Supporting Information for

Design, synthesis, biological evaluation and molecular docking of amide and sulfamide derivatives as *Escherichia coli* pyruvate dehydrogenase complex E1 inhibitors

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1. Assay of *E.coli* PDHc-E1(*in vitro*) and site-directed mutagenesis of PDHc E1

The expressing plasmid pMal-C_{2X}-PDHc E1 was transformed into *E. coli* strain TB1 and inoculated in Luria–Bertani broth containing 2% glucose and 30 mg/ml ampicillin at 37 ℃until reaching a cell density to A600 of 0.6–0.8. Then cells were induced with a final concentration of 0.5 mM IPTG for 7 h at 25℃ before harvesting. Purification of the fusion protein was carried out using a MBP affinity column attached to an AKTA purifier 10 (UPC-F920, GE Healthcare Life Sciences). The concentrations of purified proteins were determined by the method of Bradford[1] using bovine serum albumin (Tiangen) as standard. The final purity (>95%) of the sample was verified by SDS–PAGE and then the purified protein was stored in 50% (v/v) glycerol at -20℃. The inhibitory activities of synthesized compounds were measured by the enzymatic assay. PDHc-E1 activity was assayed by a modified method of N. Nemeria[2], and measured by monitoring the reduction of 2,6-DichloroPhenolindophenol (2,6-DCPIP) at 600 nm using a microplate reader (BioTek Synergy 2, USA). The total volume of 100 μL reaction mixture contained 50 mM K_3PO_4 , pH 7.2, 2.0 mM sodium pyruvate as substrate, 0.8 mM 2,6-DCPIP, 7.1 μM enzyme and different concentration of inhibitors. The reaction mixtures were incubated for 3 min at 37 ℃, then different concentrations of ThDP (ranging from 0 to 200 μM) were added to the initiate reaction. To determine the inhibitor concentration of synthesized compounds at 50% inhibition (IC_{50}), initial rate data taken at saturating substrate, fixed effectors, and systematically varied inhibitor concentrations were fit to Hill equation, $V = V_0-(V_0-V_\infty)/((IC_{50}/I)^n + 1)$.[3] Where V, V_0 , and V_∞ are the velocity, maximum velocity (at $I = 0$), and the limiting velocity (at I saturating); n is the Hill coefficient associated with the inhibitor; and IC_{50} is the inhibition concentration of synthesized compounds at 50% inhibition. Each experiment was performed at least three times. All kinetic data were fit to the growth/sigmoidal model from origin 7.0 software. One unit of activity is defined as the amount of 2,6-DCPIP reduced (μmol/min/mg of PDHc-E1).

Site-directed mutagenesis of PDHc E1 was accomplished by the introduction of specific base changes into a double-stranded DNA plasmid, as described previously. DNA encoding of the wild-type PDHc E1 cloned into the pMAL-C_{2x} -PDHc-E1 was used as a template for mutagenesis. The standard PCR mixture contained 50–100 ng of template DNA and 100–200 ng of each mutagenizing primer. The methylated plasmid was digested with DpnI, and 4 μL of each reaction was used to transform the DH5α competent cells. All mutations were confirmed by DNA sequencing. Verified plasmids containing the desired mutations were transformed into the E. coli TB1 strain. The mutant PDHc E1 proteins were purified in the same manner as the wild-type PDHc E1.

2. Fluorescence spectral analyses

Fluorescence spectral analyses were carried out as described previously[4]. All fluorescent measurements were carried out on a Fluore Max-P fluorescence spectrophotometer (HORIBAJOBIN YVON, France) equipped with a xenon lamp source and 1.0 cm quartz cell. The emission spectrum was recorded in the 305–500 nm range with excitation at 290 nm. The fluorescence quenching experiments of PDHc E1 or its mutants (2 μM) were performed at different concentrations of compounds by applying a 1 cm path length cuvette. The appropriate blank measurement corresponding to the buffer was subtracted to correct the background fluorescence. The binding constant (*K*) was calculated according to the equation: $\ln(F_0 - F)/F = \ln K + n\ln Q$, where F_0 and F are the fluorescence intensities without and with the ligand, respectively. Term [Q] denotes the concentration of the quencher. A plot of $\ln[(F_0 - F)/F]$ vs. ln[Q] gave a straight line using least squares analysis. The Y-intercept was equal to ln*K* (where *K* is equal to the binding constant).

3. Inhibitory bacterial activity and fungal activity evaluation of compounds

The antifungicidal activities of a part of compounds were tested in vitro against against *G. zeae, R. solani, B. cinerea* and *A. solani,* and antibacterial activity against *Xanthimonas oryzae pv*. *Oryzae* (*Xoo*) *Acidovorax avenae subsp.* Avenae *(Aaa)* and their relative inhibitory ratio (%) had been determined by using the mycelium growth rate method[5]. A set amount of each sample was dissolved in dimethylsulfoxide to which a drop of emulsifier, Tween 80, was added. The solution was then diluted in water until it reached the concentrations required. The final concentration of the compounds **5** and **9** in the medium was tested at 100 μgmL^{-1} and 500 μgmL^{-1}. The solutions (1.5 mL) were mixed rapidly with thawed potato glucose agar culture medium (9 cm) under 50℃The mixtures were poured into Petri dished. After the dishes were cooled, the solidified plates were incubated with 5 mm mycelium disk, inverted, and incubated at 28 ℃ for 48 h. The mixed edium without sample was used as the blank control. Three replicates of each test were carried out. The mycelial elongation radium (mm) of fungi settlements was measured after 48 h of culture. The growth inhibition rates were calculated with the following equation: I = [(C-T)/C]*100%. Here, I is the growth inhibition rate (%), C is average diameter of mycelia in the blank control, T is the average diameter of mycelia in the presence of those compounds. The inhibition ratio of those compounds was summarized in **Table 2 and 3**.

References:

[1] M. M. Bradford, Anal. *Biochem*. 1976, **72**, 248-254..

[2] N. Nemeria, Y. Yan, Z. Zhang, A. M. Brown, P. Arjunan, W. Furey, J. R. Guest, F. Jordan, *J. Biol. Chem*. 2001, **276**, 45969-45978.

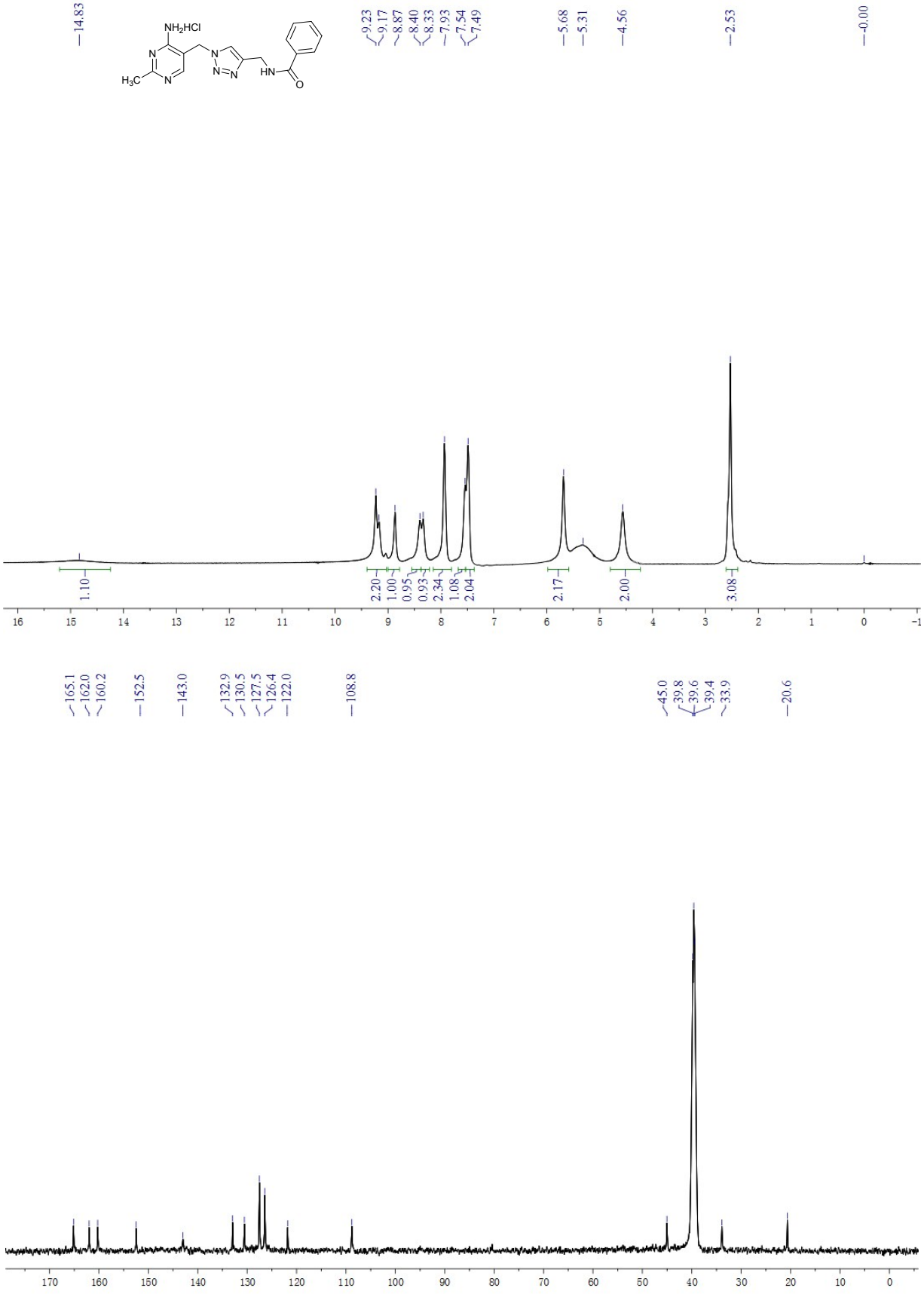
[3] J. K. Hines, C. E. Kruesel, H. J. Fromm, R. B. Honzatko, *J. Biol. Chem*. 2007, **282**, 24697.

[4] Y. Sun, X. Liao, D. Li, L. L. Feng, J. Li, X. F. Wang, J. Jin, F. Yi, L. Zhou and J. Wan, *Spectrochim. Acta, Part A*, 2012, **89**, 337-344.

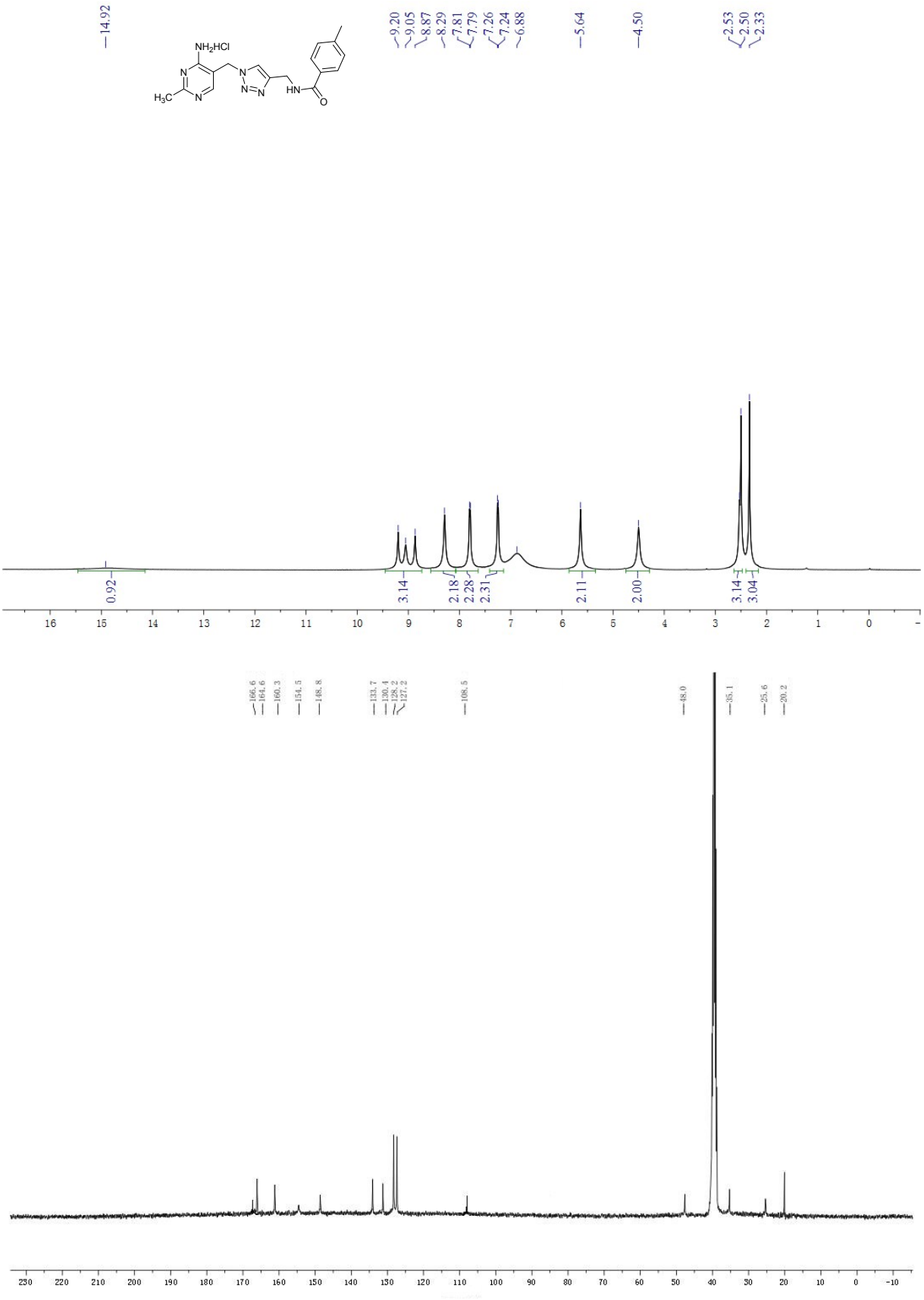
[5] N. C. Chen, Bioassay of Pesticides; Beijing Agricultural University Press: Beijing, China, 1991. 161–162.

4. ^{1}H and ^{13}C NMR Spectra of 5a-e, 9a-k and 13

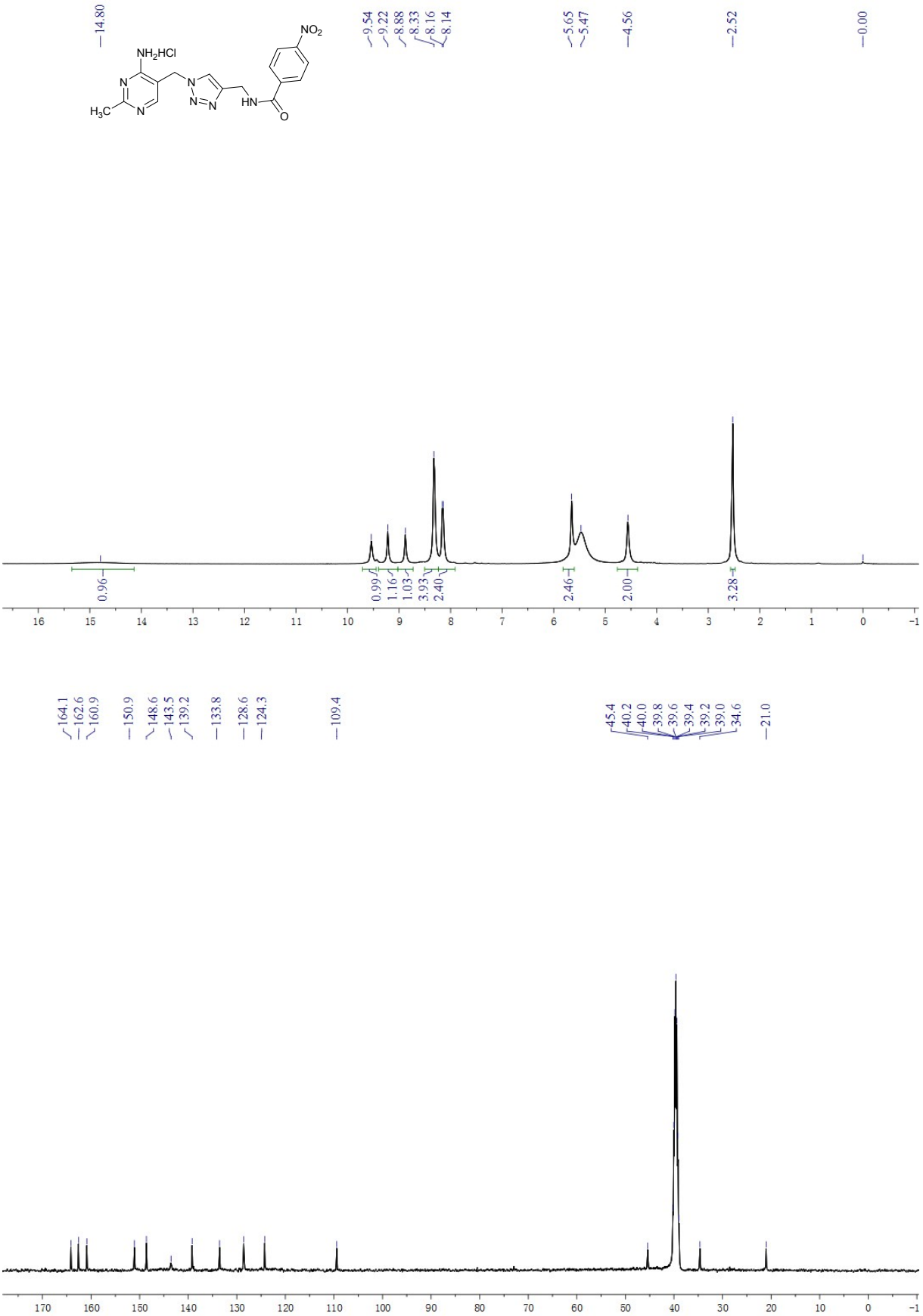
^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 5a



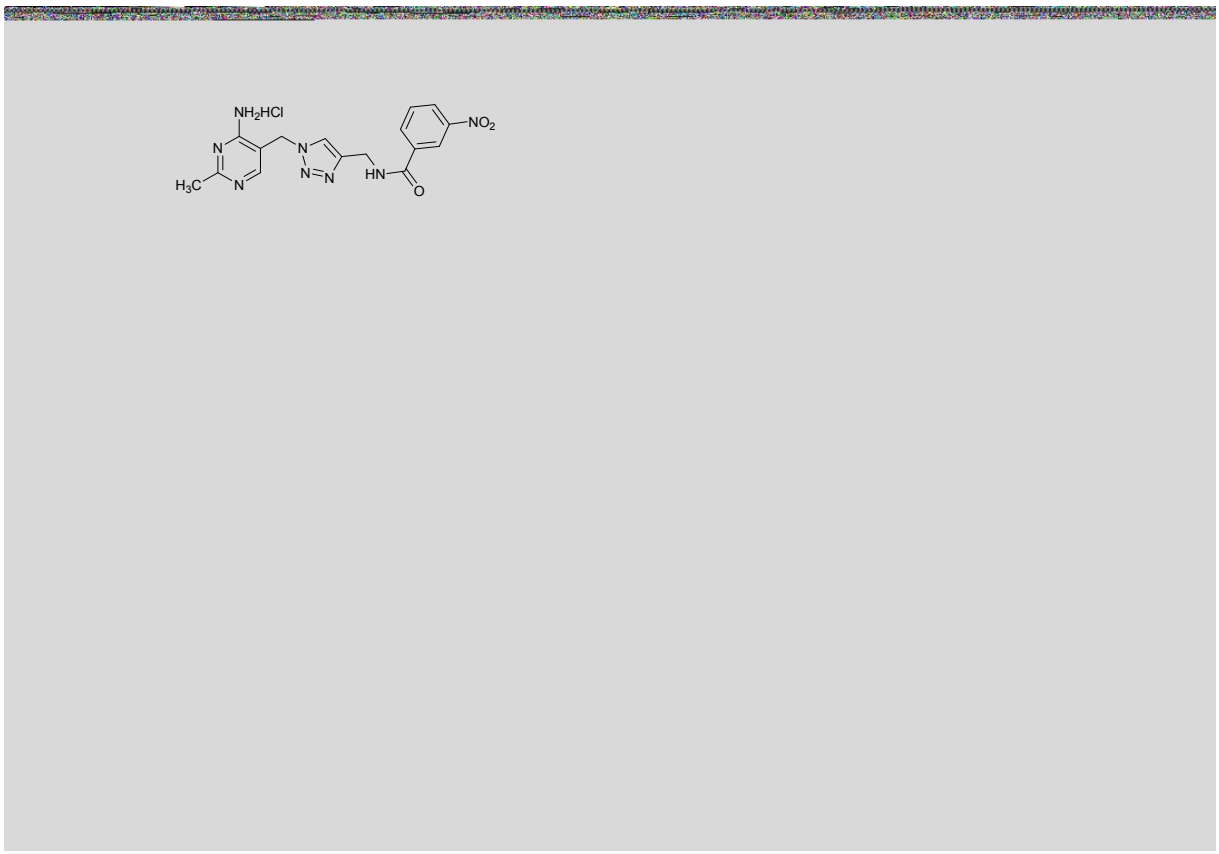
^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 5b

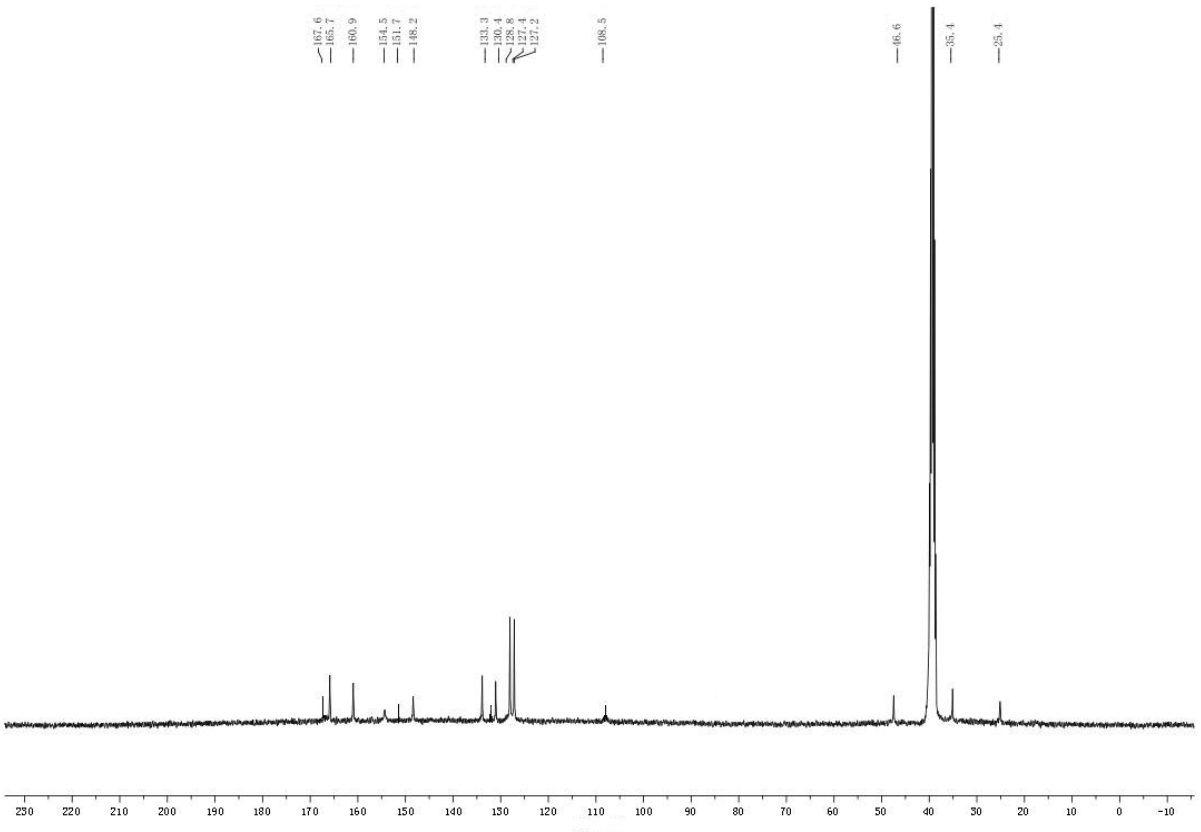


^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 5c



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 5d





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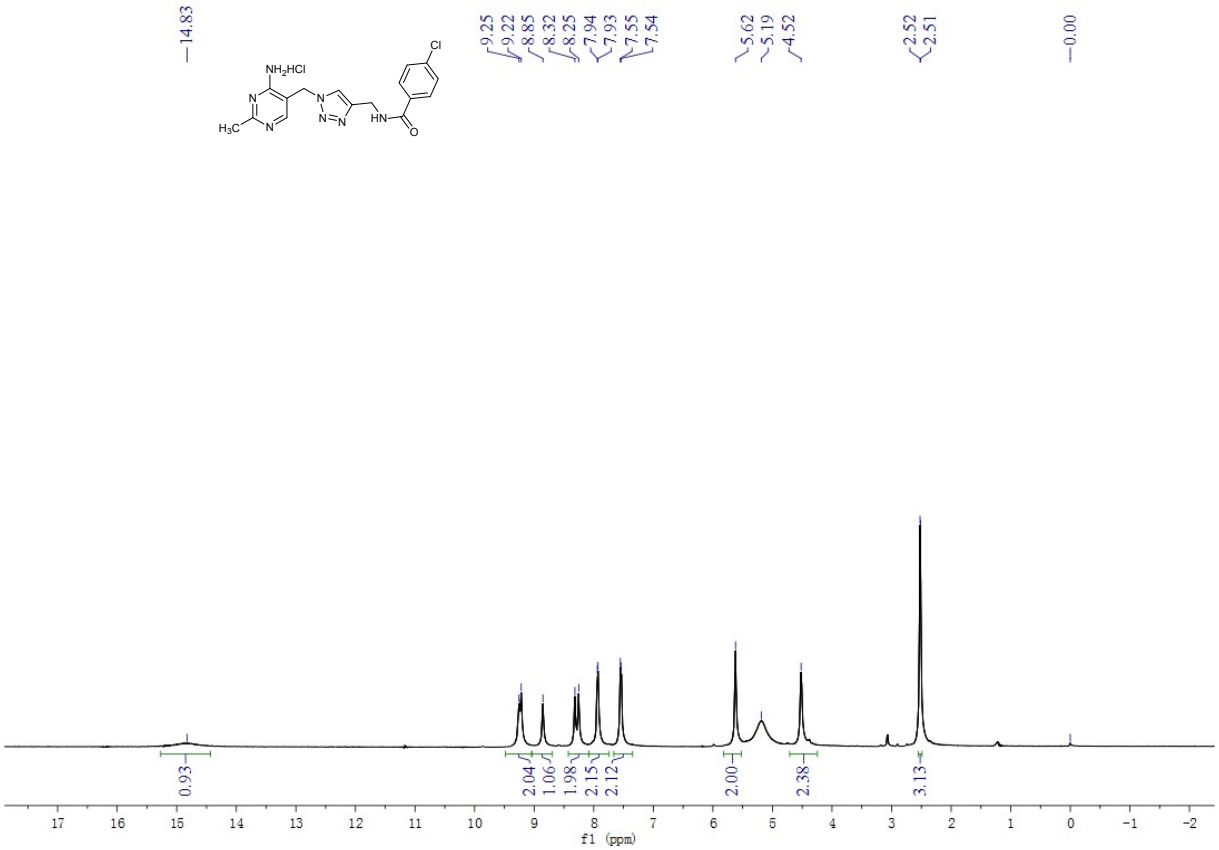
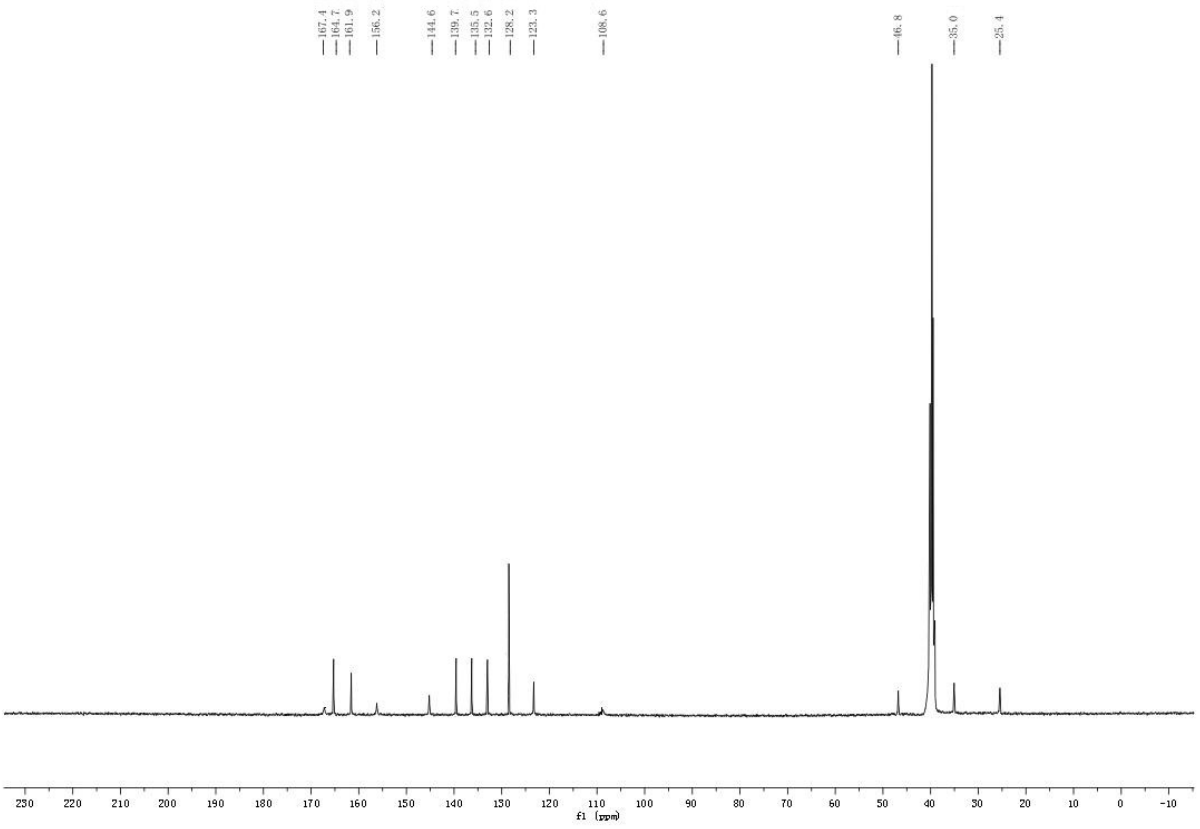
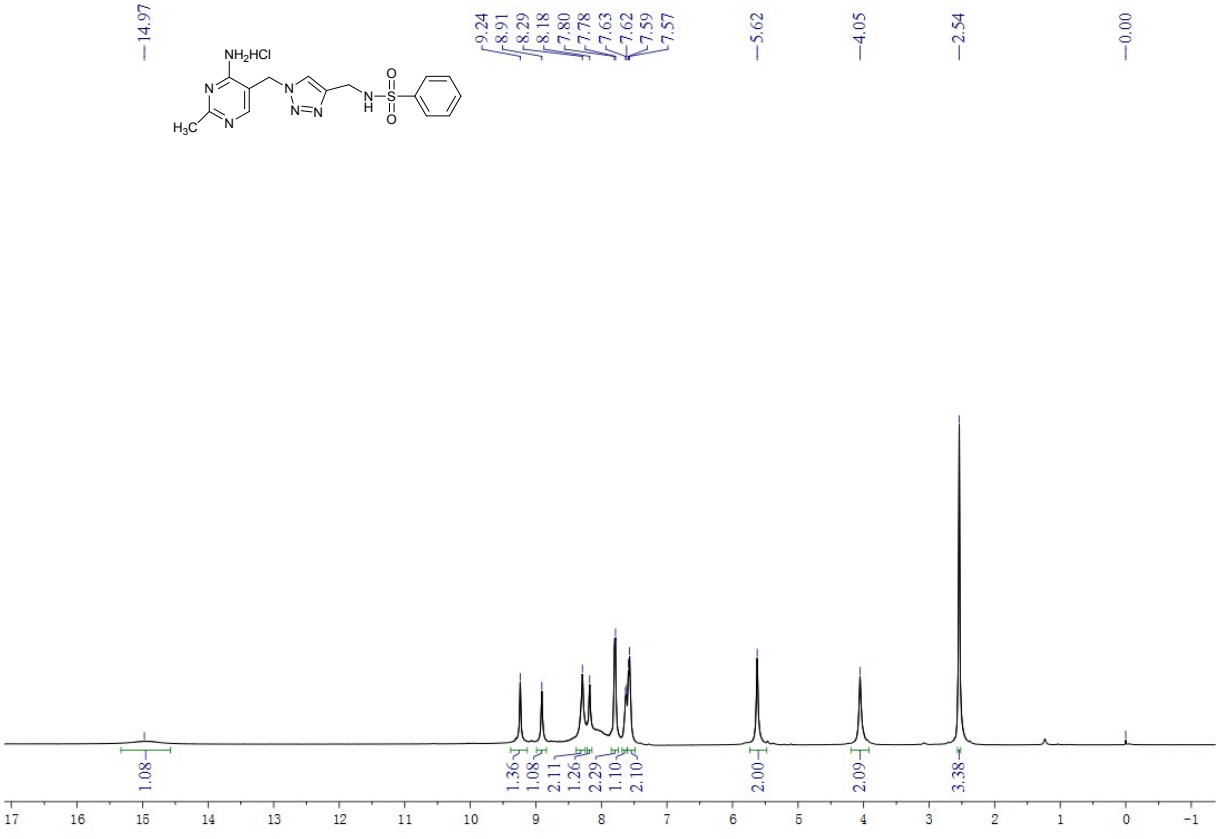
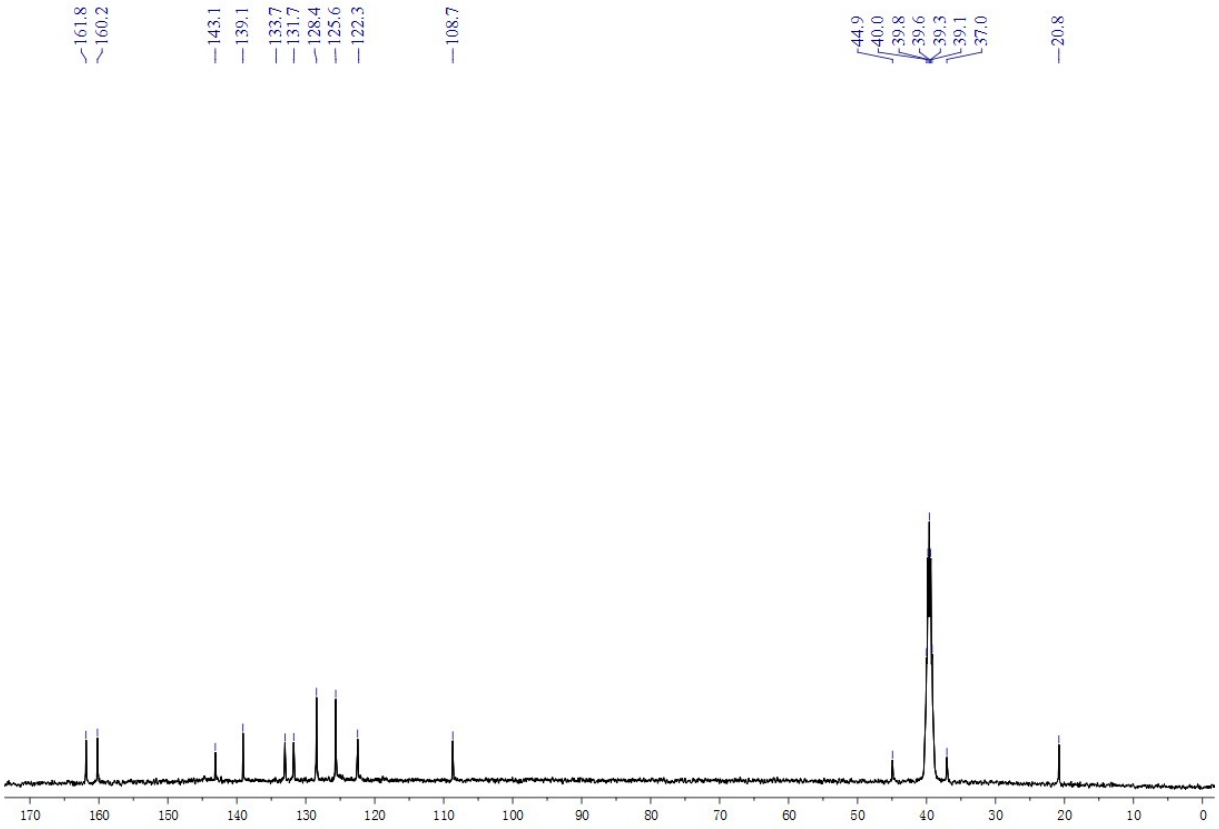


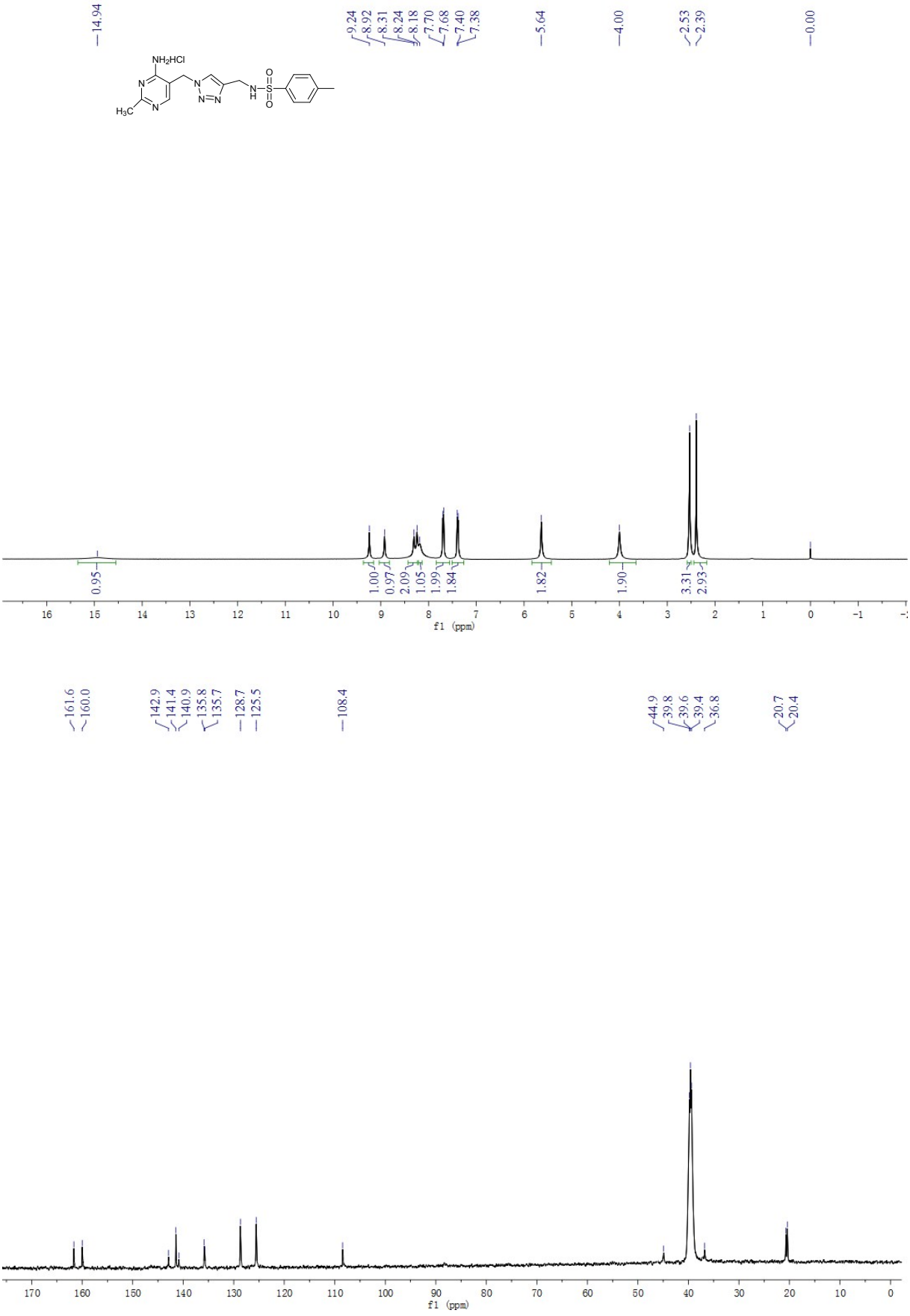


^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 9a

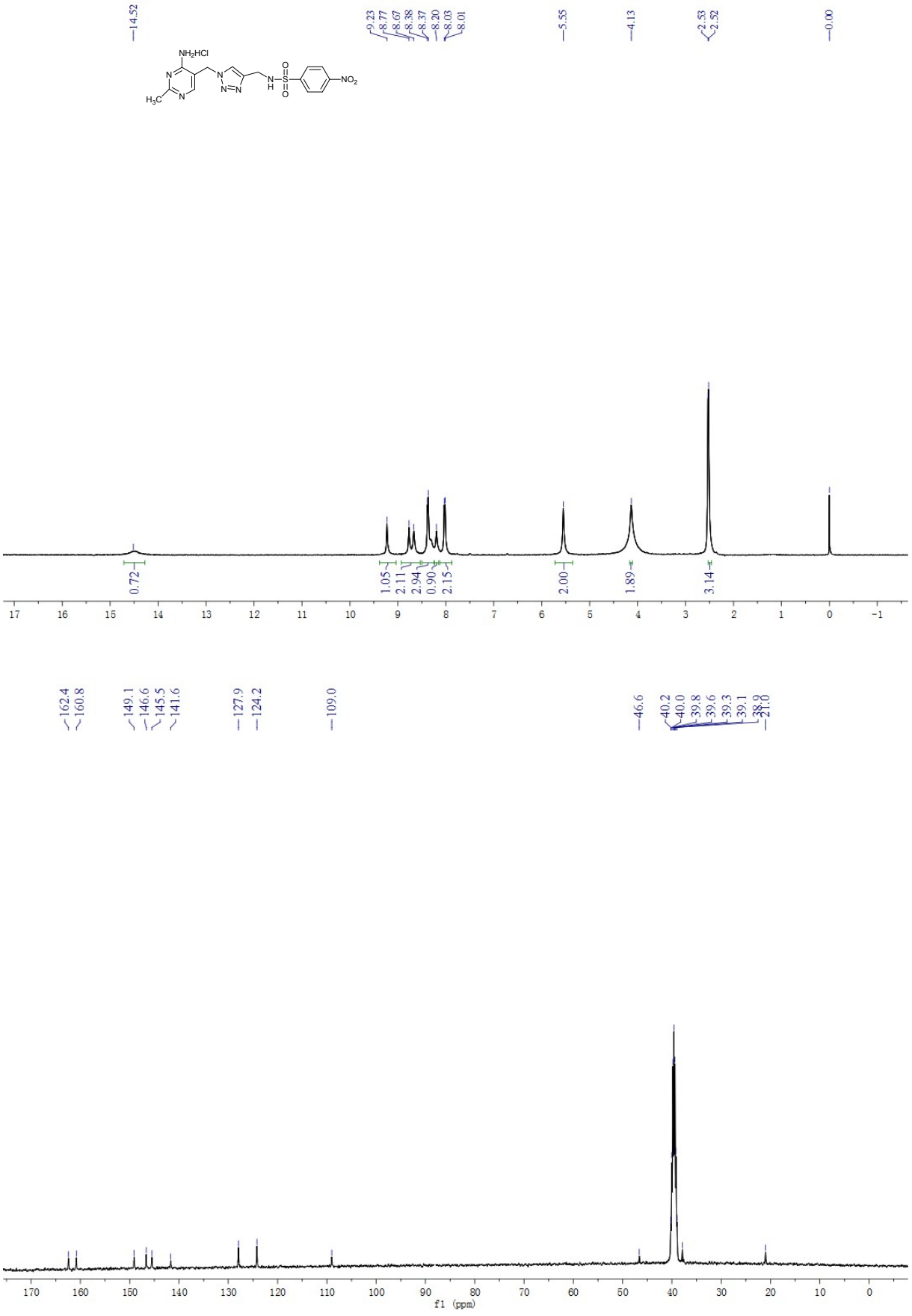




^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 9b



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 9c



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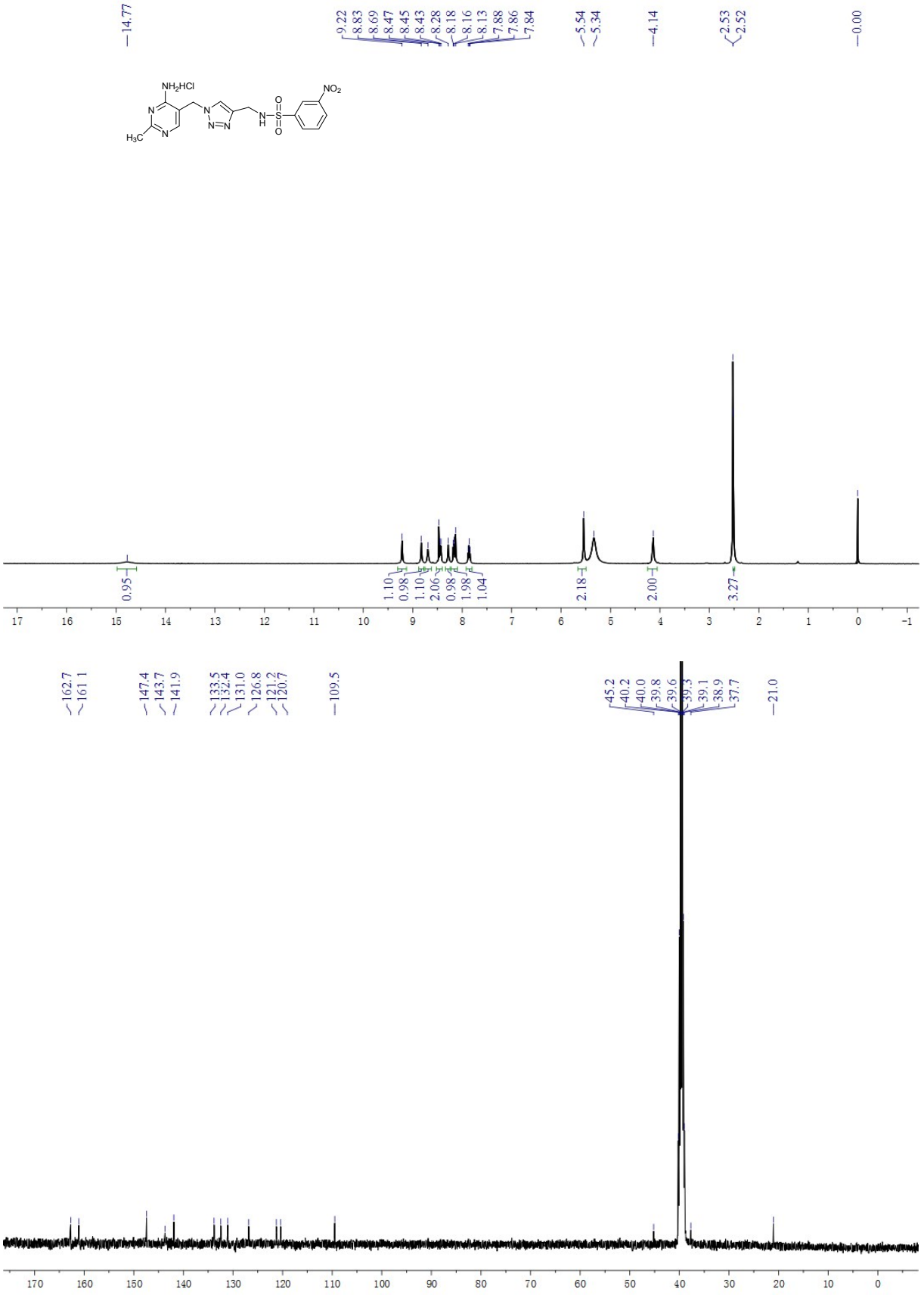
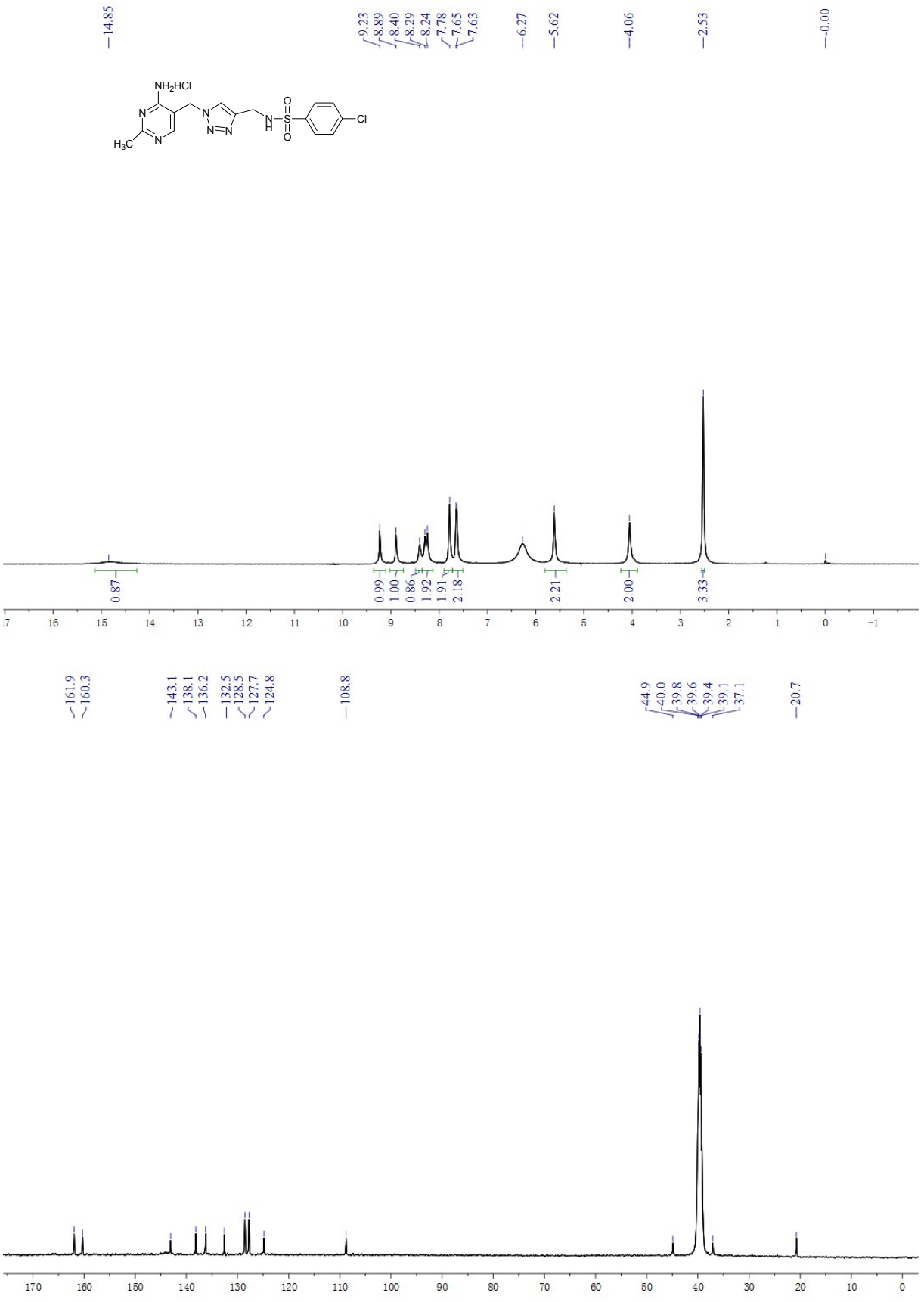
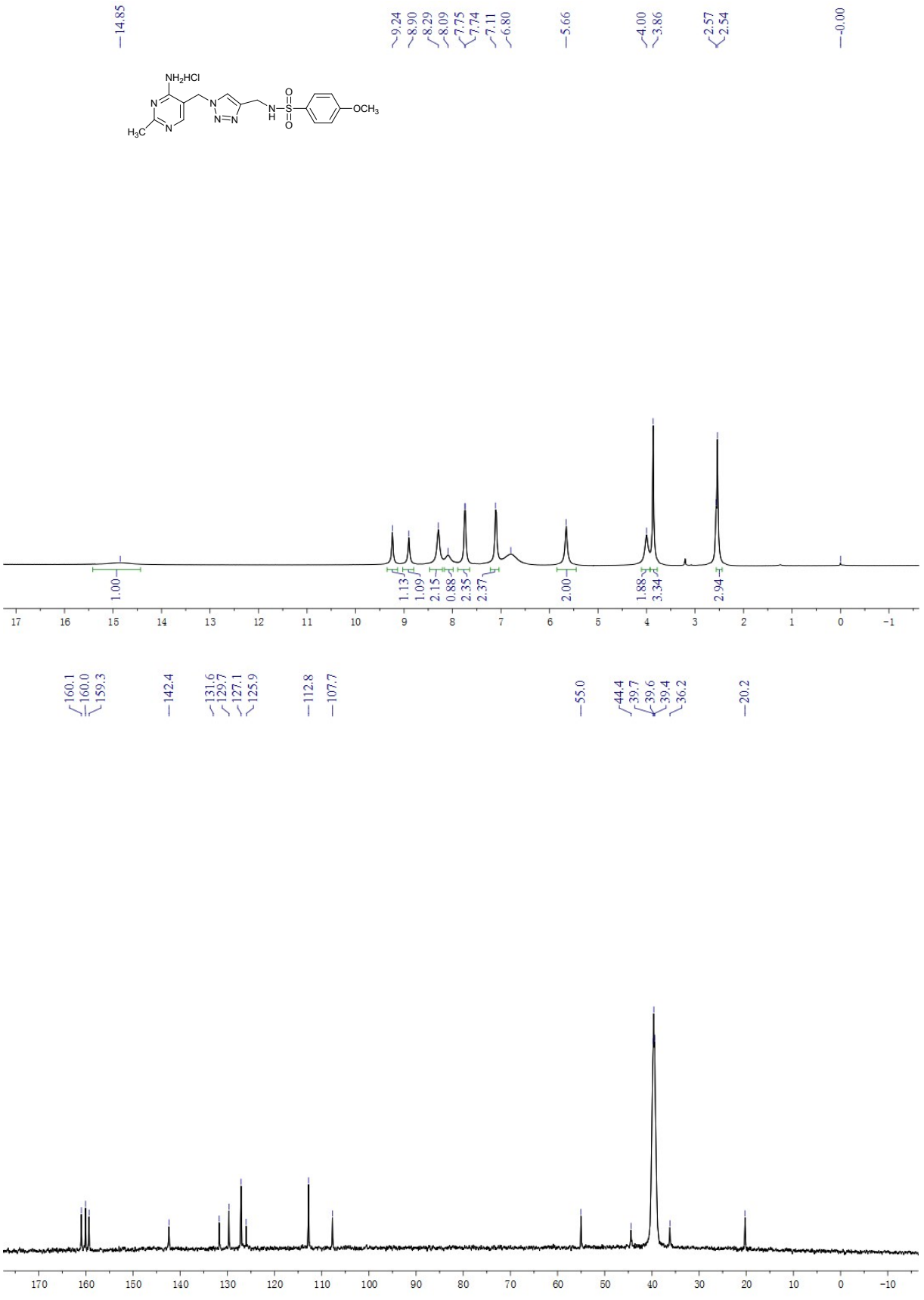


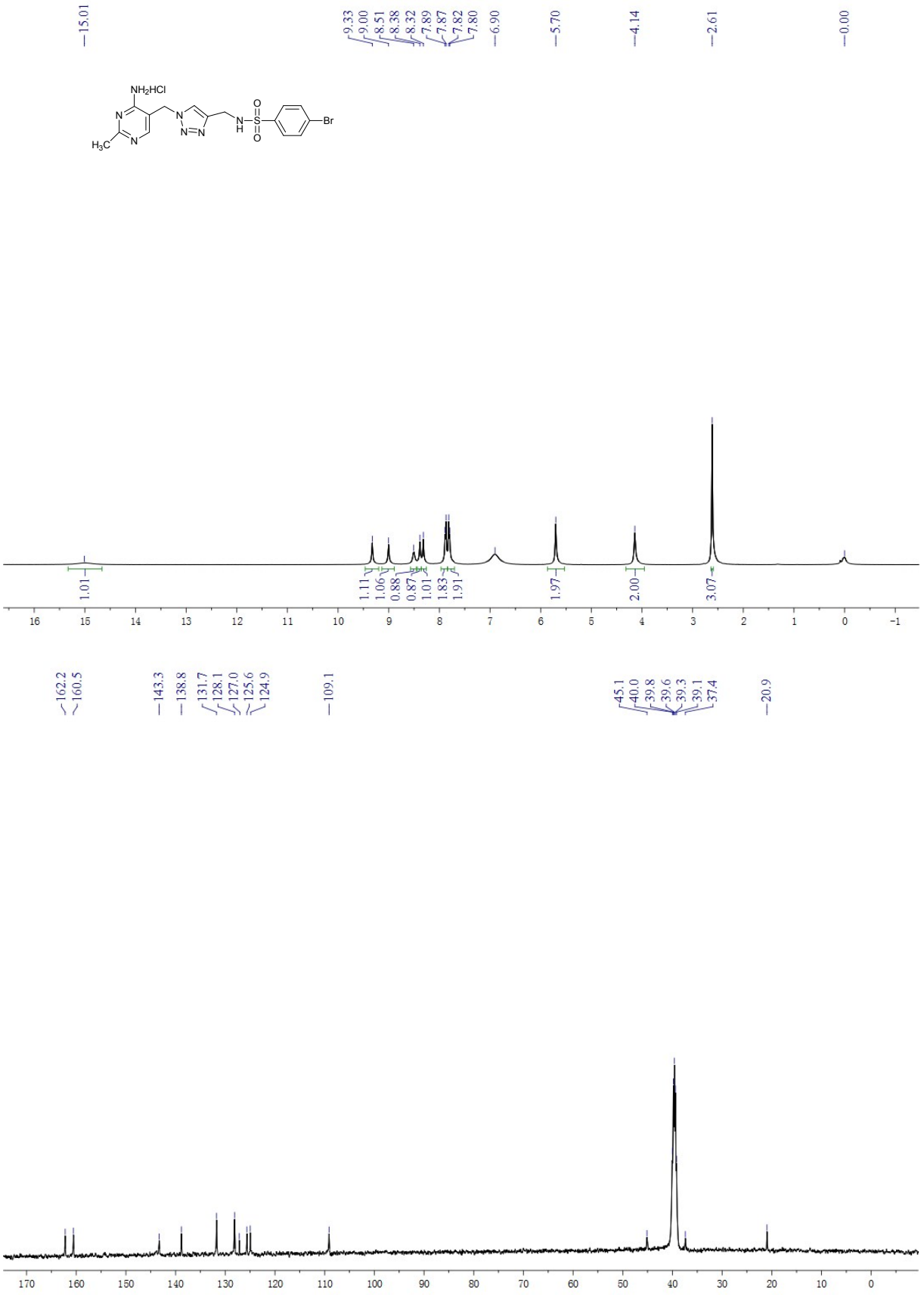
^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}CNMR (100 MHz, DMSO-d_6) spectrum of 9e



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 9f



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 9g



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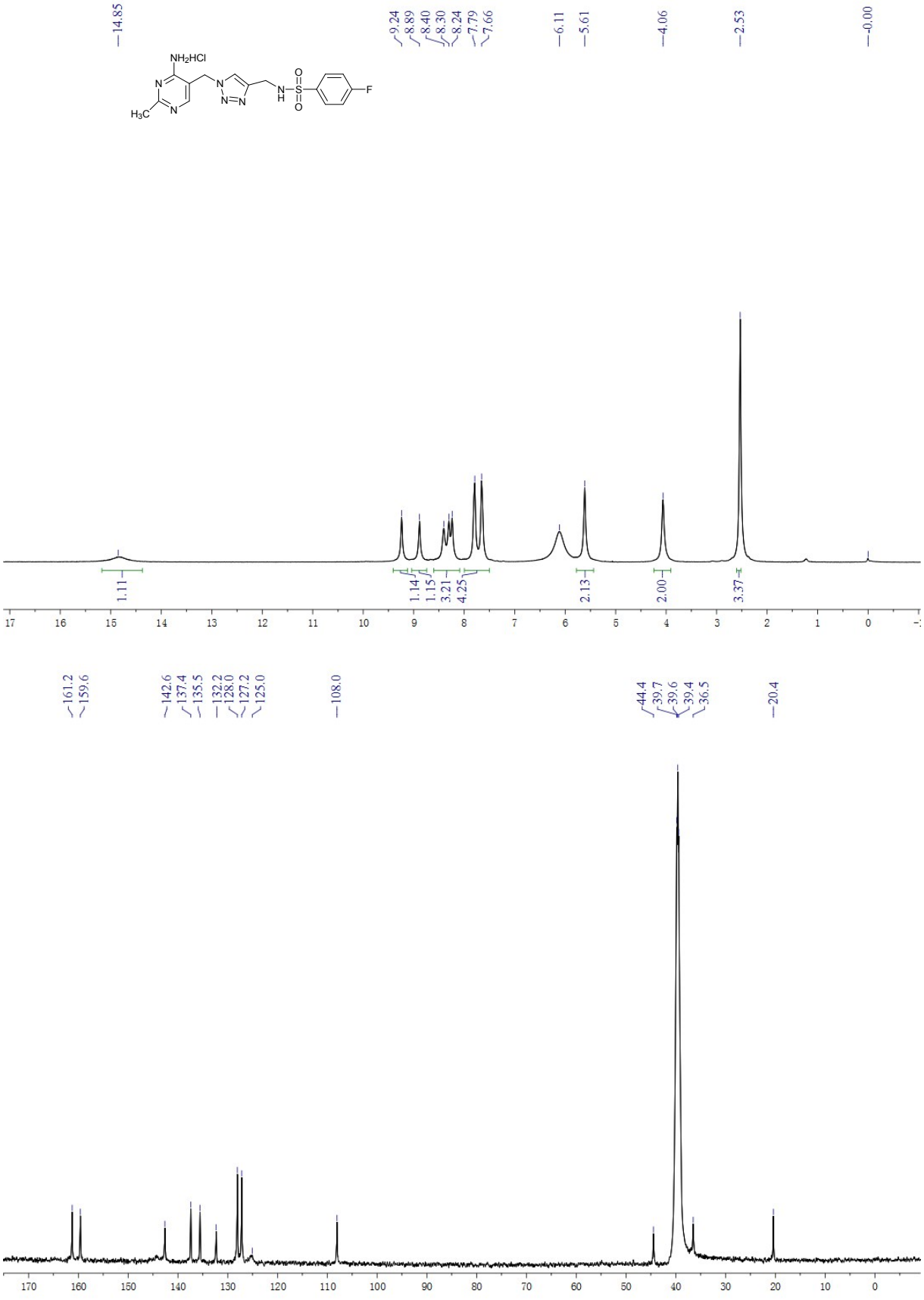
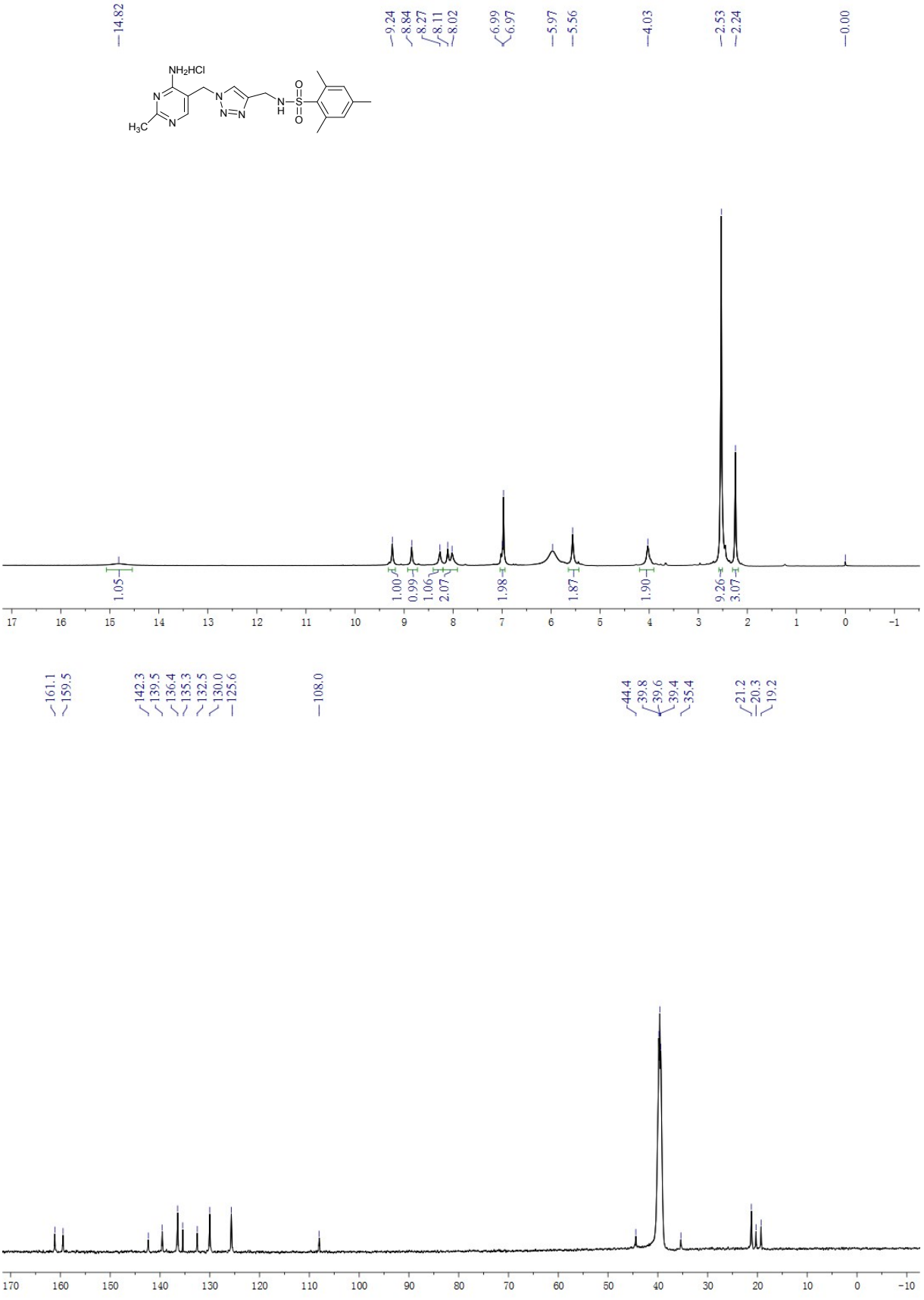
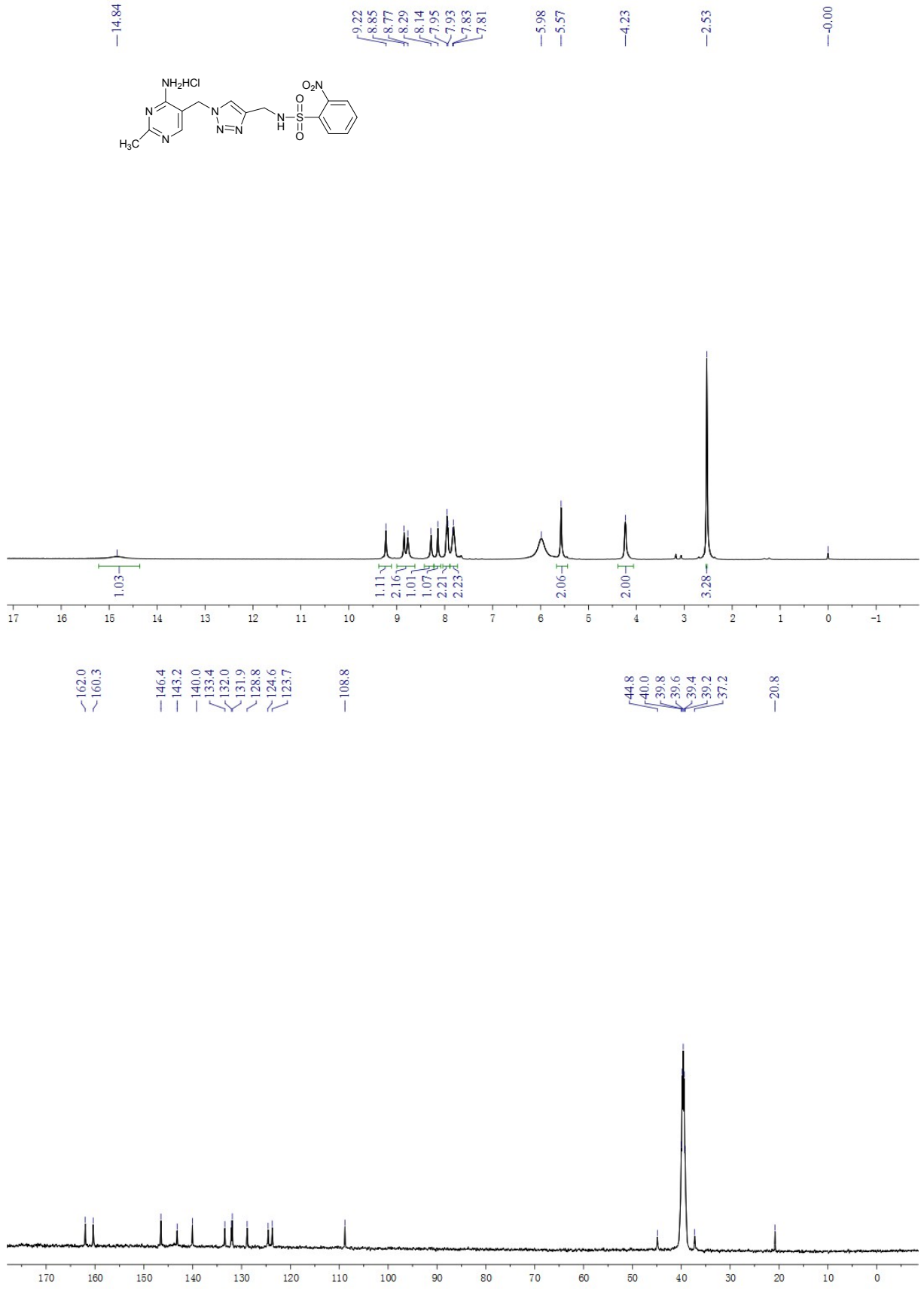


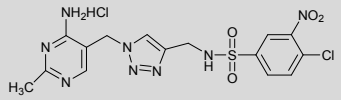
^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 9i



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 9j



^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 9k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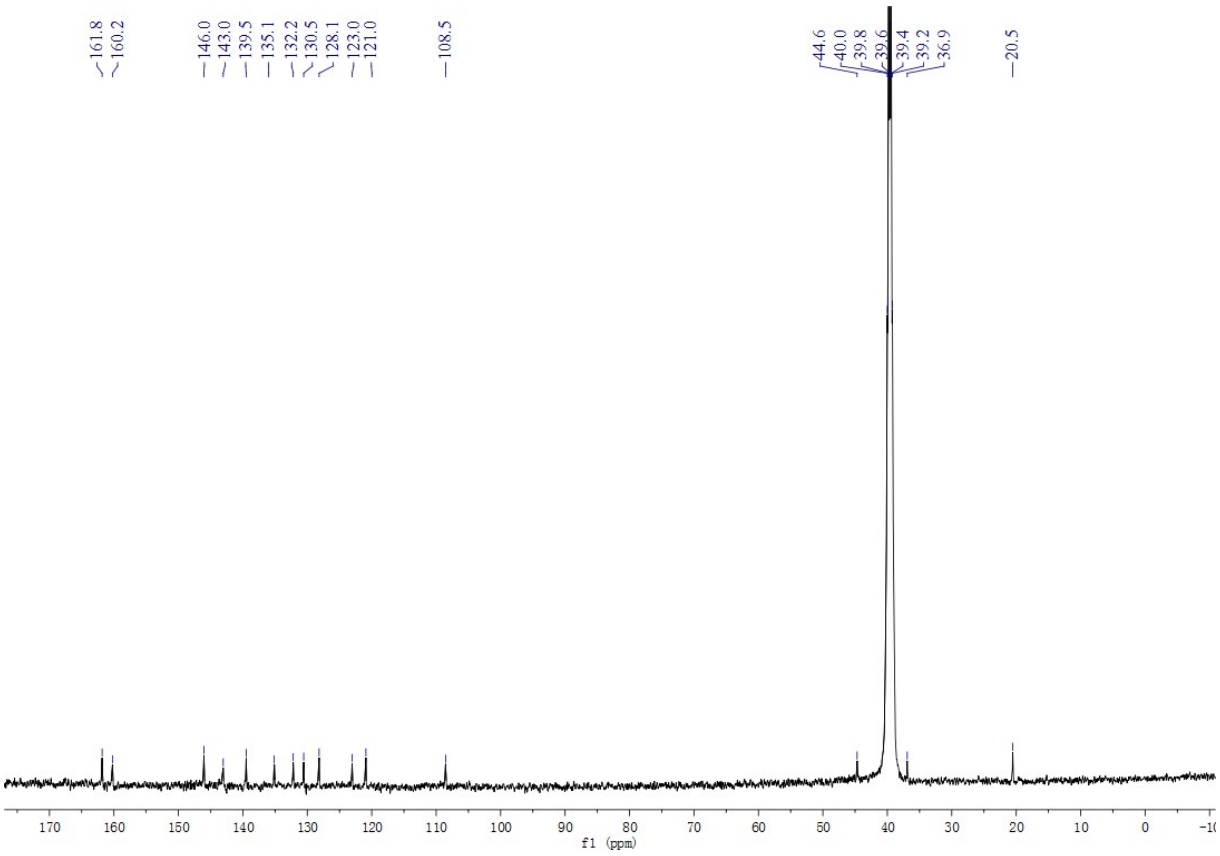
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40.0
39.8
39.6
39.4
39.2
36.9
20.5
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160
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140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of 13