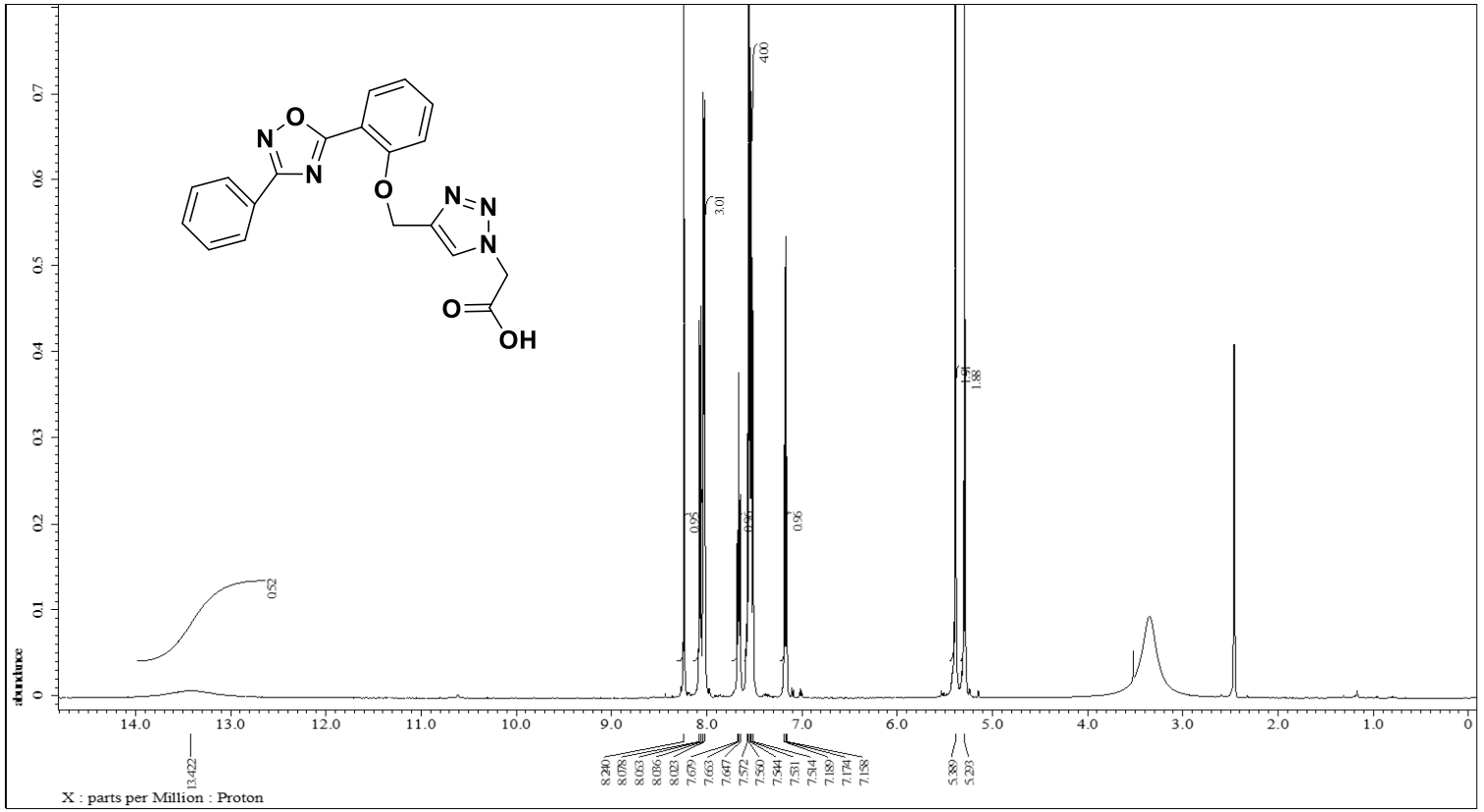

Figure S13. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **7a**

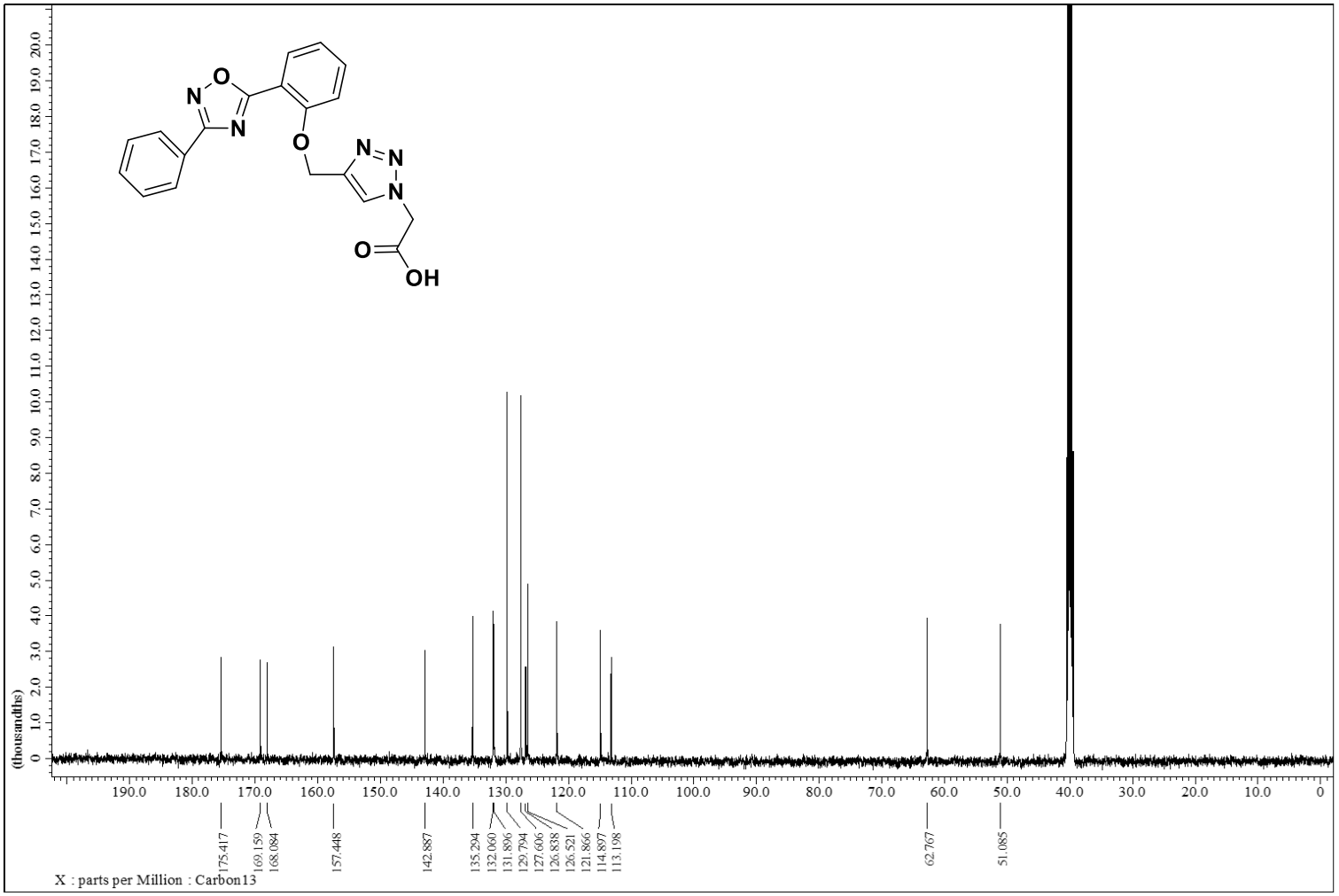

Figure S14. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **7a.**

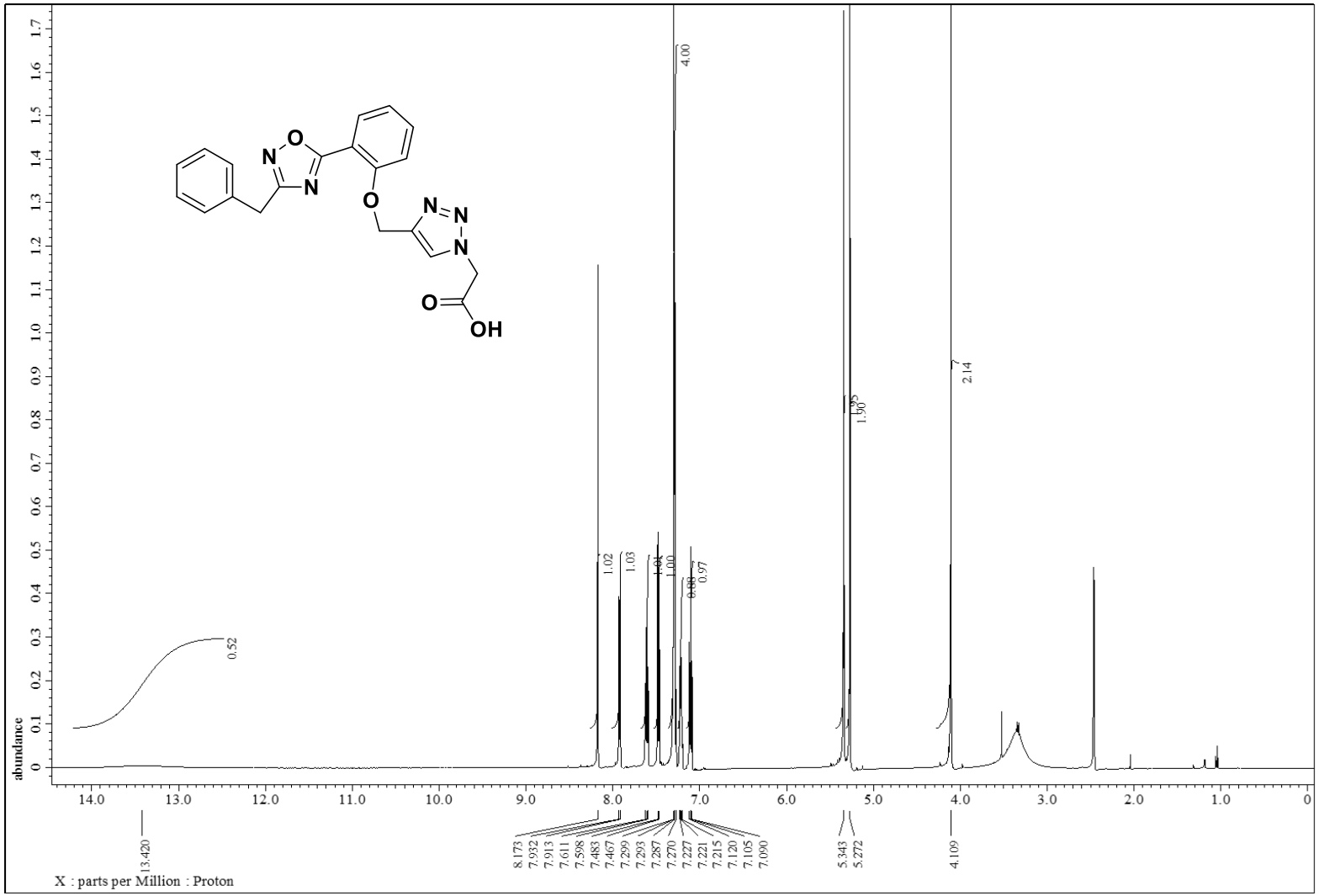

Figure S15. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **7b**

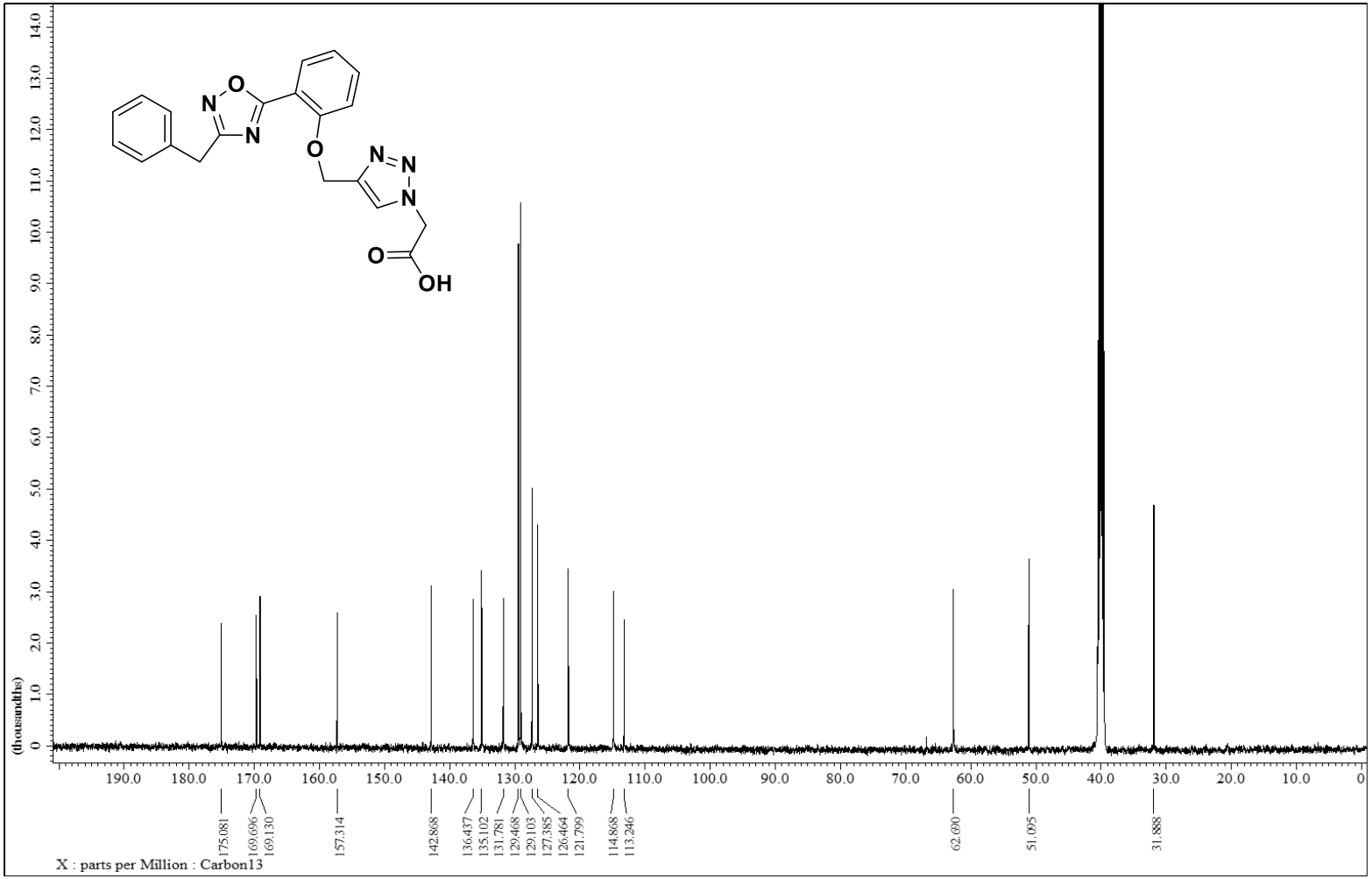

Figure S16. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **7b.**

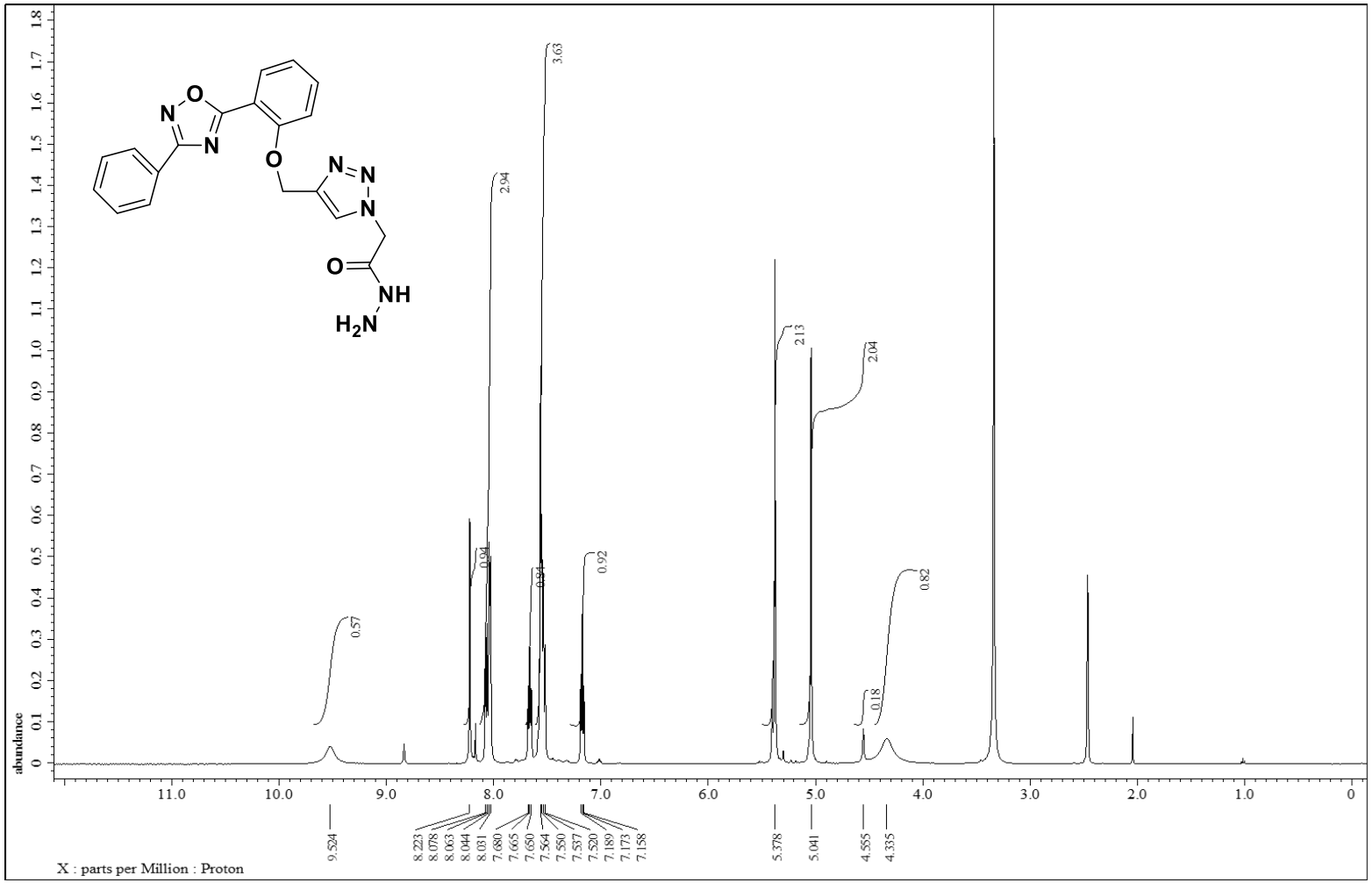

Figure S17. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **8a**

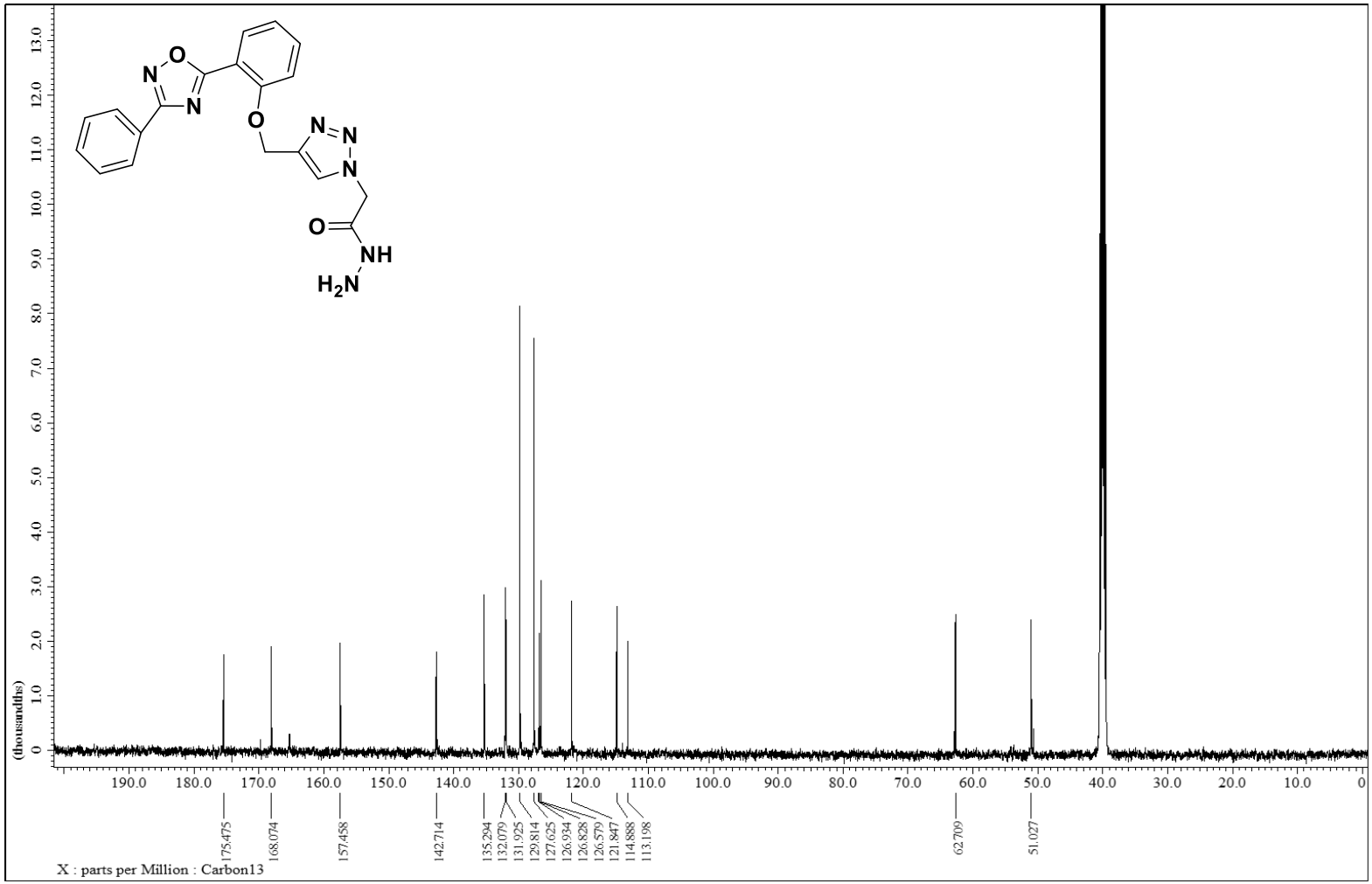

Figure S18. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **8a.**

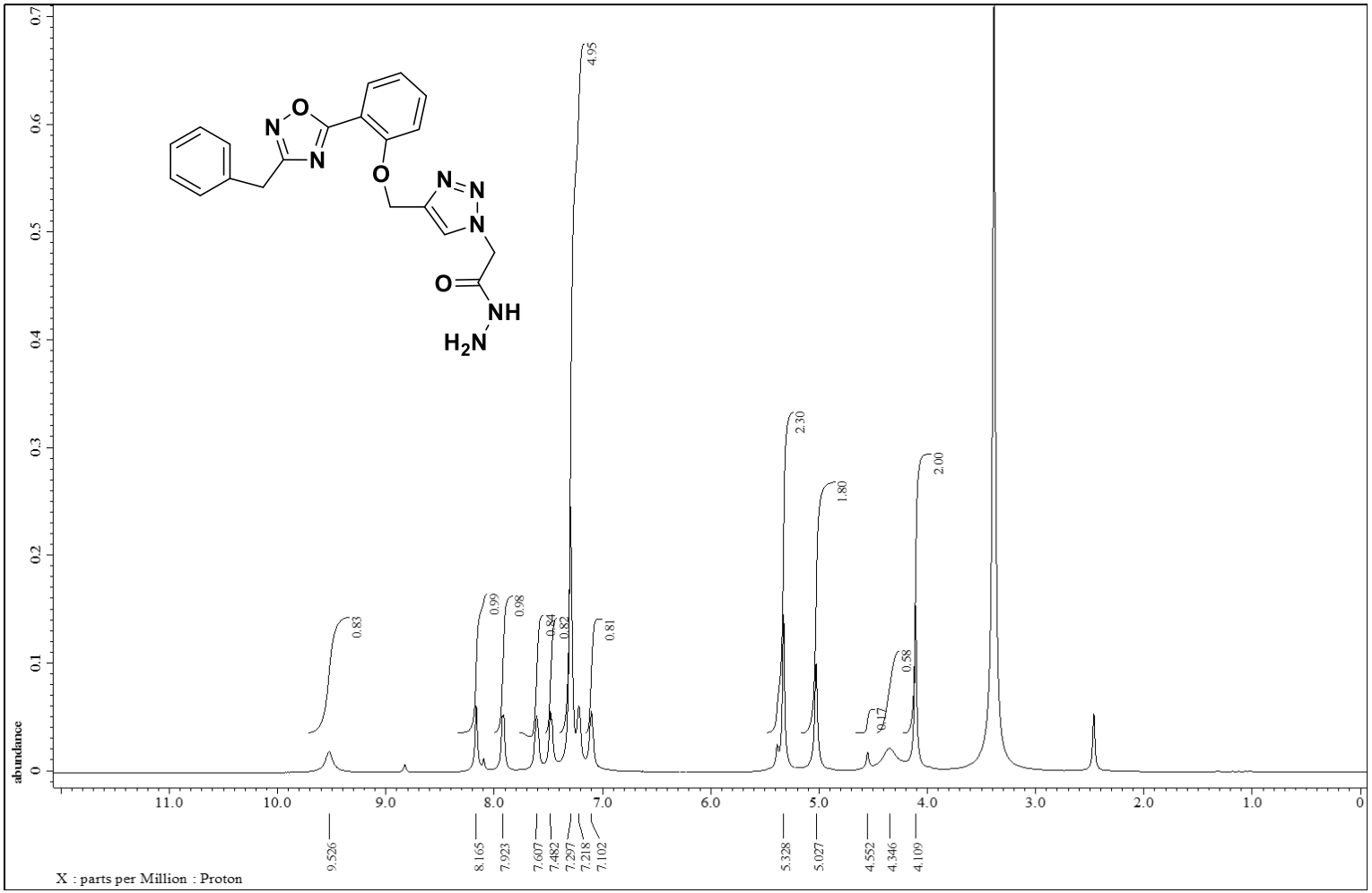

Figure S19. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **8b.**

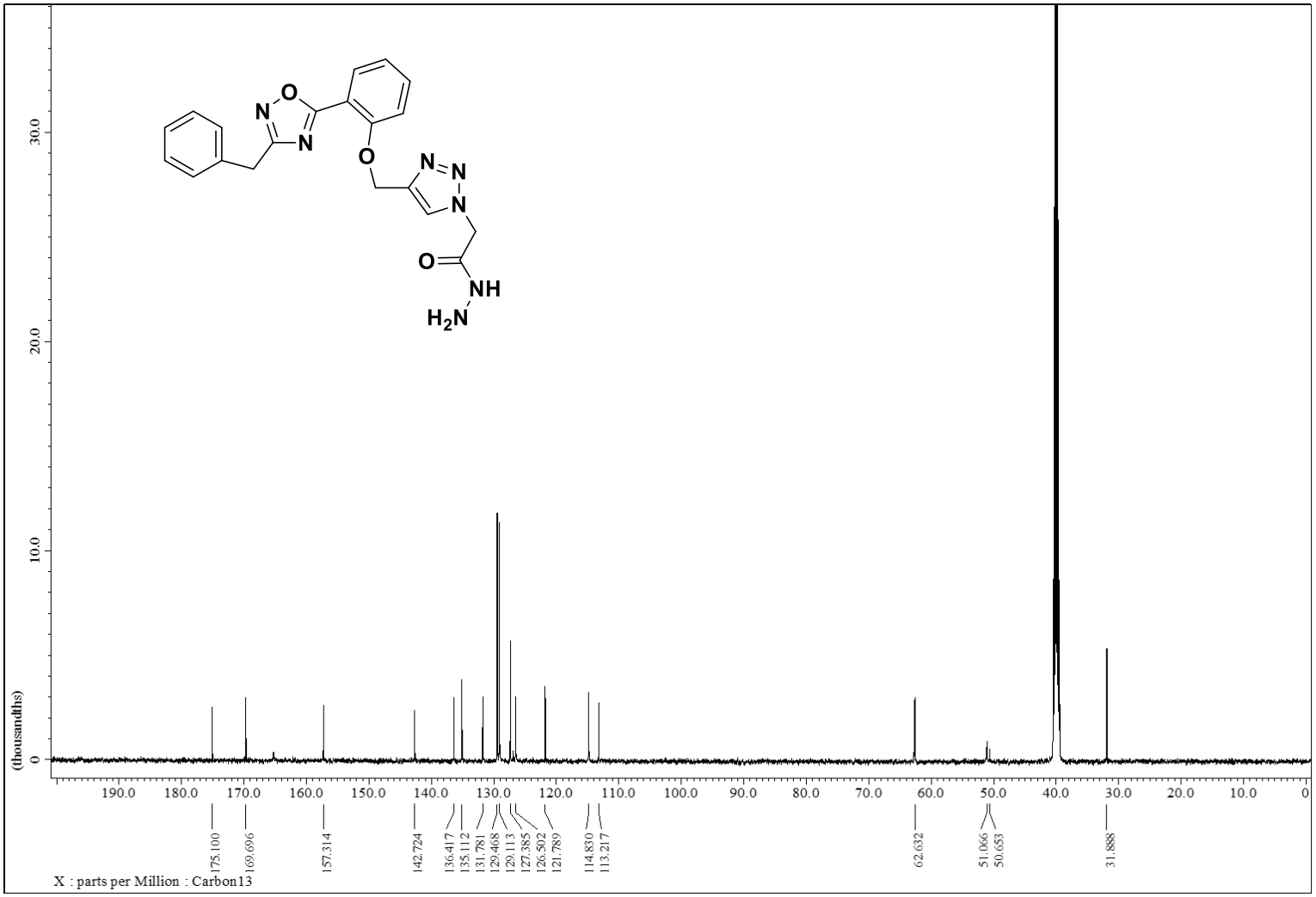

Figure S20. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **8b.**

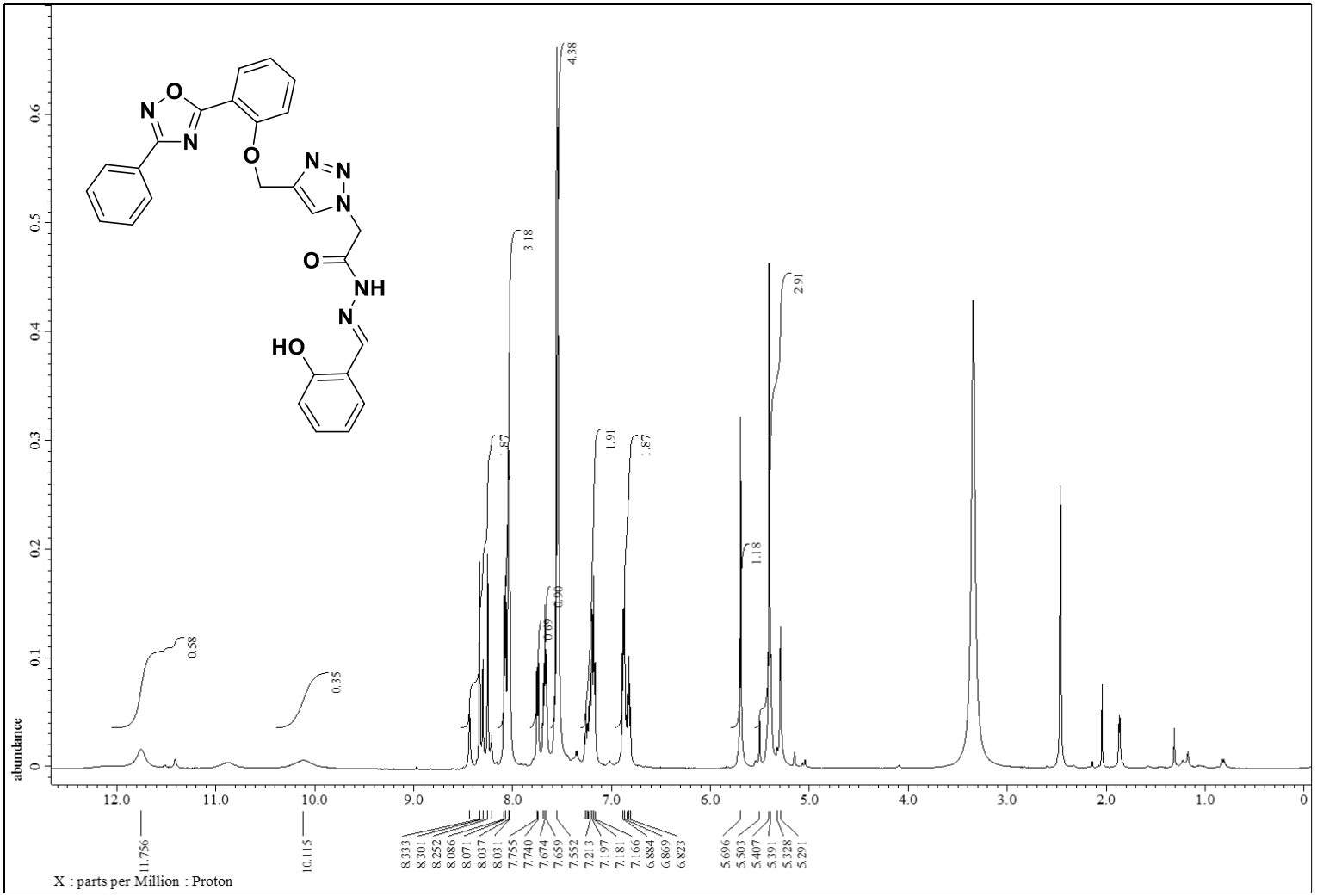

Figure S21. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **9a**

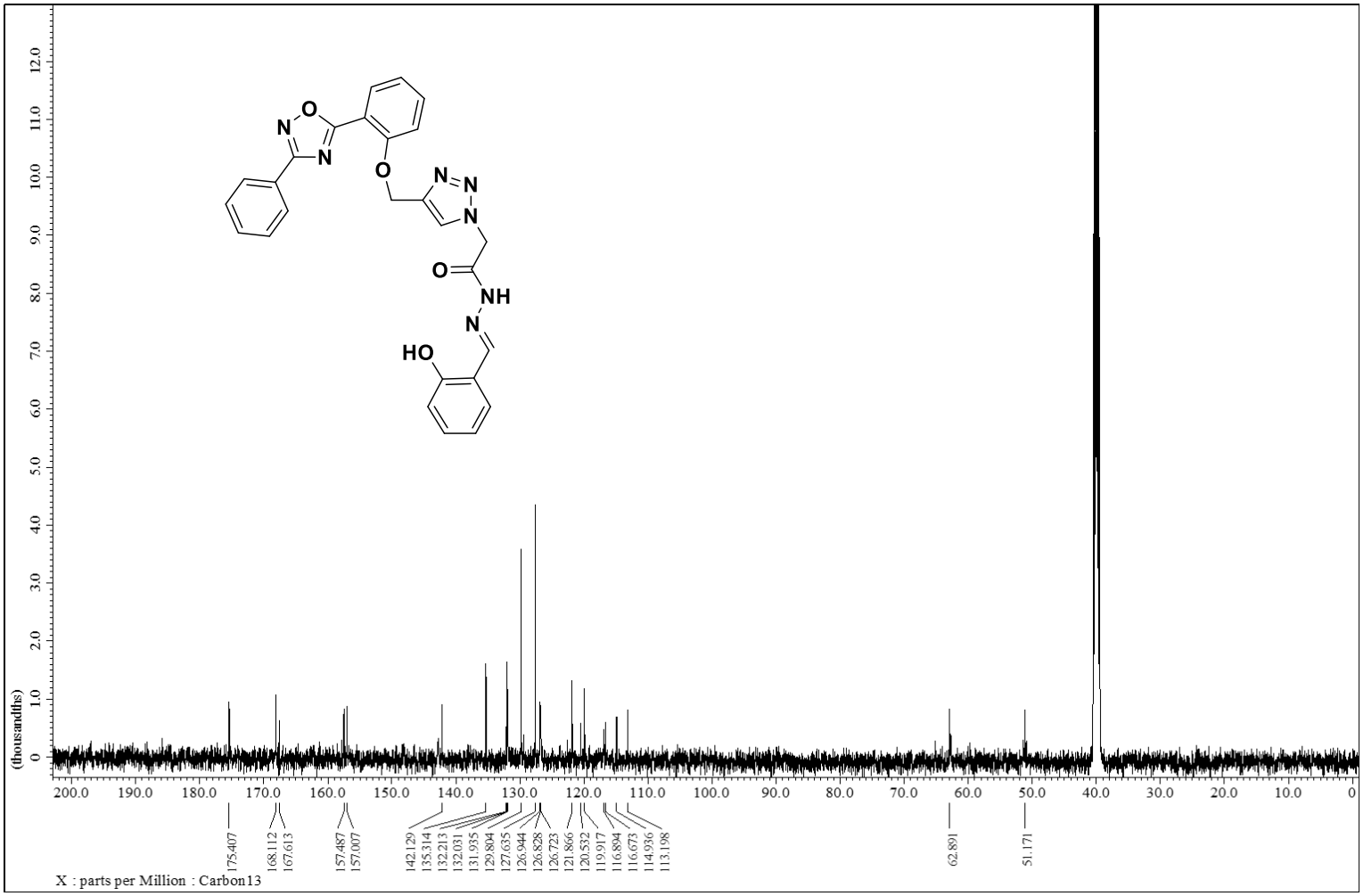

Figure S22. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **9a.**

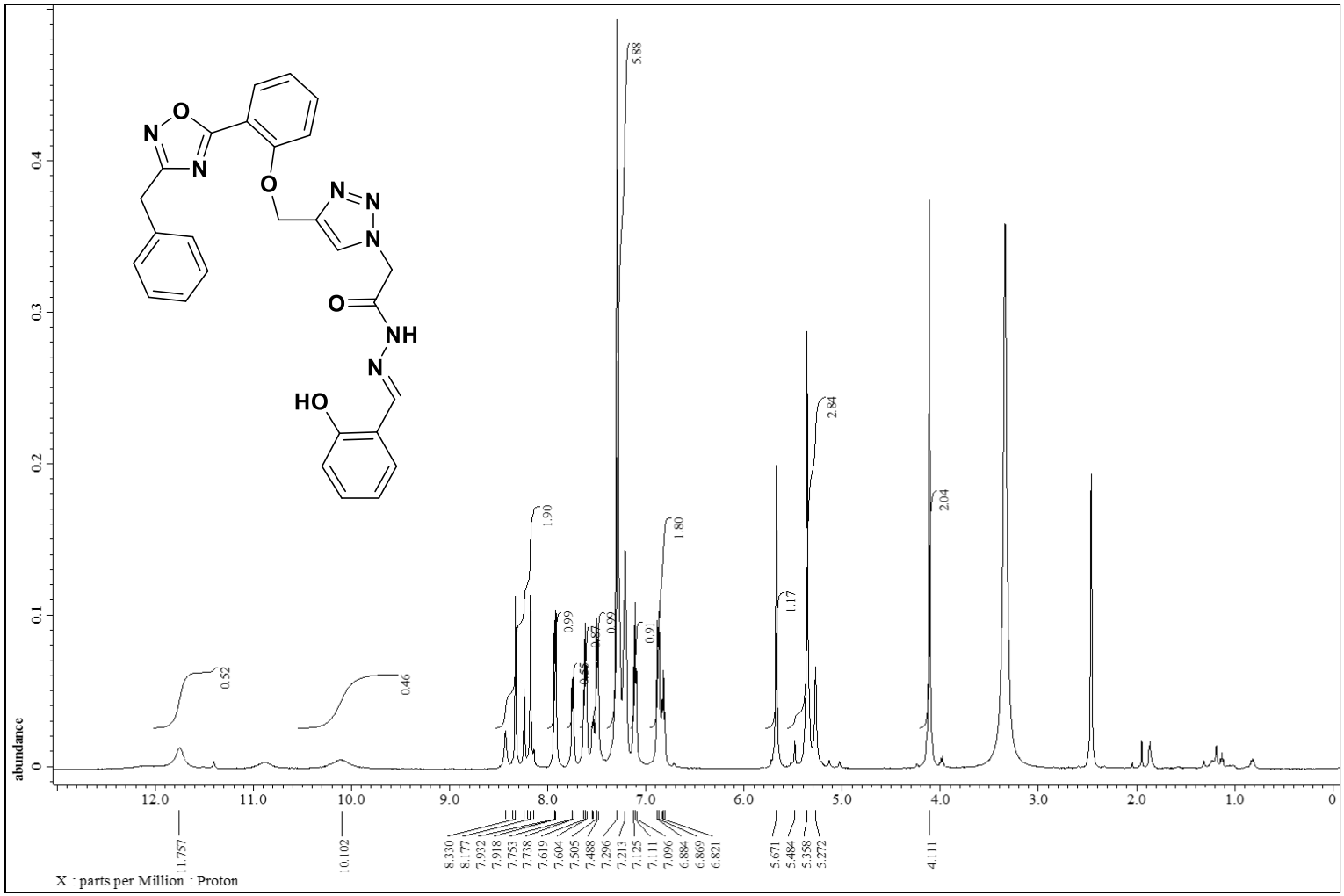

Figure S23. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **9b**

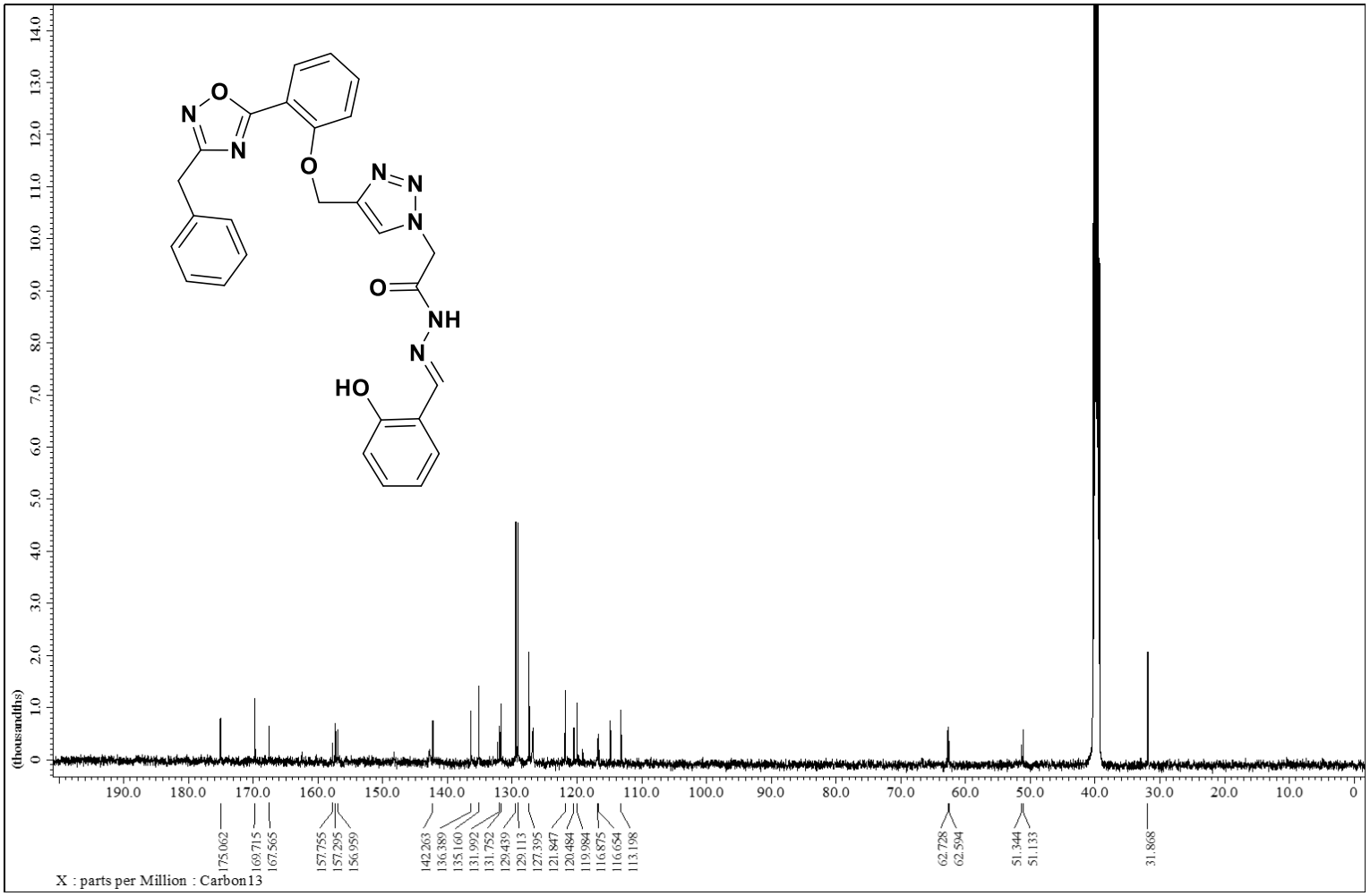

Figure S24. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **9b.**

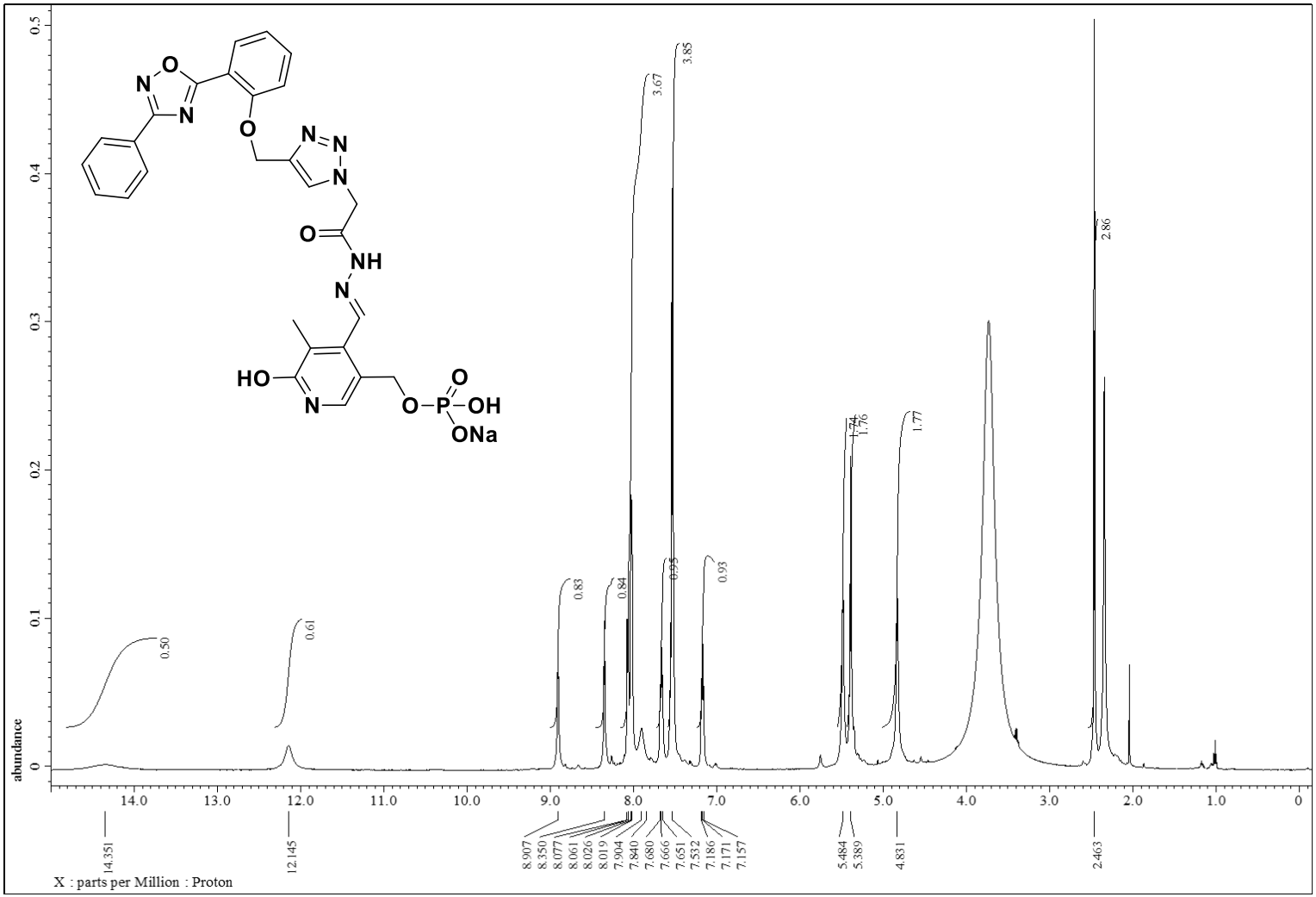

Figure S25. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **10a**

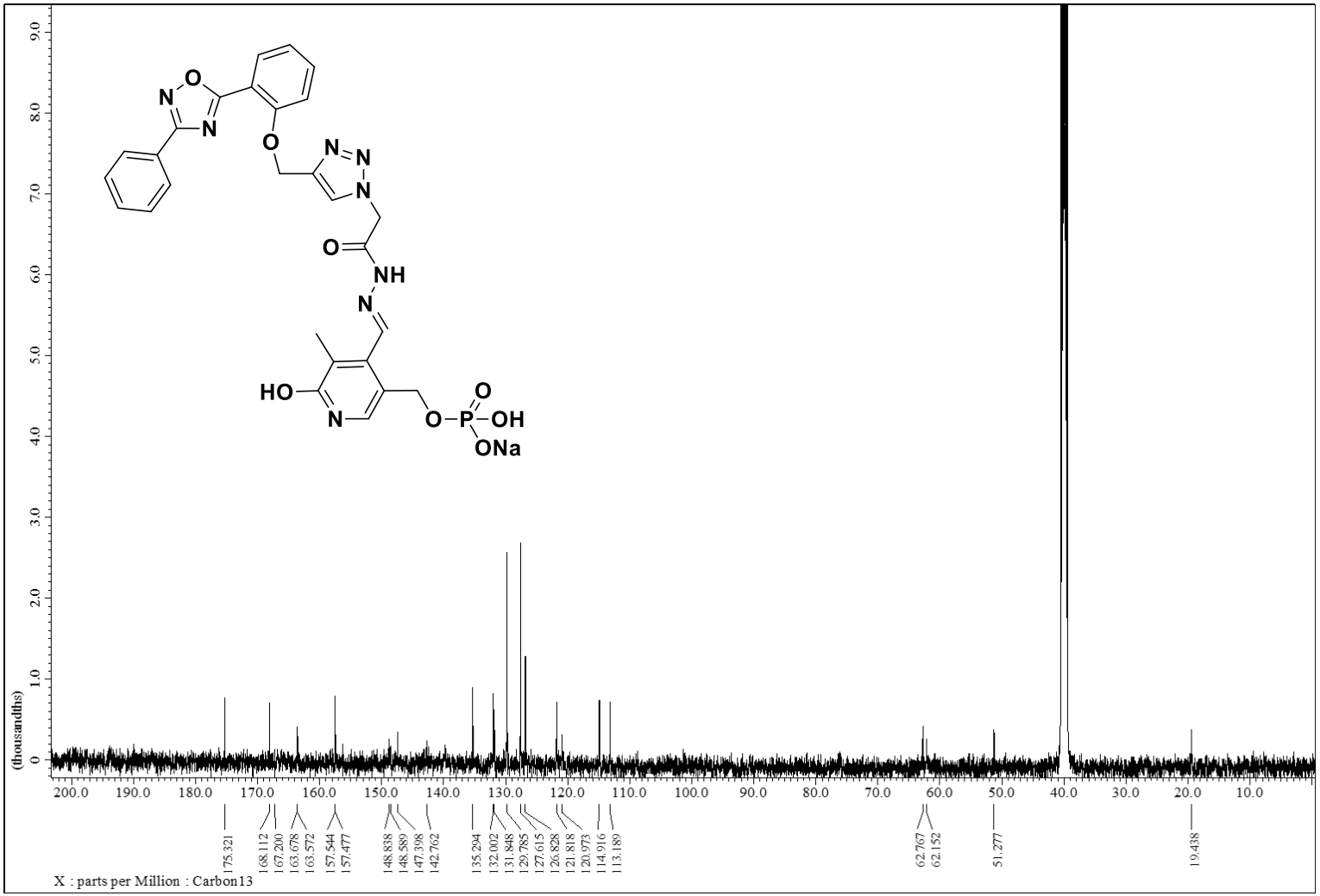

Figure S26. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **10a.**

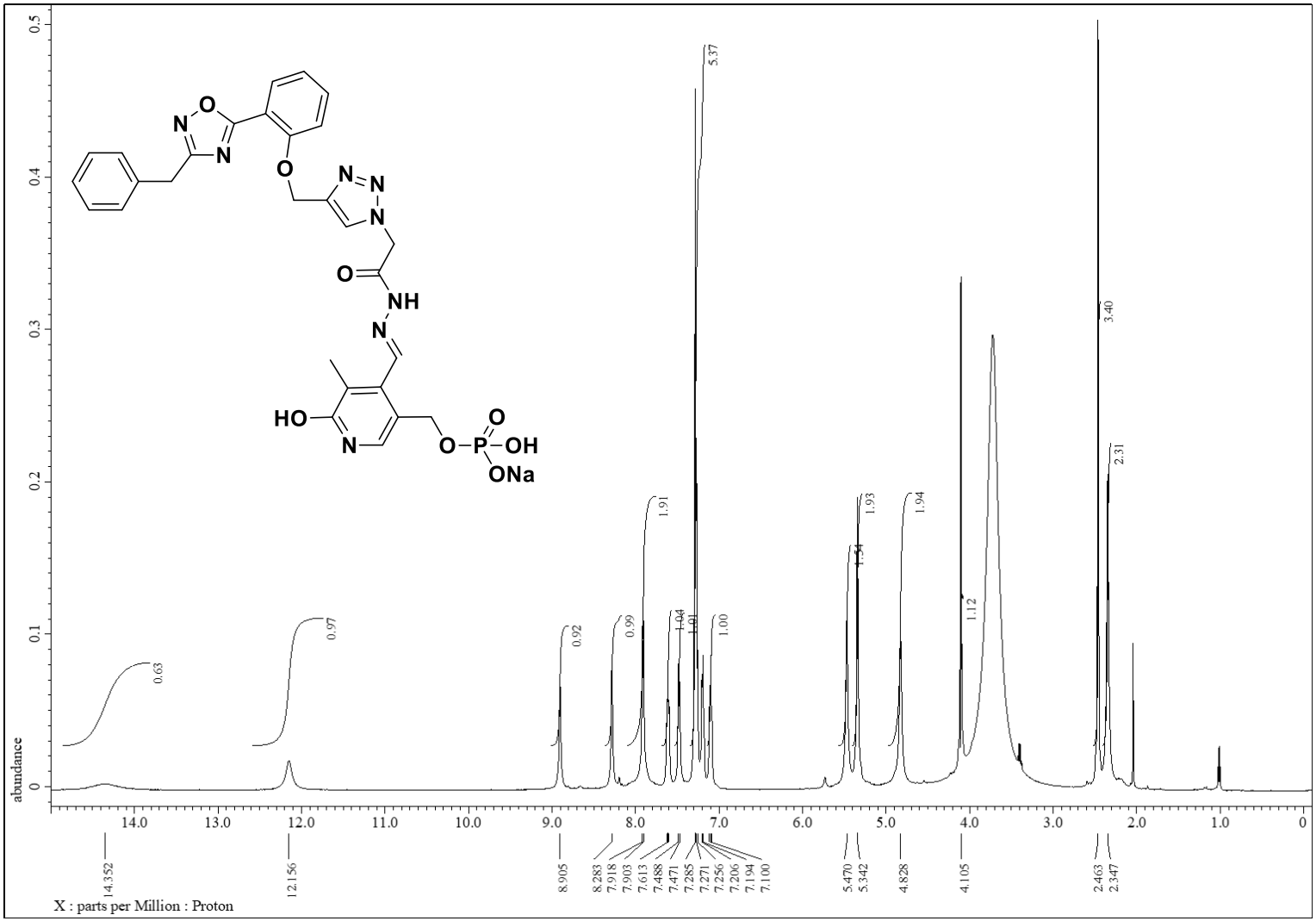

Figure S27. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **10b**

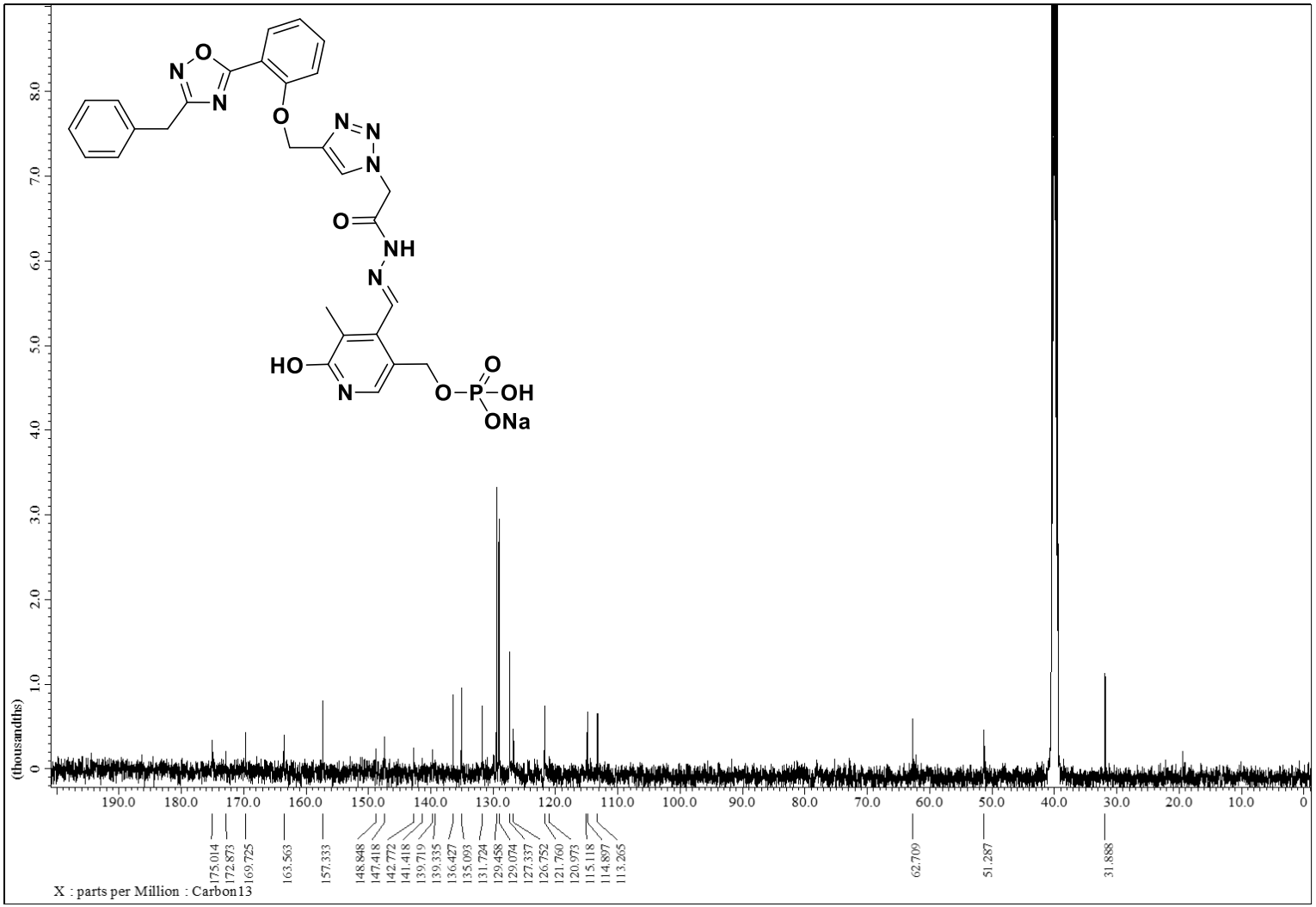

Figure S28. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **10b.**

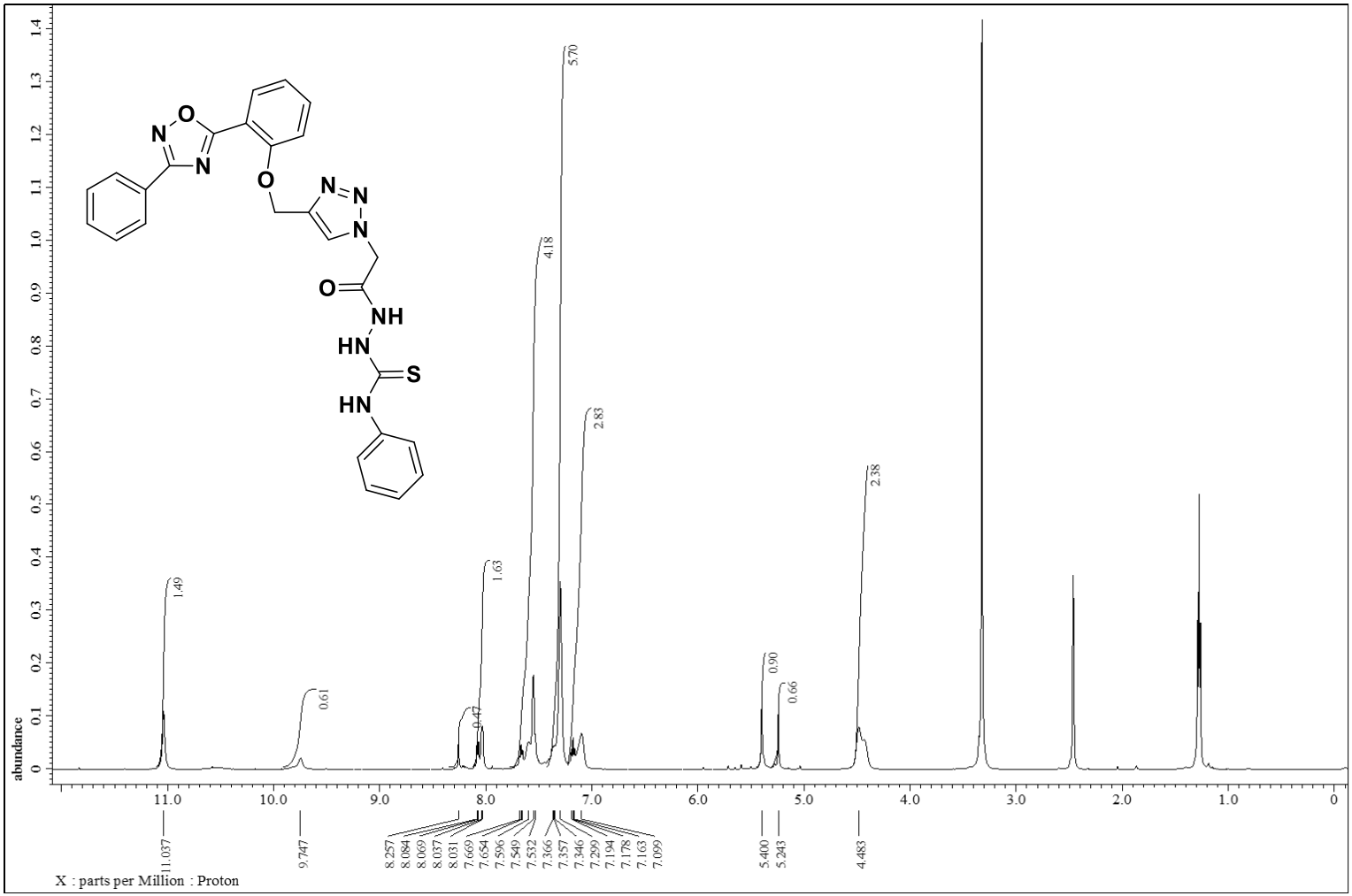

Figure S29. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **11a**

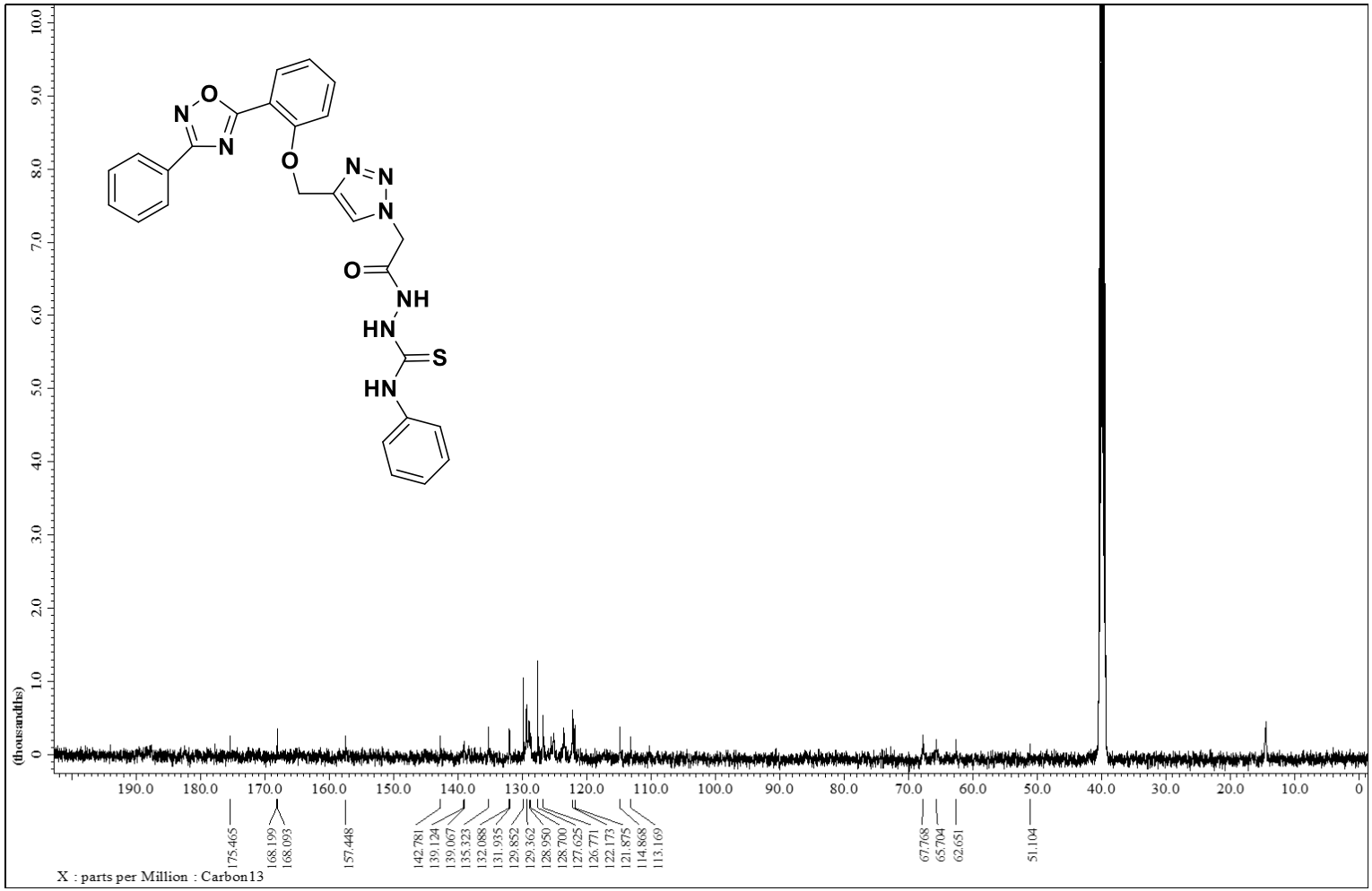

Figure S30. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **11a.**

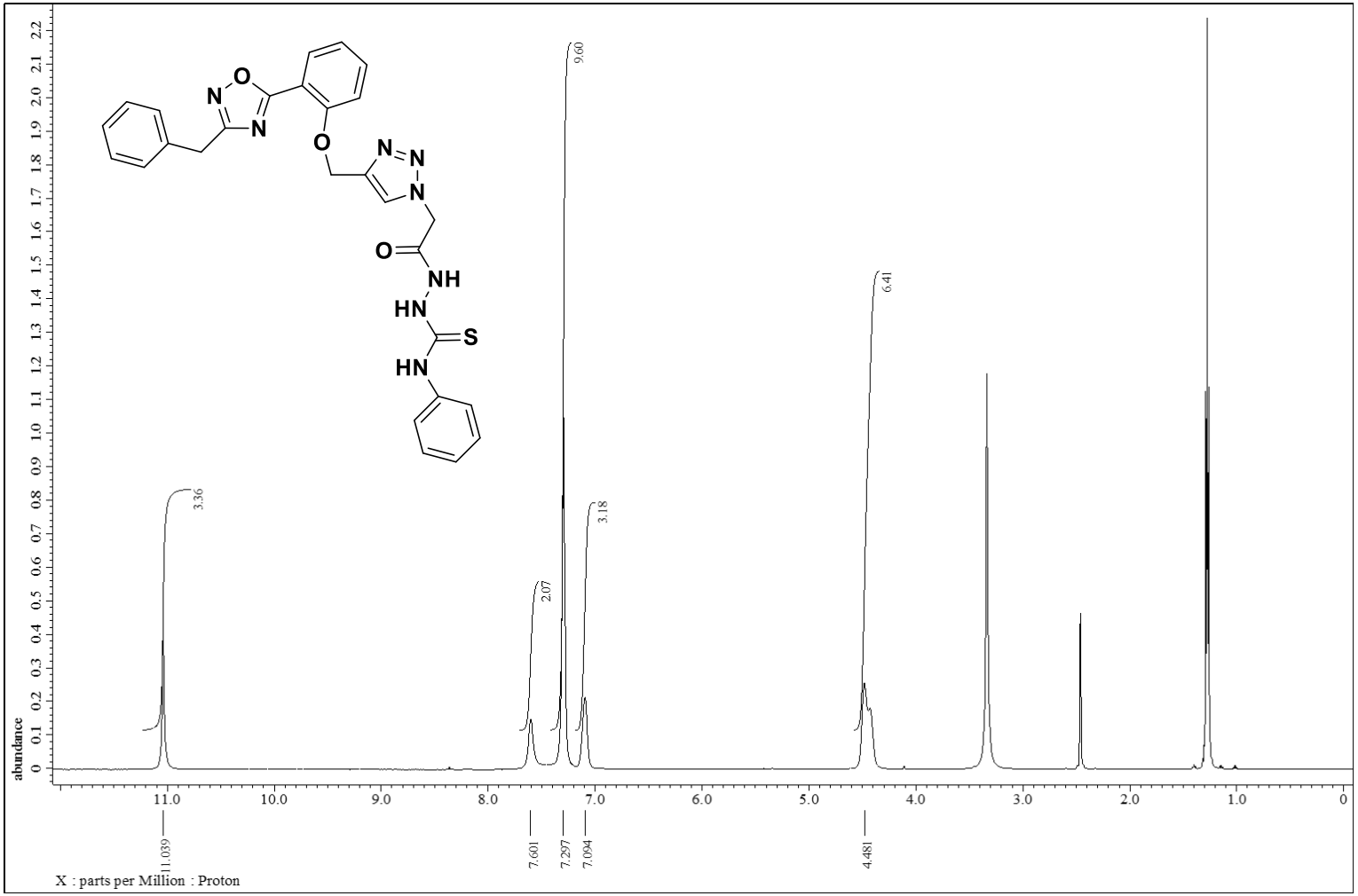

Figure S31. ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) of **11b**

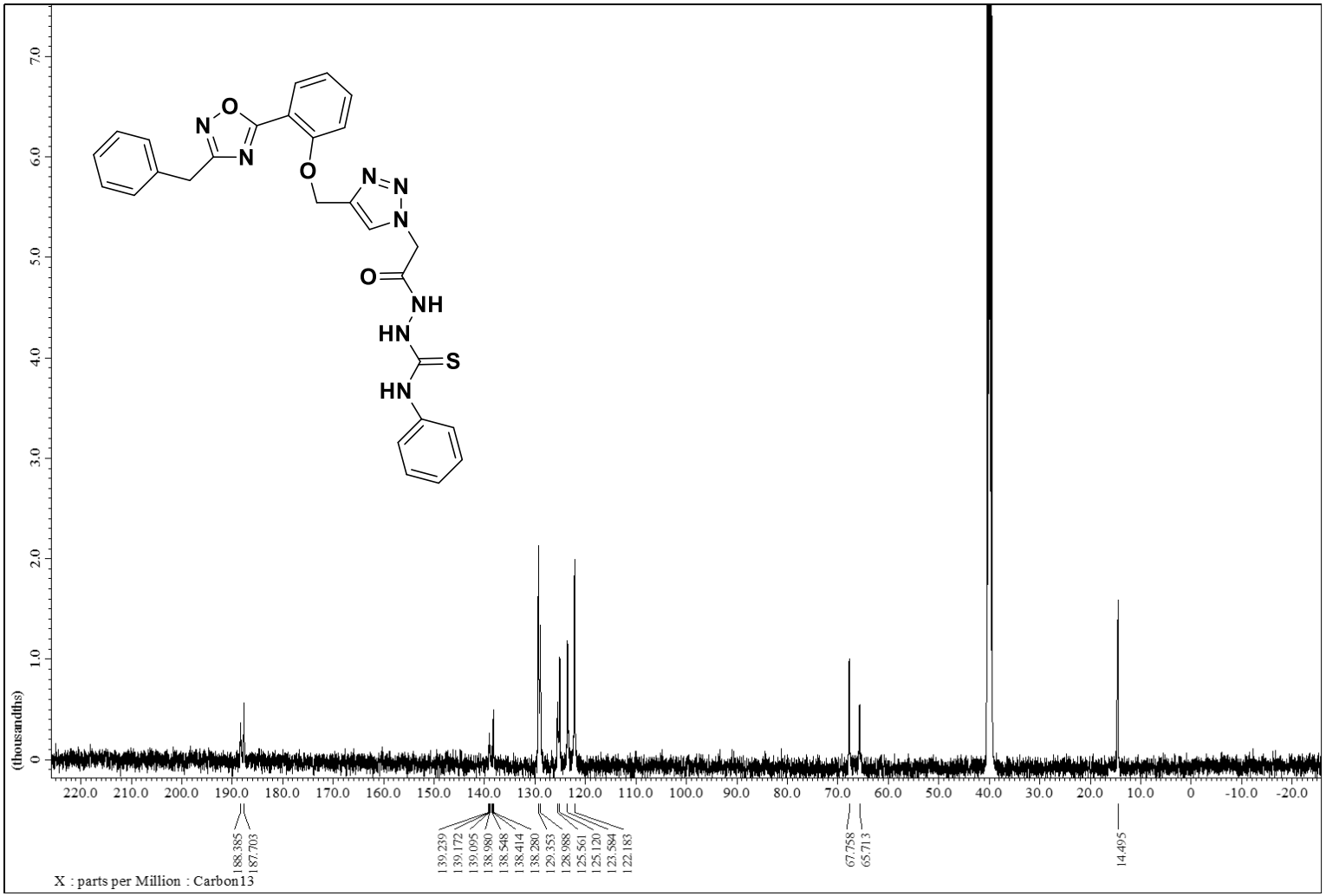

Figure S32. ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of **11b.**

2. Materials and equipments

All reactions were carried out in dried glasswares. Chemicals were purchased NMR spectra were measured using a JEOLJNM ECA 500. The deuterated solvent was used as an internal deuterium lock. ^{13}C NMR spectra were recorded using the UDEFT pulse sequence and broad band proton decoupling at 125 MHz. All chemical shifts (δ) are stated in units of parts per million (ppm) and presented using TMS as the standard reference point. An Alpha Bruker spectrophotometer (Billerica, Massachusetts, USA) was used to measure the FTIR spectra in KBr pellets. Melting points were recorded using Thermo Scientific, Model NO: 1002D, 220-240v; 200 W; 50/60 Hz and are uncorrected. Reaction time was monitored by TLC on Merck silica gel aluminum cards (0.2 mm thickness) with a fluorescent indicator at 254 nm. Visualization of the TLC during monitoring of the reaction was done by UV VILBER LOURMAT 4w-365 nm or 254 nm tube. Elemental analysis was carried out using Perkin-Elmer 2400 Elemental Analyzer: CHN mode.

3. Determination of cytotoxicity of synthetic compounds on normal and cancer human cell line

The cytotoxicity of the studied compounds **5-11** were investigated on Normal human lung fibroblast Wi-38 cell line, human lung cancer cell line A549 (ATCC: CCL-185), or colon cancer cell line Caco-2 (ATCC-HTB-37). Wi-38 cell line was cultured in DMEM medium-contained 10% fetal bovine serum (FBS), seeded as $5x10^3$ cells per well in 96-well cell culture plate and incubated at 37ºC in 5% CO_2 incubator. After 24 h for cell attachment, serial concentrations of the synthetic compounds and standard chemotherapy (doxorubicin) were incubated with Wi-38 cells for 72 h. Cell viability was assayed by MTT method [1, 2]. Twenty microliters of 5 mg/ml MTT (Sigma, USA) was added to each well and the plate was incubated at 37ºC for 3 h. Then MTT solution was removed, 100 µl DMSO was added and the absorbance of each well was measured with a microplate reader (BMG LabTech, Germany) at 570 nm. The dose (EC50 and EC_{100}) value (at 50% and100% cell viability, respectively) of the tested compounds was estimated by the Graphpad Instat software.

References

[1] M. Ferrari, M.C. Fornasiero, A.M. Isetta, *J. Immunol. Methods*, **1990**, *131*, 165.

[2] T. Mosmann, *J. Immunol. Methods*, **1983**, *65*, 55.