SUPPLEMENTAL MATERIALS

for

Novel 4-Oxoquinazoline-Based N-Hydroxypropenamides as Histone Deacetylase Inhibitors: Design, Synthesis and Biological evaluation

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1. Chemistry

Thin layer chromatography which was performed using Whatman® 250 μm Silica Gel GF Uniplates and visualized under UV light at 254 and 365 nm, was used to check the progress of reactions and preliminary evaluation of compounds' homogeneity. Melting points were measured using a Gallenkamp Melting Point Apparatus (LabMerchant, London, United Kingdom) and are uncorrected. Purification of compounds was carried out using crystallization methods and/or open silica gel column flash chromatography employing Merck silica gel 60 (240 to 400 mesh) as stationary phase. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 500 MHz spectrometer with DMSO-d_6 as solvent unless otherwise indicated. Tetramethylsilane was used as an internal standard. Chemical shifts are reported in parts per million (ppm), downfield from tetramethylsilane. Mass spectra with different ionization modes including electron ionization (EI), Electrospray ionization (ESI), were recorded using PE Biosystems API2000 (Perkin Elmer, Palo Alto, CA, USA) and Mariner® (Azco Biotech, Inc. Oceanside, CA, USA) mass spectrometers, respectively. The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. All reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were used directly as purchased unless otherwise indicated.

2. HDAC enzymes assay

The HDAC enzymes (Hela cell nuclear extract) were purchased from Enzo Life Sciences Inc. (Farmingdale, New York, USA). The HDAC enzymatic assay was performed using a Fluorogenic HDAC Assay Kit (Enzo Life Sciences Inc.) according to the manufacturer's instructions. Briefly, HDAC enzymes were incubated with vehicle or various concentrations of the assayed samples or SAHA for 30 min at 37^{o}C in the presence of an HDAC fluorimetric substrate. The HDAC assay developer (which produces a fluorophore in reaction mixture) was added, and the fluorescence was measured using VICTOR (PerkinElmer, Waltham, MA, USA) with excitation at 360 nm and emission at 460 nm. The measured activities were subtracted by the vehicle-treated control enzyme activities and IC_{50} values were calculated using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

3. HDAC6 enzymes assay

The HDAC6 enzyme assay Kit were purchased from BPS Bioscience (San Diego, CA, USA). The enzymatic HDAC assay was performed according to the manufacturer's guidance. In brief, the HDAC6 enzymes were incubated with vehicle or various concentrations of test samples or SAHA for 30 min at 37^{o}C in the presence of an HDAC fluorimetric substrate. The HDAC assay developer (which produces a fluorophore in reaction mixture) was then added,

and the fluorescence was measured using VICTOR3 (PerkinElmer, Waltham, MA, USA) with excitation at 360 nm and emission at 460 nm. The measured activities were subtracted by the vehicle-treated control enzyme activities. Calculation of IC_{50} values were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

4. Molecular docking studies

London function express binding energy and affinity scoring:

The London function express binding energy (E_score1) as a sum of interaction contributions, including the average gain or loss of rotational-translational entropy, the energy term describing the loss of ligand flexibility, H-bond energy, metal ligation energy, and the difference in desolvation energies for each atom. Meanwhile, the affinity scoring function estimate the binding energy (E_score2) by a linear combination of the enthalpic contributions of the hydrophobic, ionic, metal ligation, atom pair and H-bond contacts. Molecular Operating Environment (MOE), Version 2009.10, Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, 2016. <http://www.chemcomp.com>

5. Spectra

ALL ^{1}H & ^{13}C NMR SPECTRA OF THE COMPOUNDS

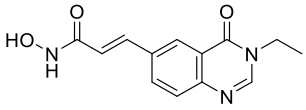


Figure S1. ^{1}H NMR of compound 9a

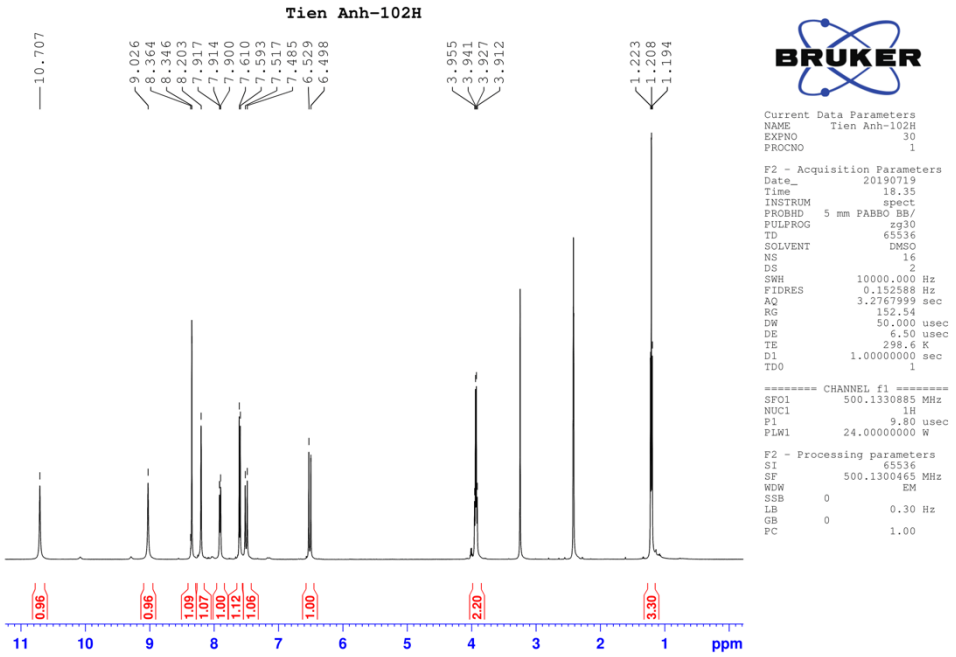
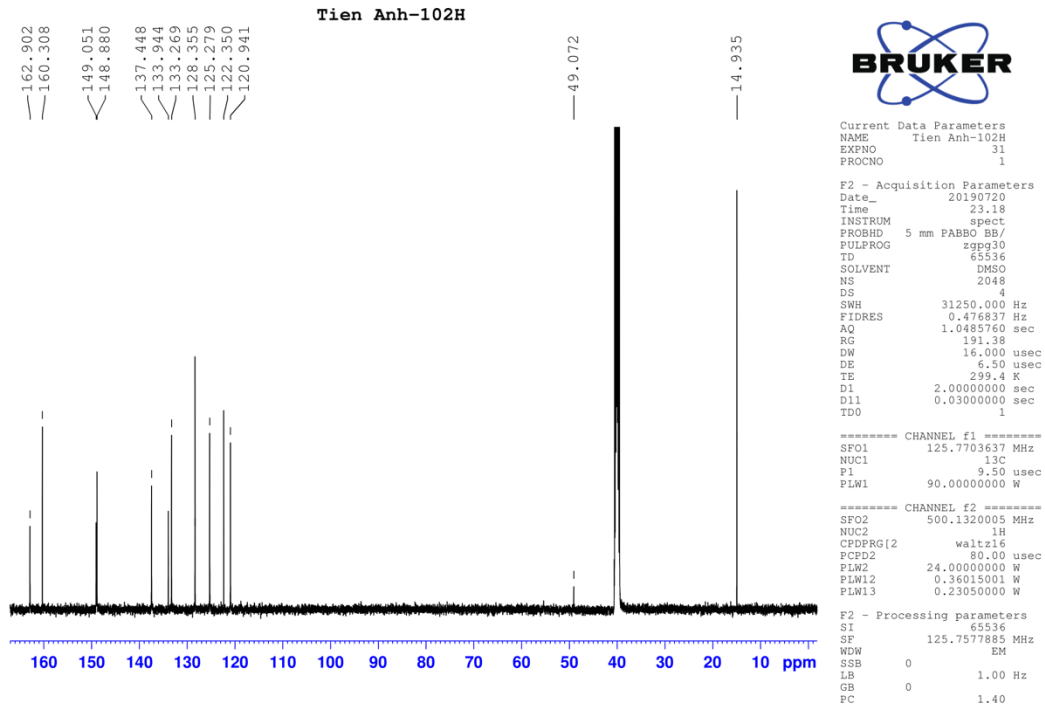


Figure S2. ^{13}C NMR of compound 9a



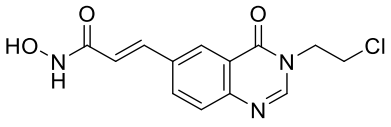


Figure S3. ^{1}H NMR of compound 9b

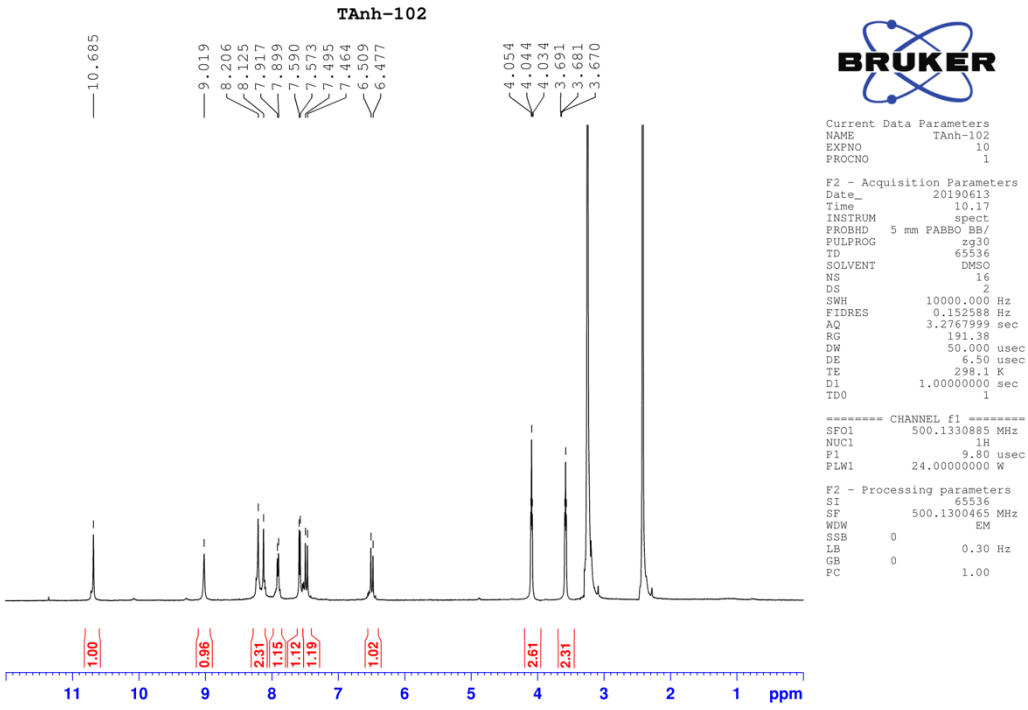
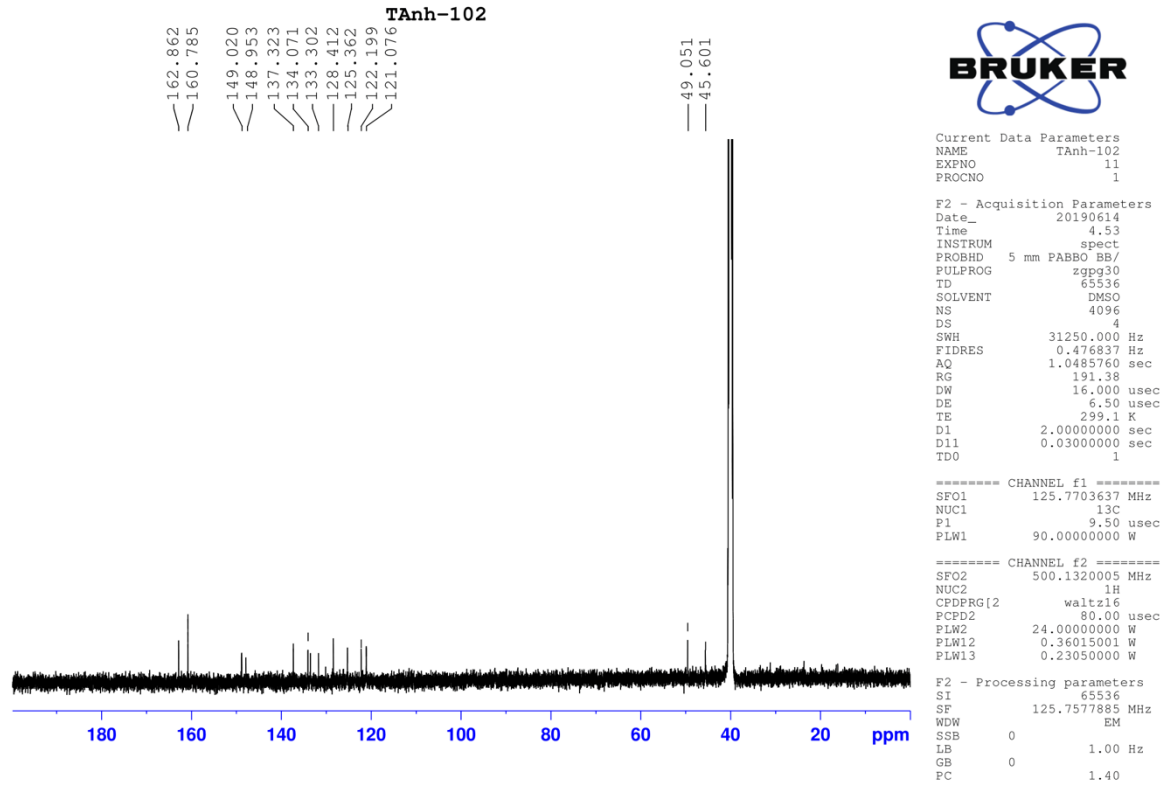


Figure S4. ^{13}C NMR of compound 9b



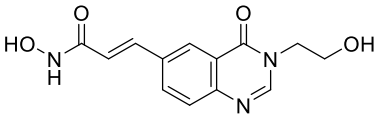


Figure S5. ^{1}H NMR of compound 9c

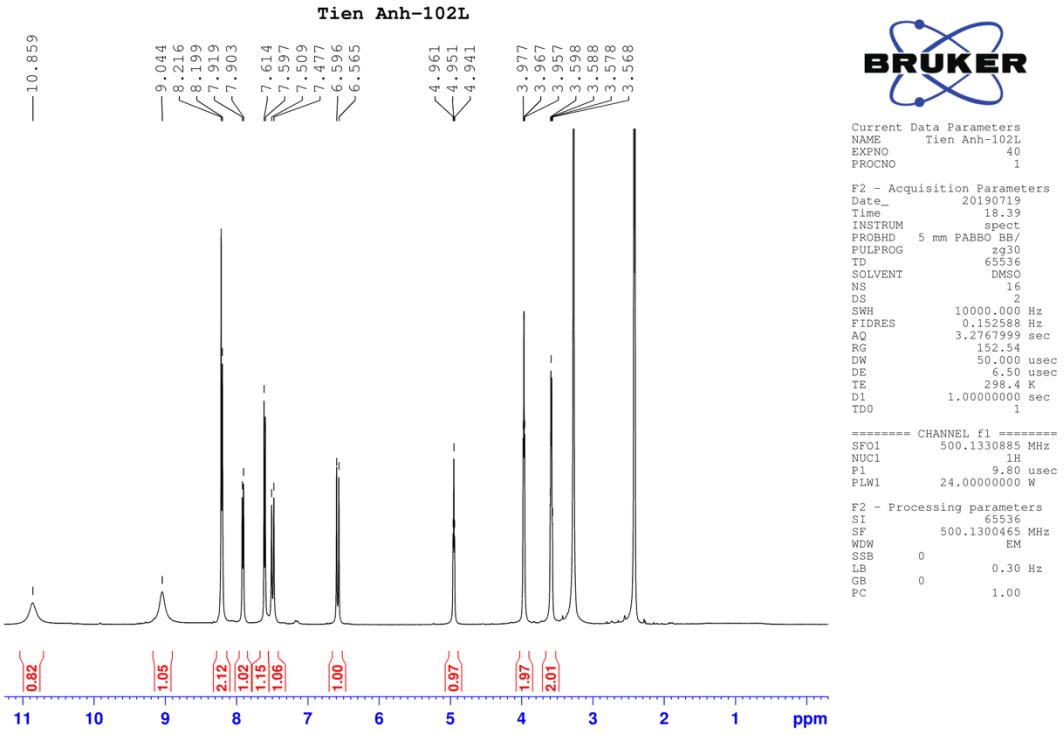
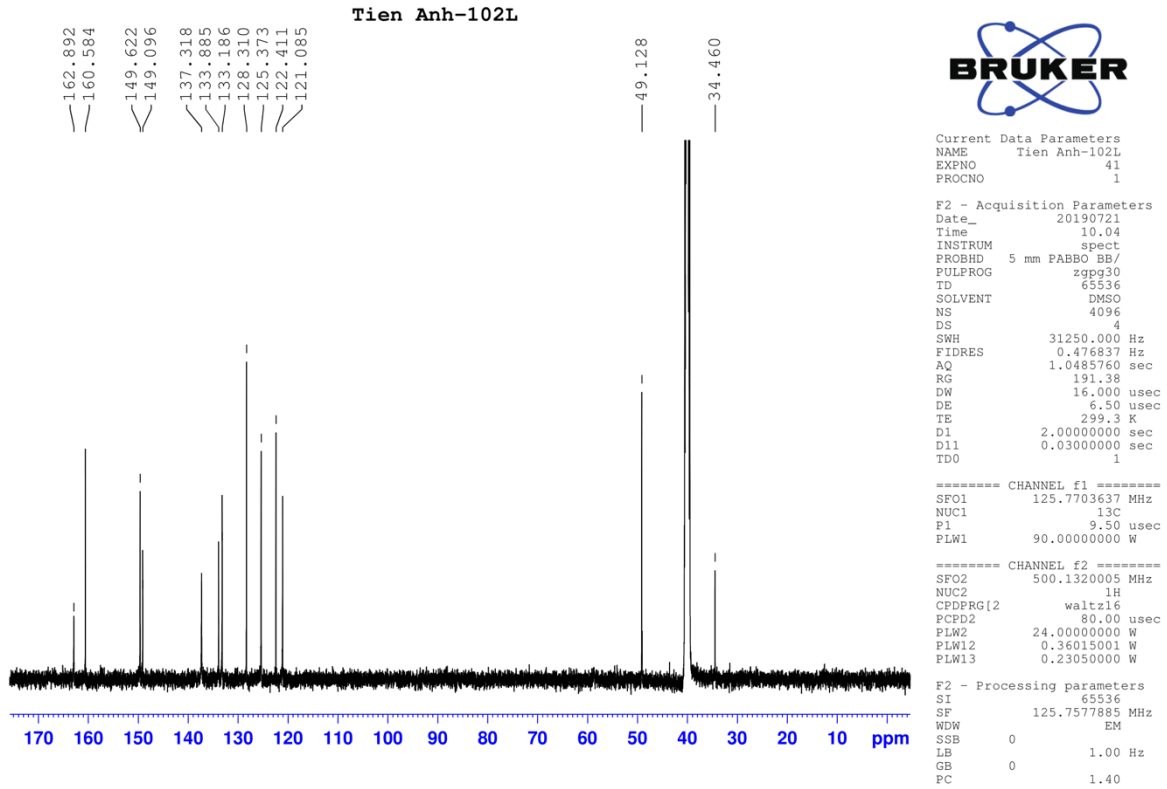


Figure S6. ^{13}C NMR of compound 9c



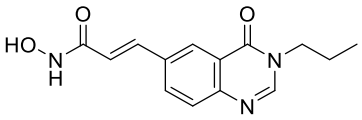


Figure S7. ^{1}H NMR of compound 9d

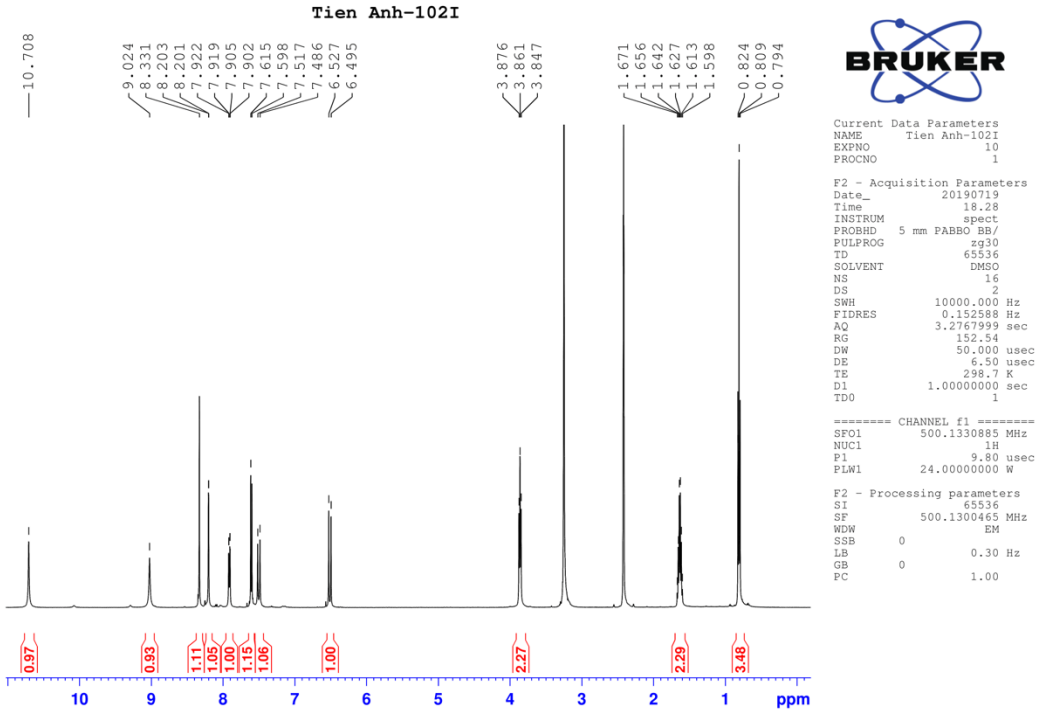
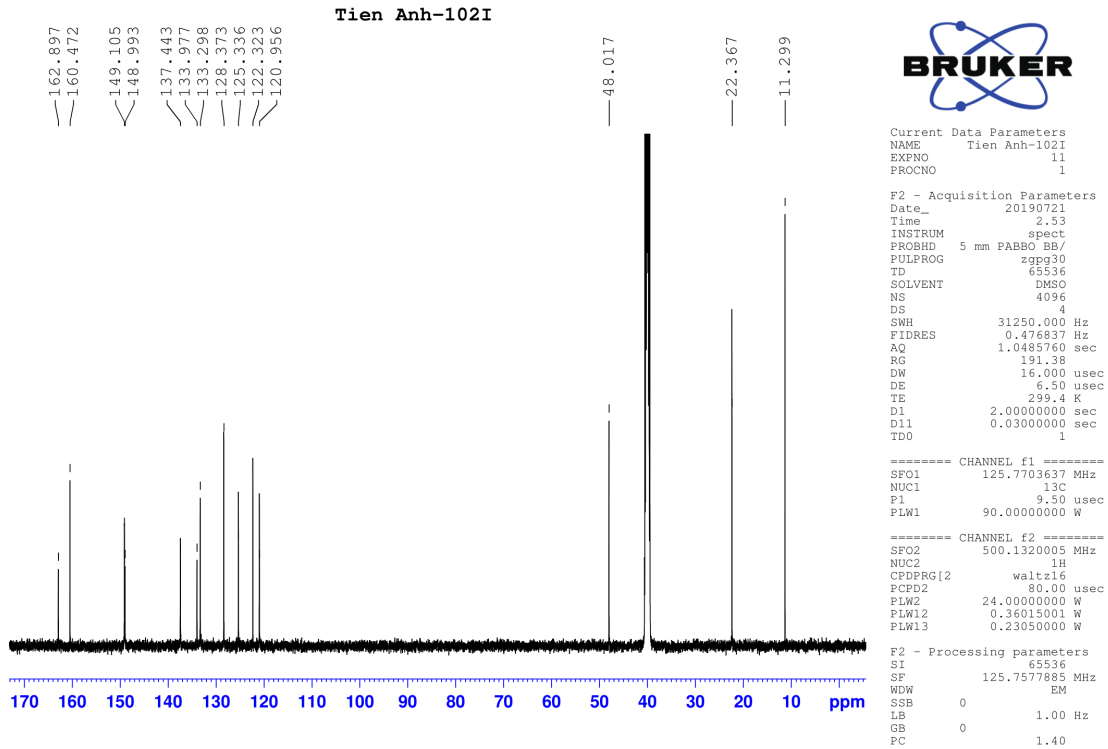


Figure S8. ^{13}C NMR of compound 9d



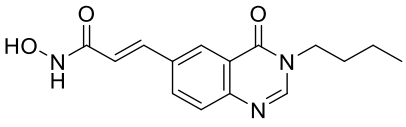


Figure S9. ^{1}H NMR of compound 9e

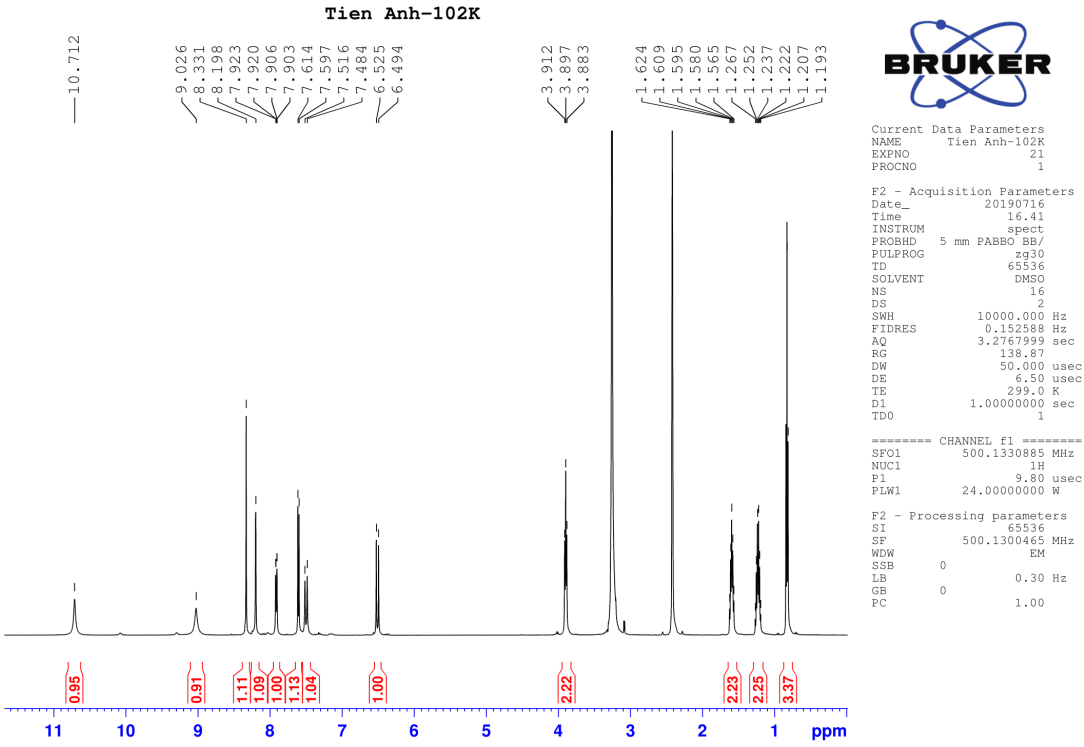
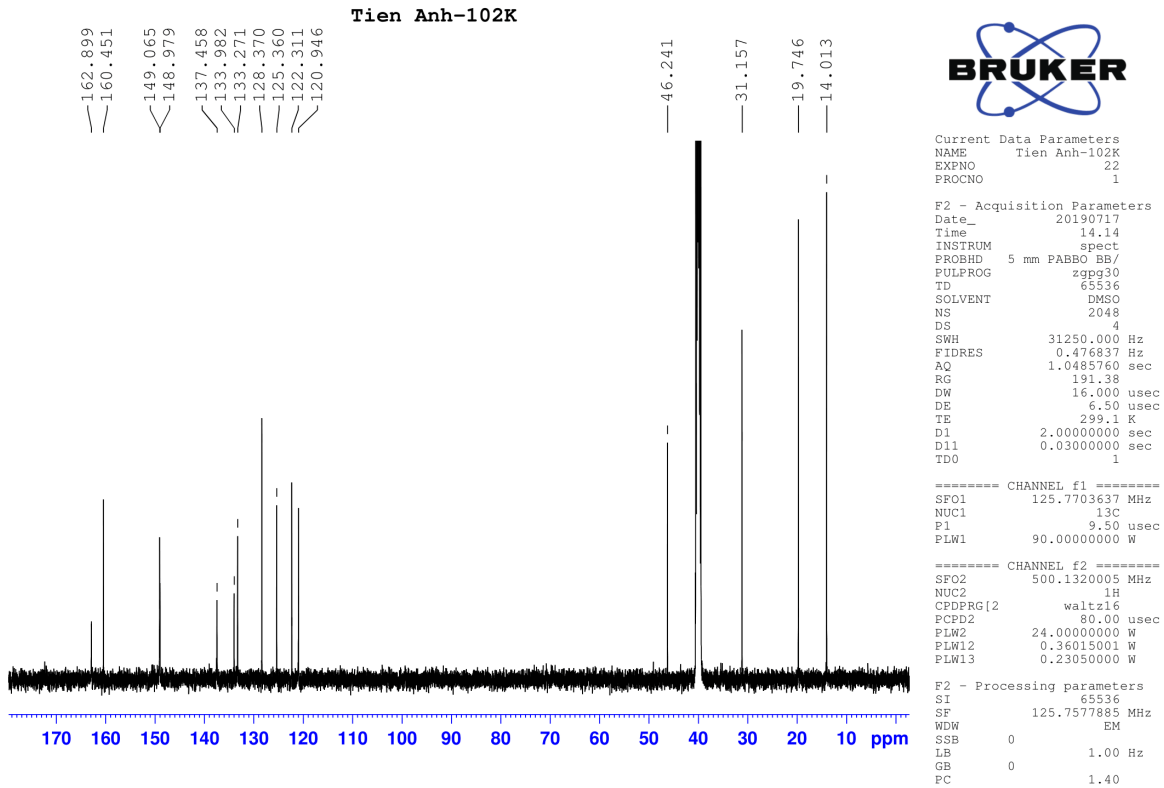


Figure S10. ^{13}C NMR of compound 9e



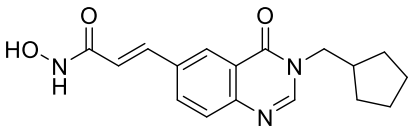


Figure S11. ^{1}H NMR of compound 9f

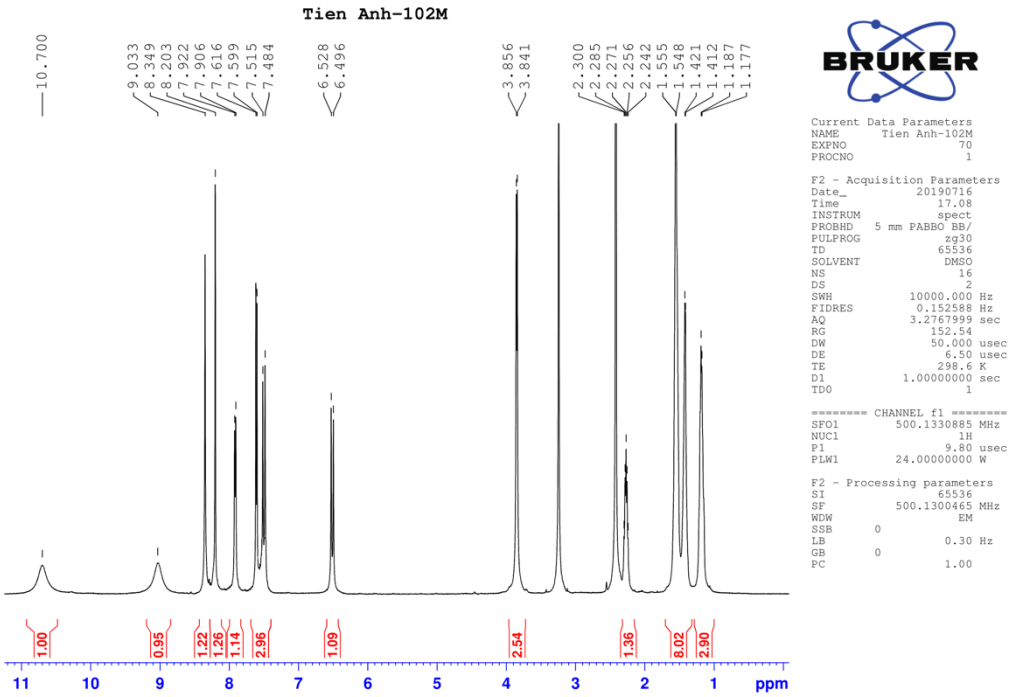
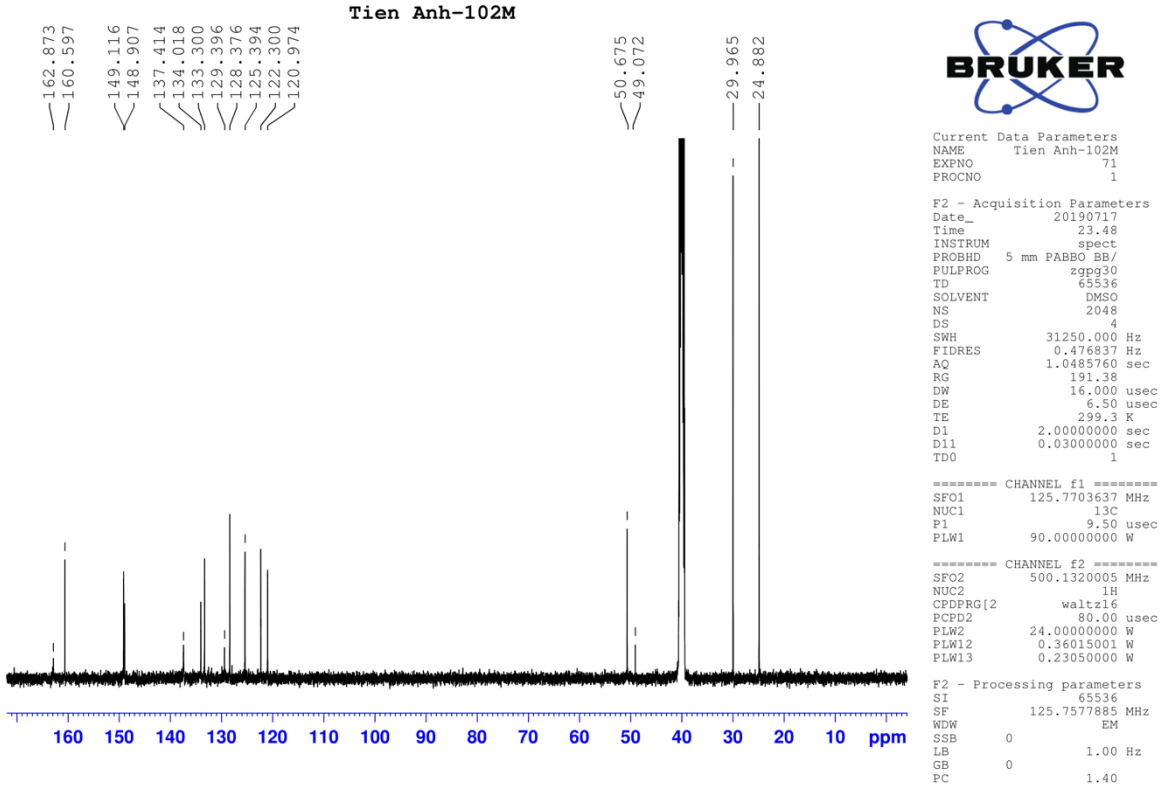


Figure S12. ^{13}C NMR of compound 9f



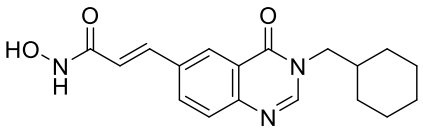


Figure S13. ^{1}H NMR of compound 9g

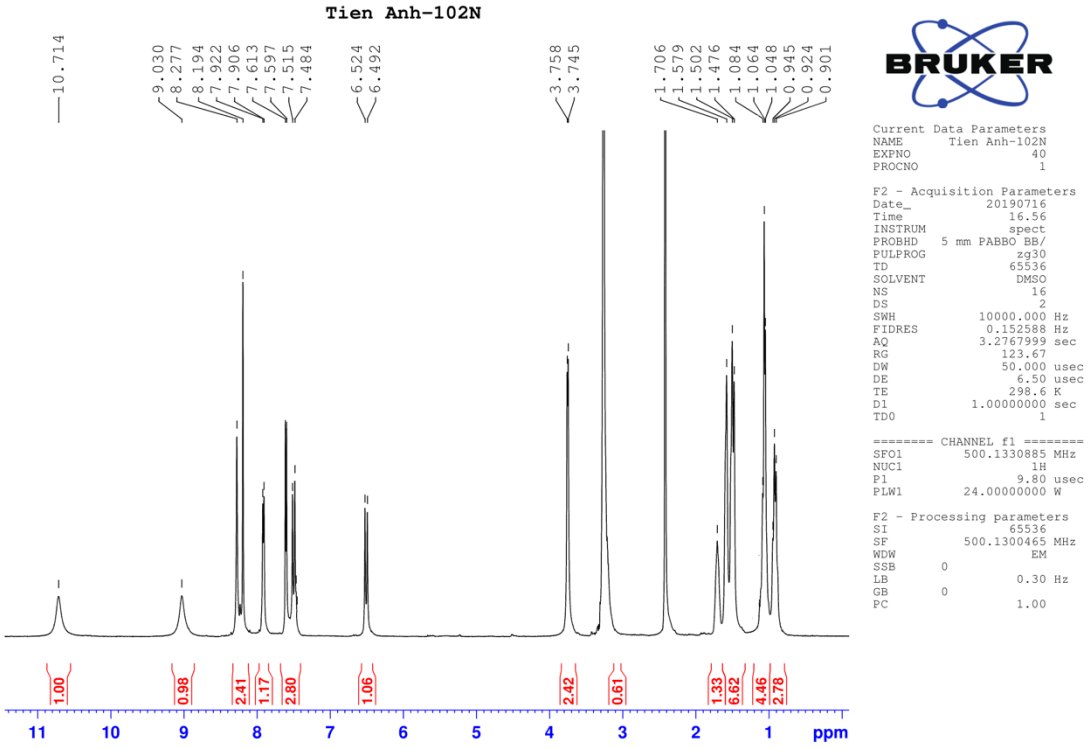
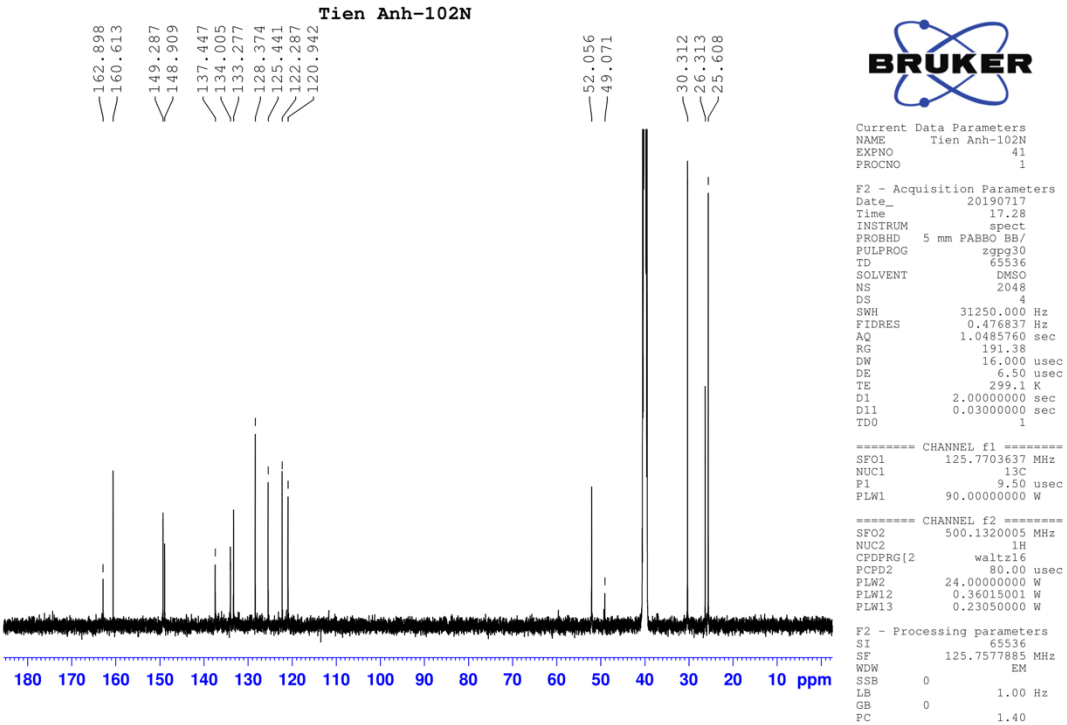


Figure S14. ^{13}C NMR of compound 9g



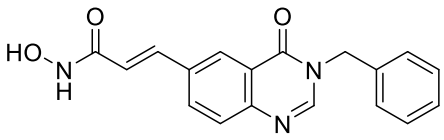
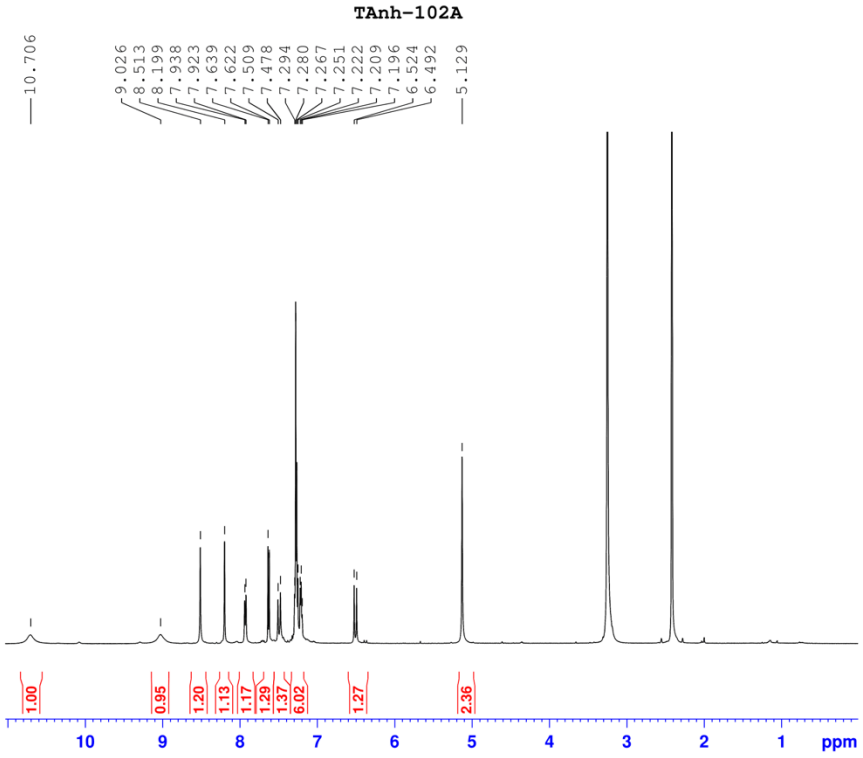


Figure S15. ^{1}H NMR of compound 9h



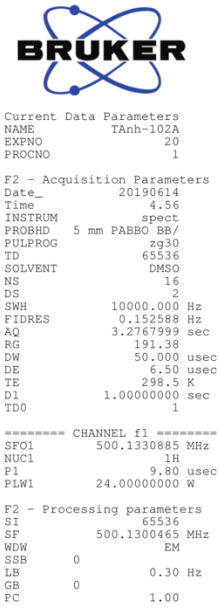
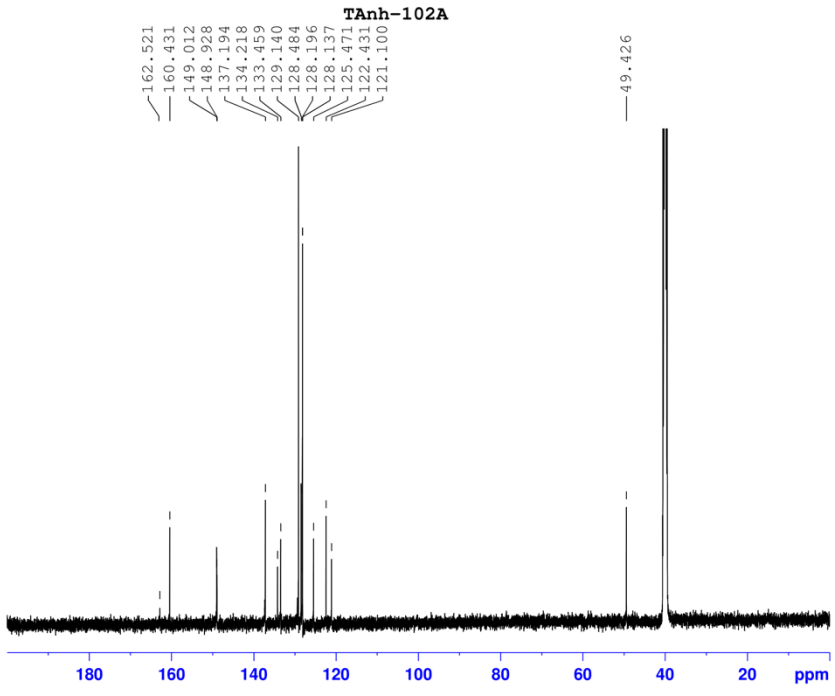
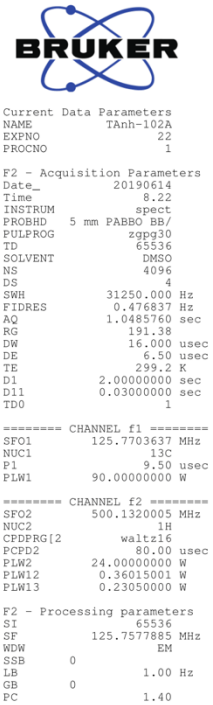


Figure S16. ^{13}C NMR of compound 9h





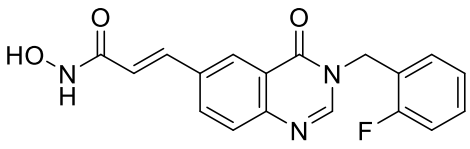


Figure S17. ^{1}H NMR of compound 9i

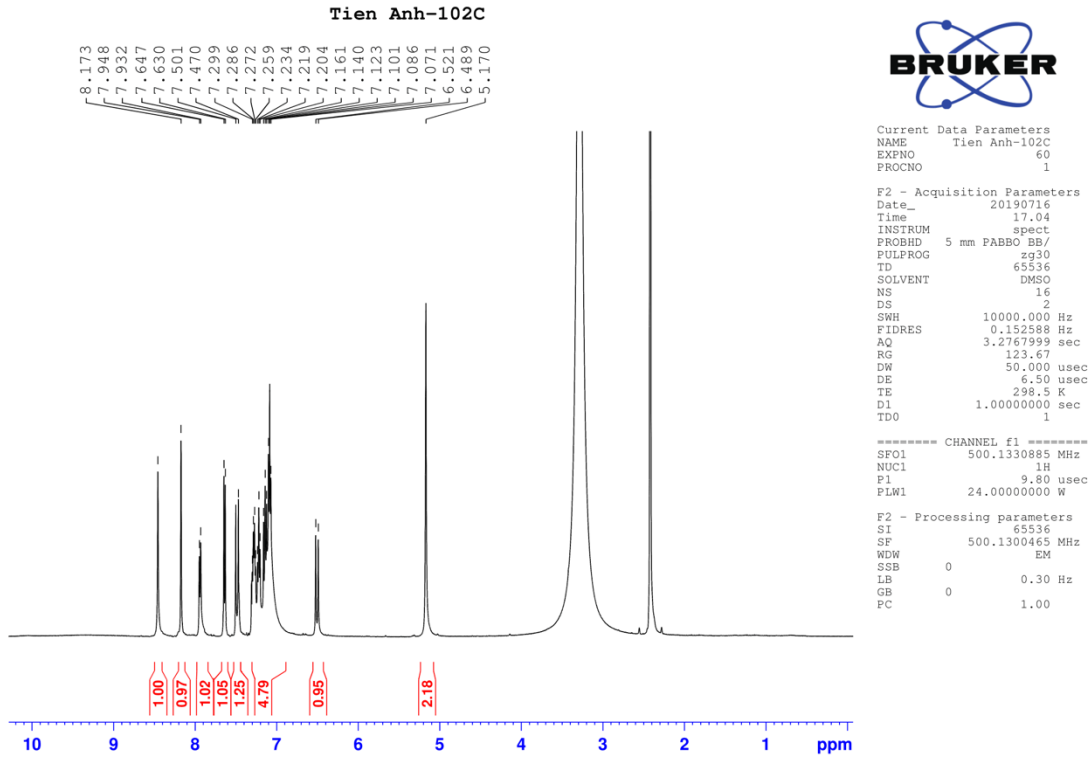
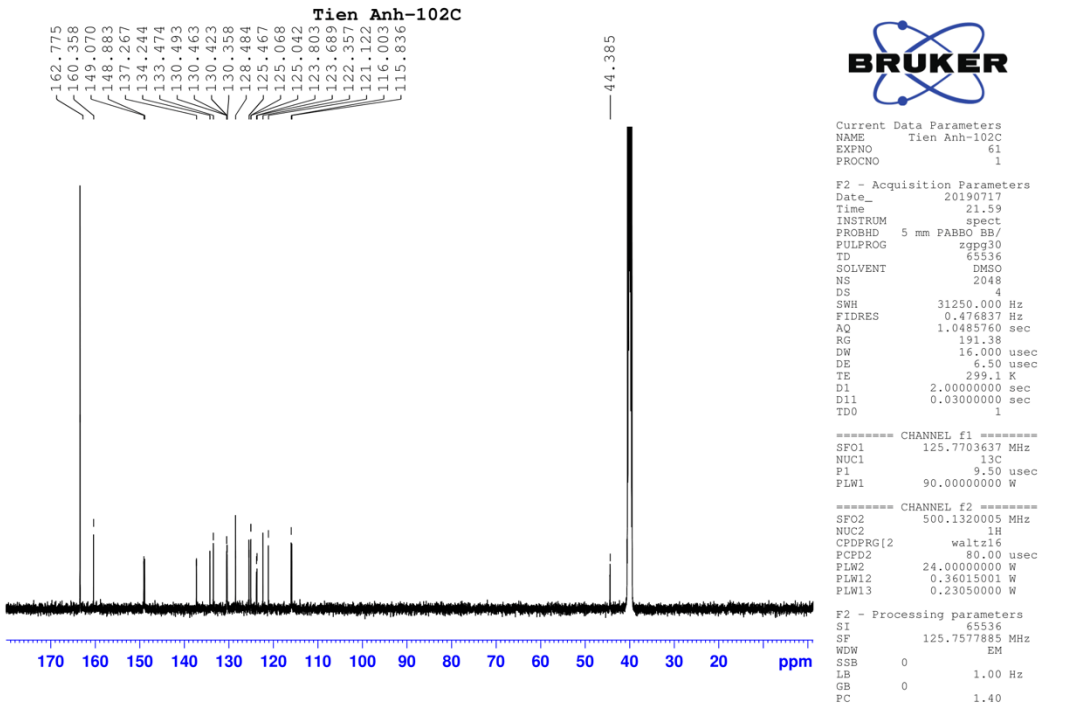


Figure S18. ^{13}C NMR of compound 9i



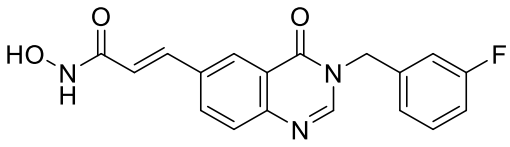
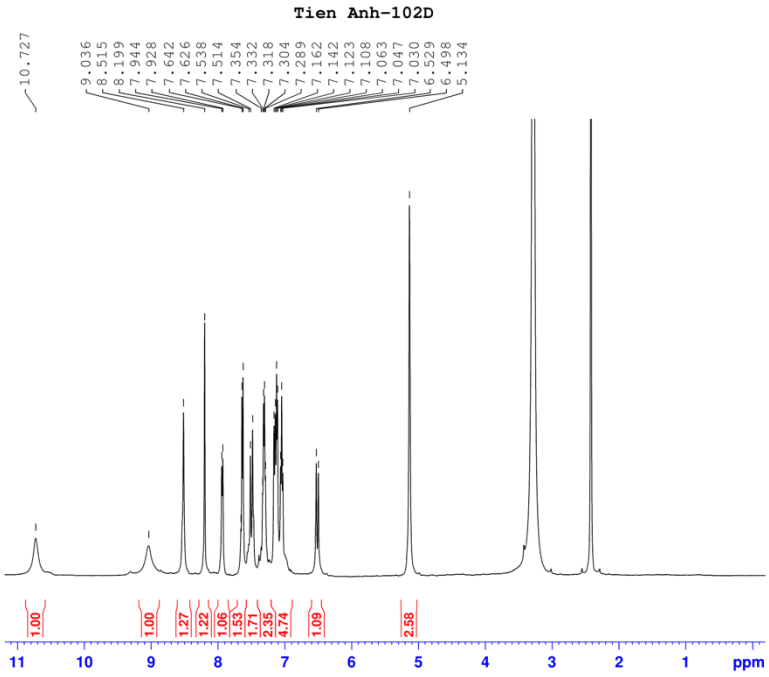


Figure S19. ^{1}H NMR of compound 9j



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TE              298.6 K
D1          1.00000000 sec
TD0                   1

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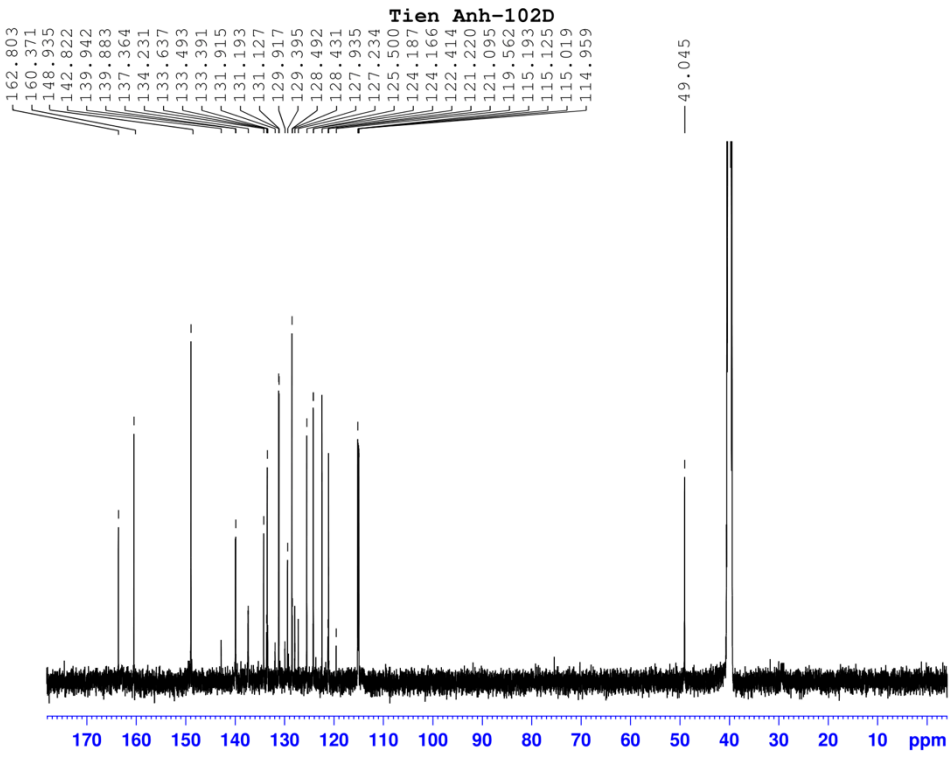
Figure S20. ^{13}C NMR of compound 9j



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D11         0.03000000 sec
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PLW13        0.23050000 W

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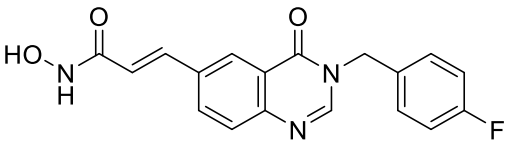


Figure S21. ^{1}H NMR of compound 9k

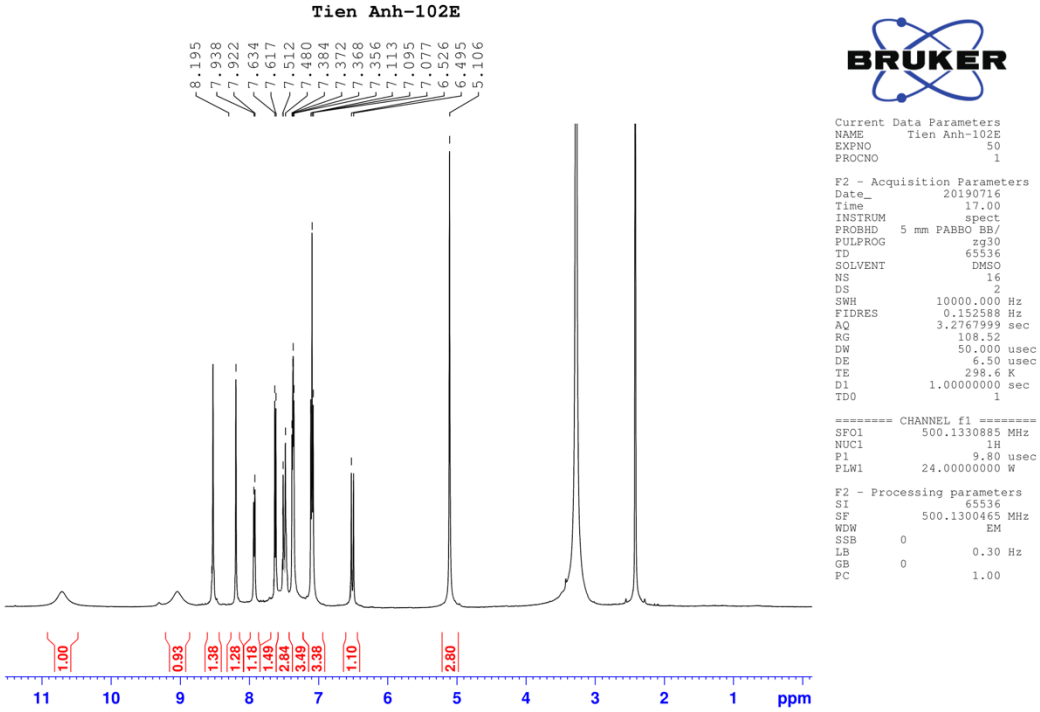
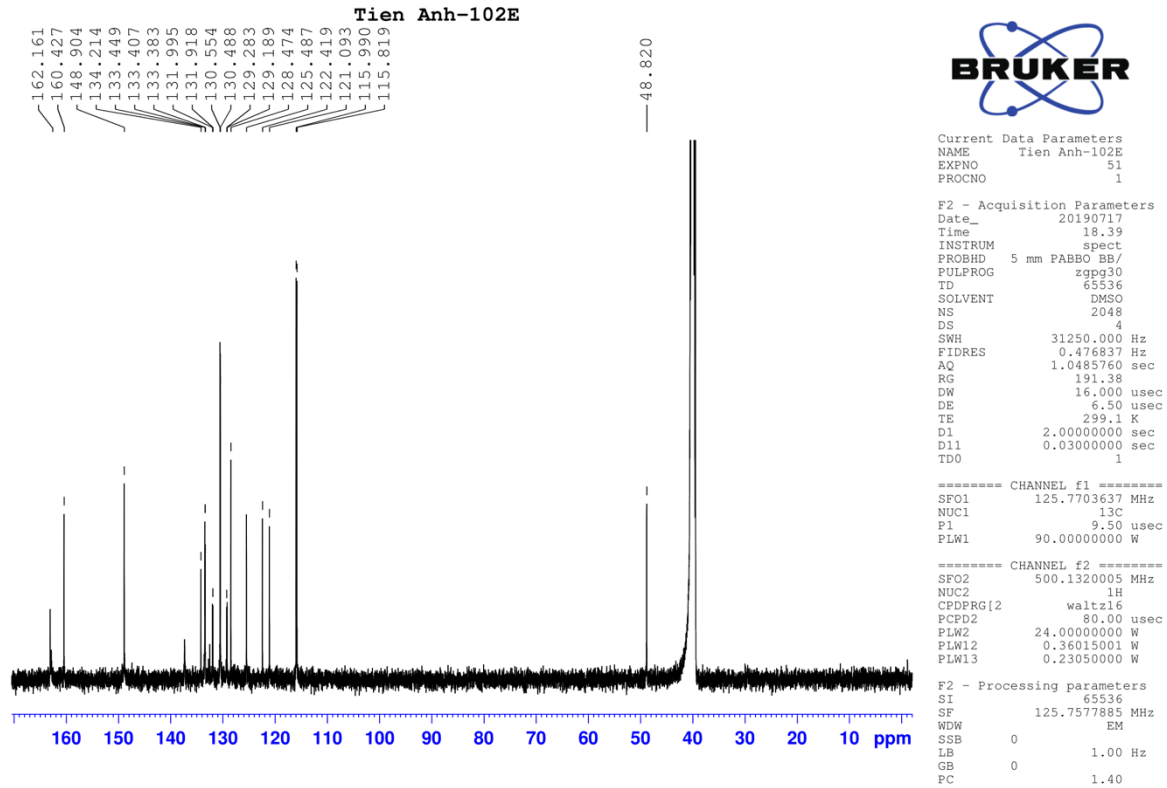


Figure S22. ^{13}C NMR of compound 9k



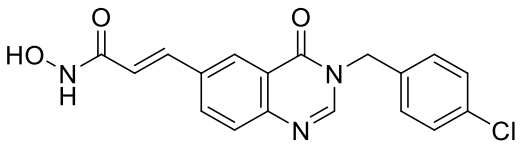


Figure S23. ^{1}H NMR of compound 9l

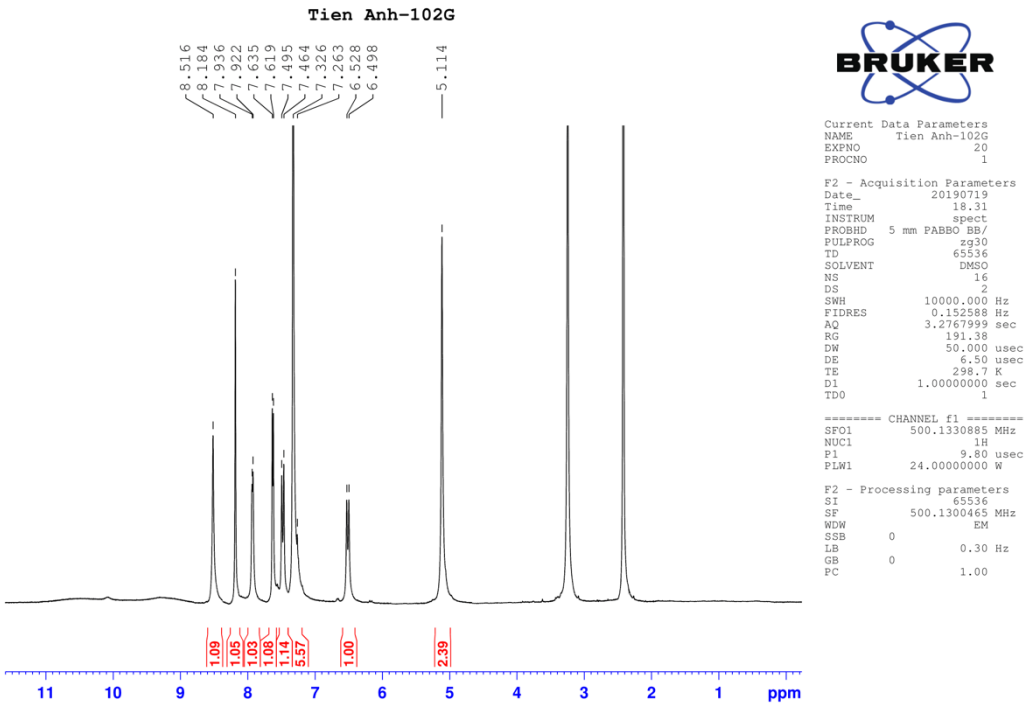
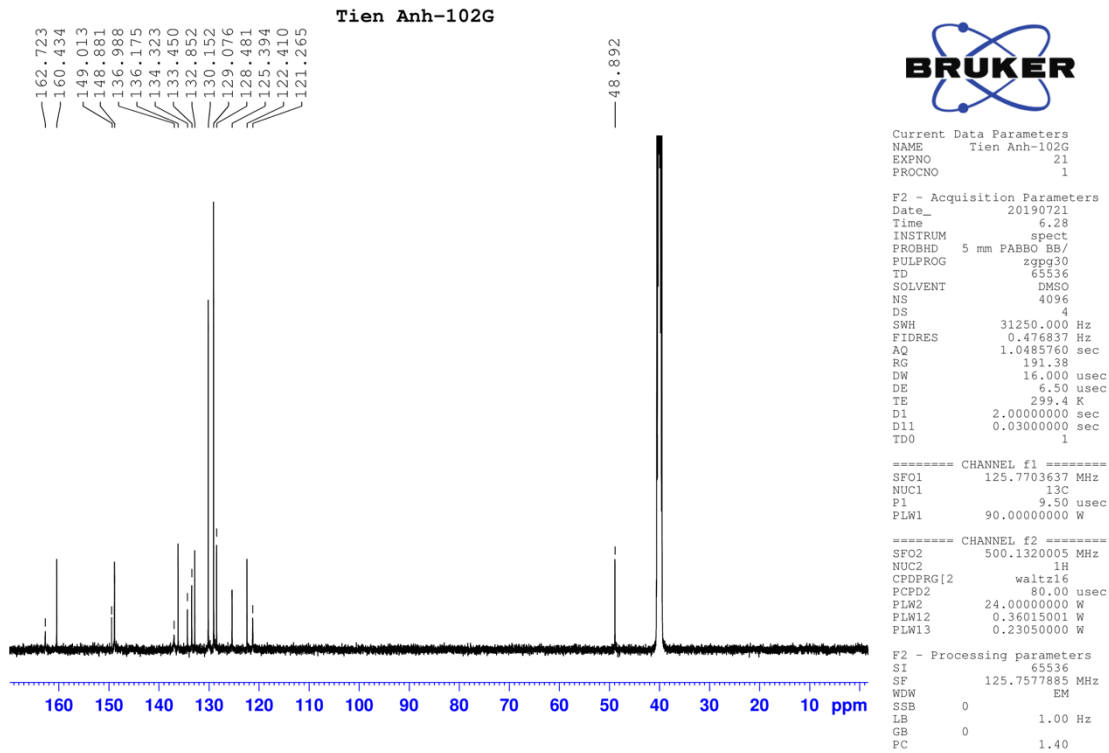


Figure S24. ^{13}C NMR of compound 9l



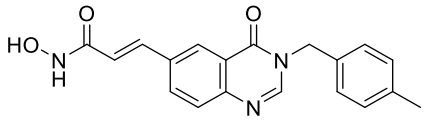
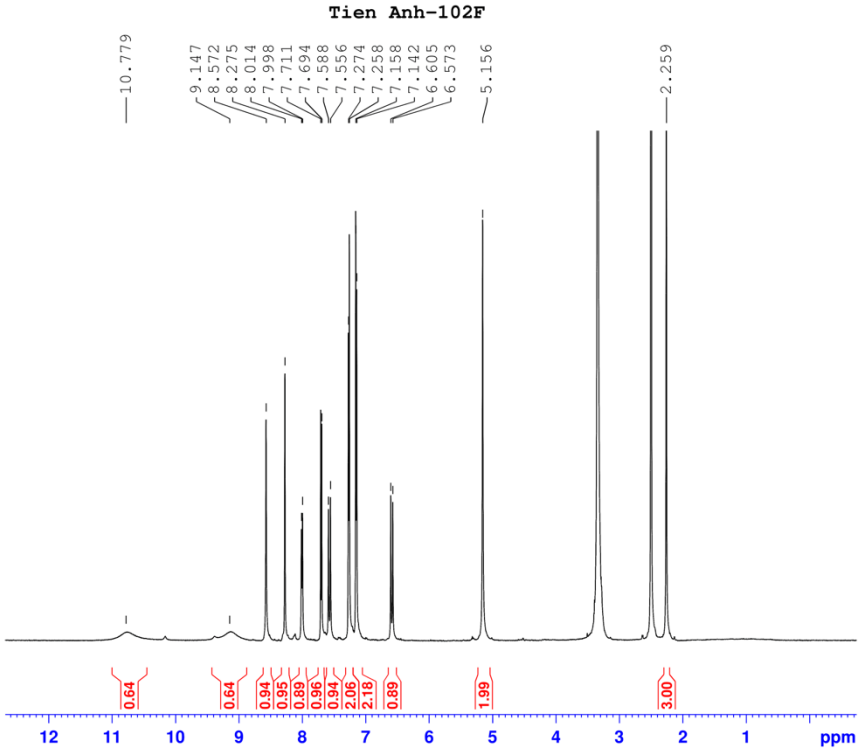


Figure S25. ^{1}H NMR of compound 9m

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D1            1.00000000 sec
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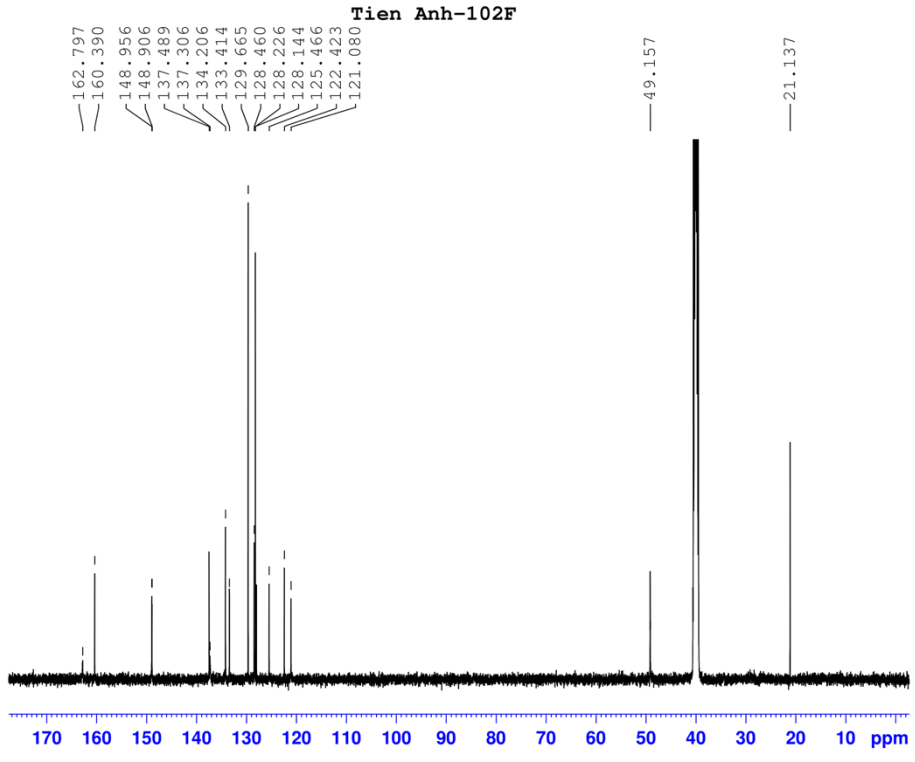
Figure S26. ^{13}C NMR of compound 9m

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FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                191.38
DW                16.000 usec
DE                 6.50 usec
TE                299.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
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NUC1                  13C
P1                  9.50 usec
PLW1        90.00000000 W

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NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2        24.00000000 W
PLW12        0.36015001 W
PLW13        0.23050000 W

F2 - Processing parameters
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WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
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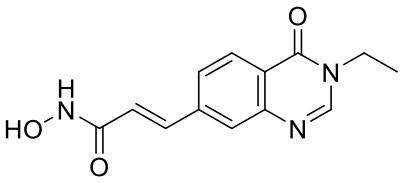
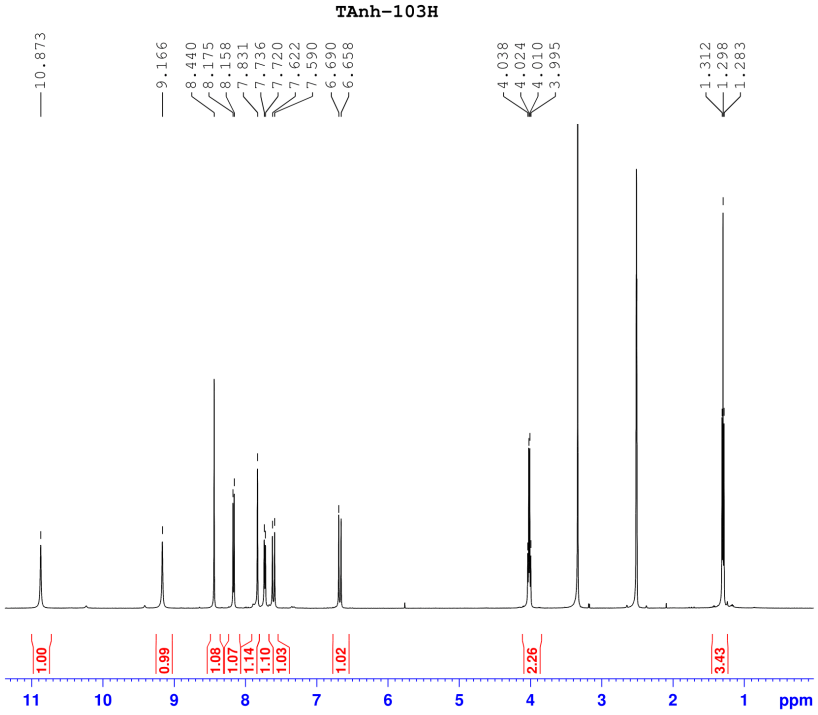


Figure S27. ^{1}H NMR of compound 10a



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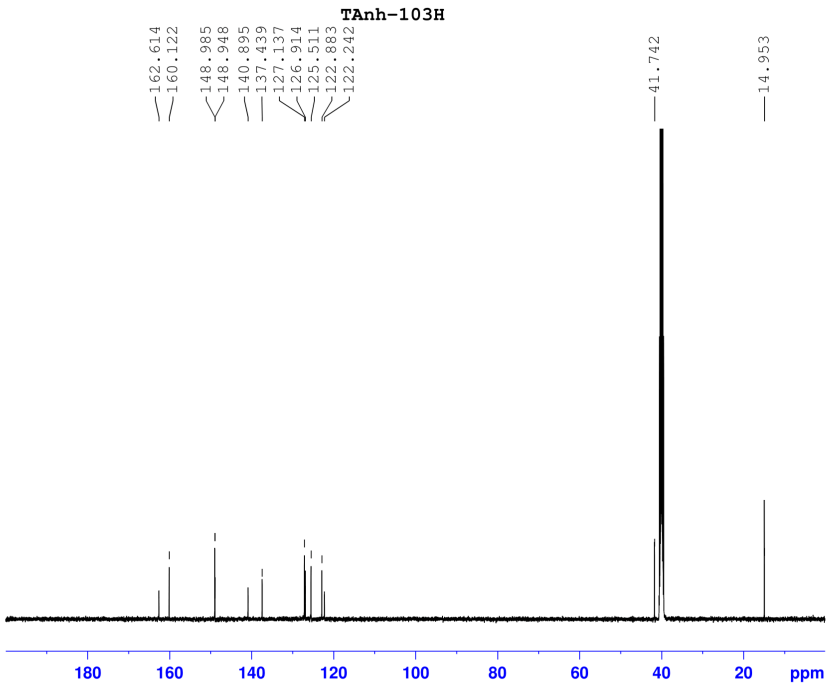
Current Data Parameters
NAME                  TAnh-103H
EXPNO                        20
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20190819
Time                      0.24
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                  zg30
TD                      65536
SOLVENT                  DMSO
NS                         16
DS                          2
SWH                 10000.000 Hz
FIDRES                0.152588 Hz
AQ                    3.2767999 sec
RG                       191.38
DW                     50.000 usec
DE                      6.50 usec
TE                       298.8 K
D1                  1.00000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                500.1330885 MHz
NUC1                        1H
P1                        9.80 usec
PLW1               24.00000000 W

F2 - Processing parameters
SI                          65536
SF                  500.1300000 MHz
WDW                          EM
SSB          0
LB                        0.30 Hz
GB           0
PC                        1.00
```

Figure S28. ^{13}C NMR of compound 10a



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Current Data Parameters
NAME                  TAnh-103H
EXPNO                        22
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20190819
Time                      2.11
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                 zgpg30
TD                      65536
SOLVENT                  DMSO
NS                        2048
DS                           4
SWH                 31250.000 Hz
FIDRES                0.476837 Hz
AQ                    1.0485760 sec
RG                       191.38
DW                     16.000 usec
DE                      6.50 usec
TE                       299.2 K
D1                  2.00000000 sec
D11                 0.03000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                125.7703637 MHz
NUC1                        13C
P1                        9.50 usec
PLW1                90.00000000 W

======== CHANNEL f2 ========
SFO2                500.1320005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     80.00 usec
PLW2               24.00000000 W
PLW12               0.36015001 W
PLW13               0.23050000 W

F2 - Processing parameters
SI                          65536
SF                  125.7577885 MHz
WDW                          EM
SSB          0
LB                        1.00 Hz
GB           0
PC                        1.40
```

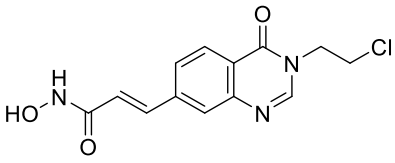


Figure S29. ^{1}H NMR of compound 10b

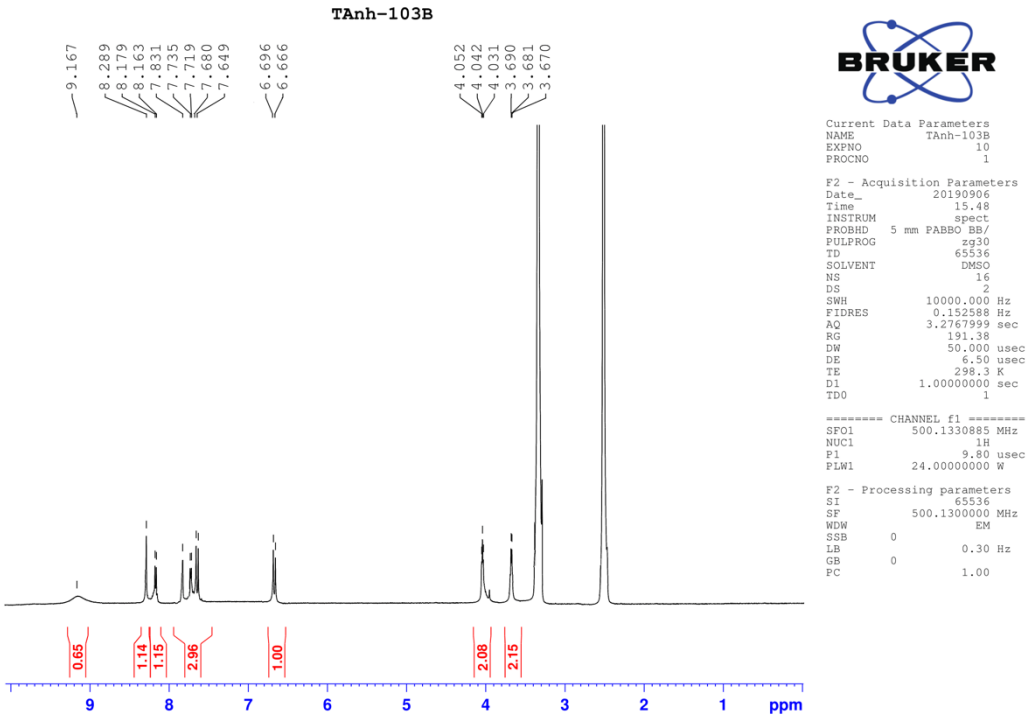
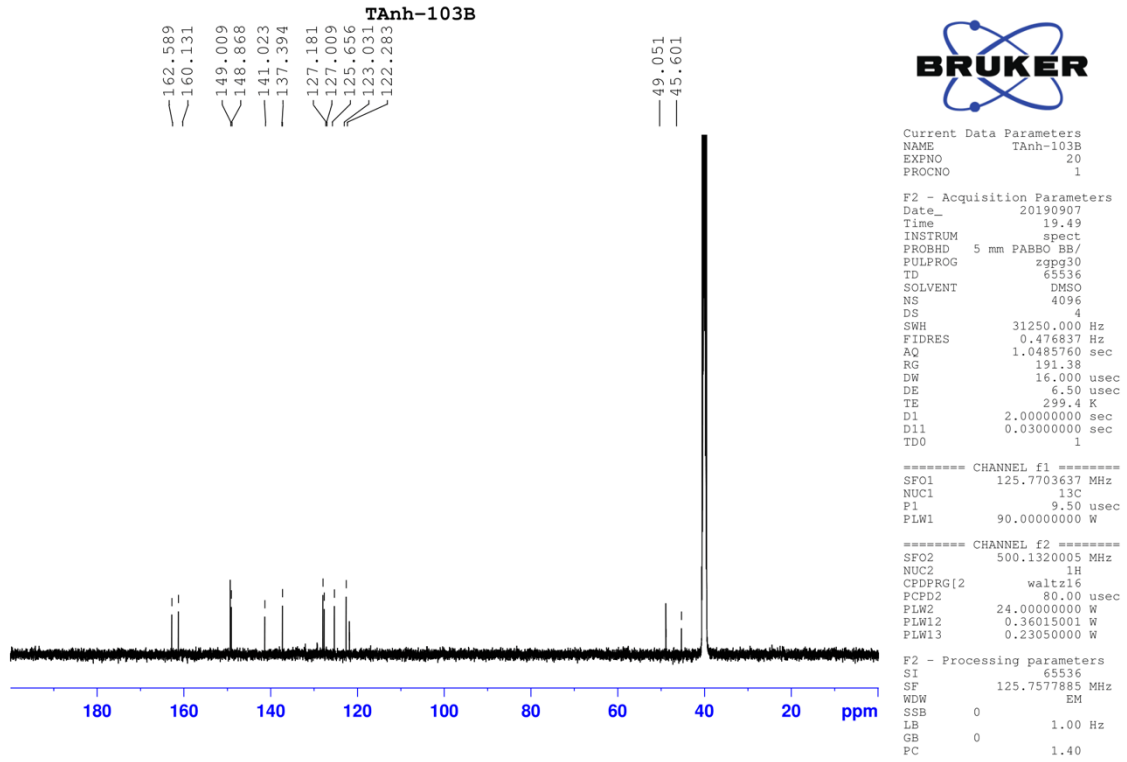


Figure S30. ^{13}C NMR of compound 10b



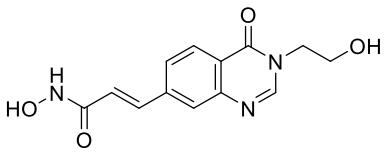


Figure S31. ^{1}H NMR of compound 10c

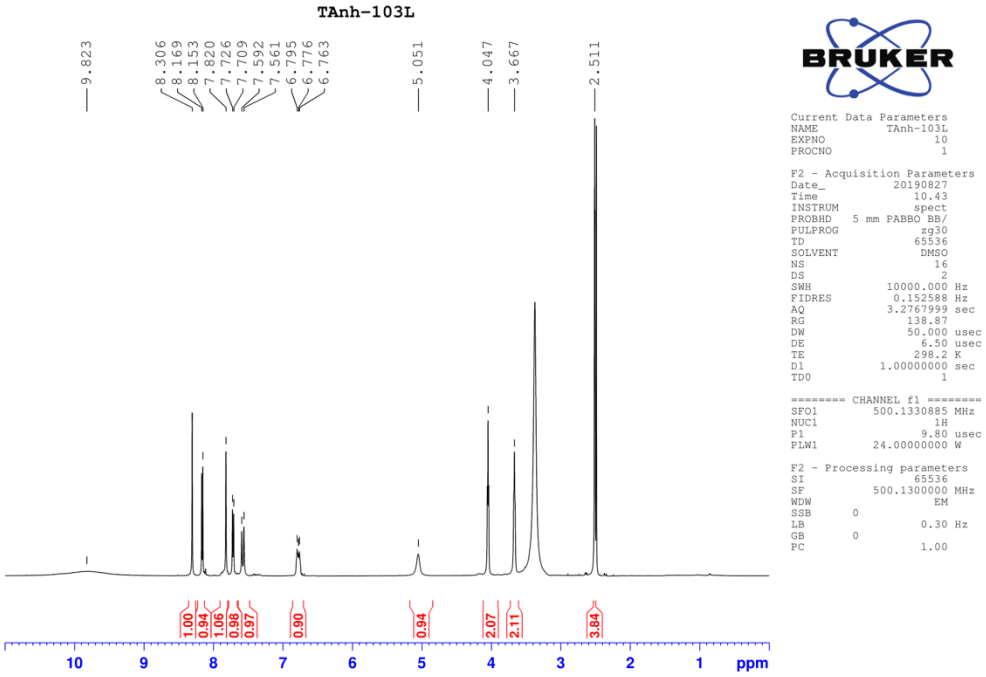
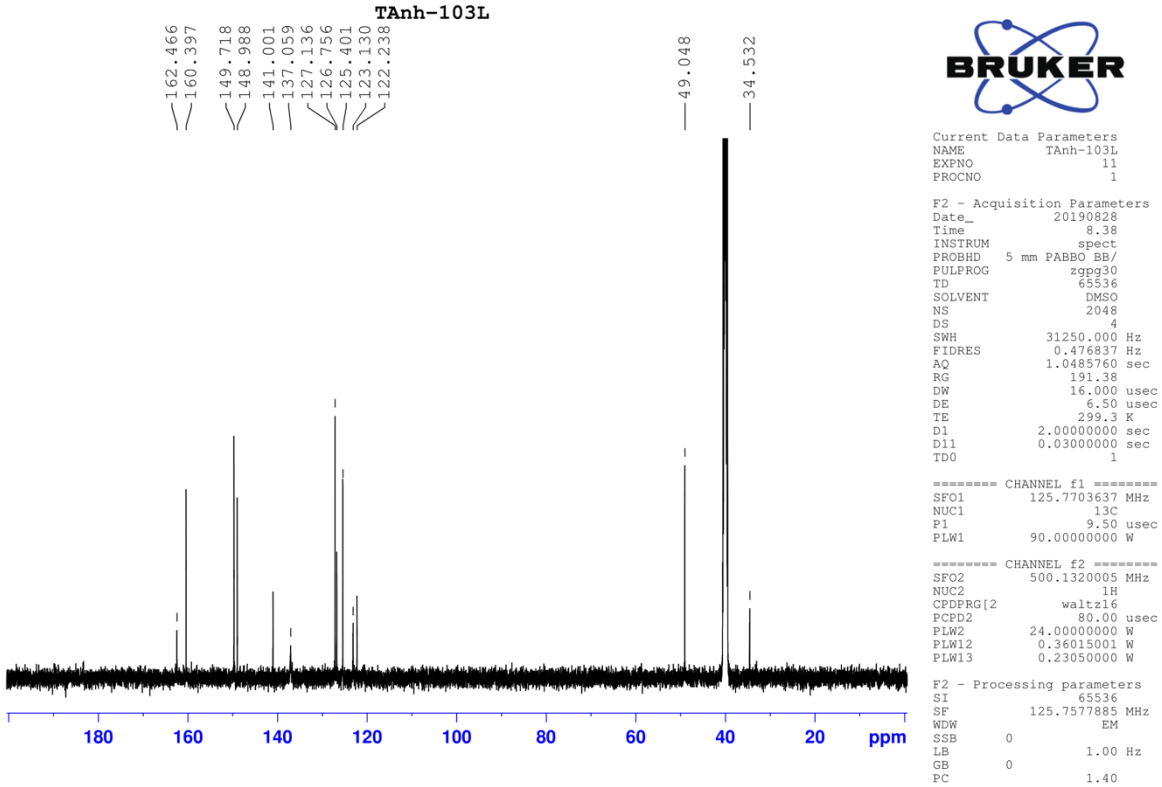


Figure S32. ^{13}C NMR of compound 10c



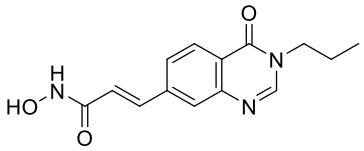


Figure S33. ^{1}H NMR of compound 10d

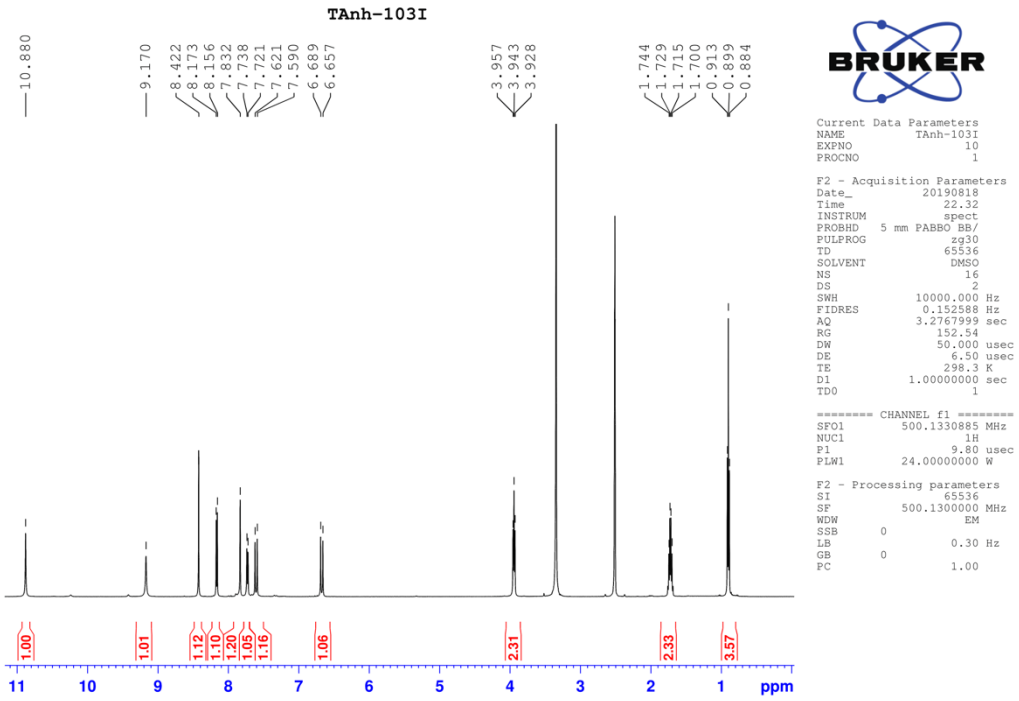
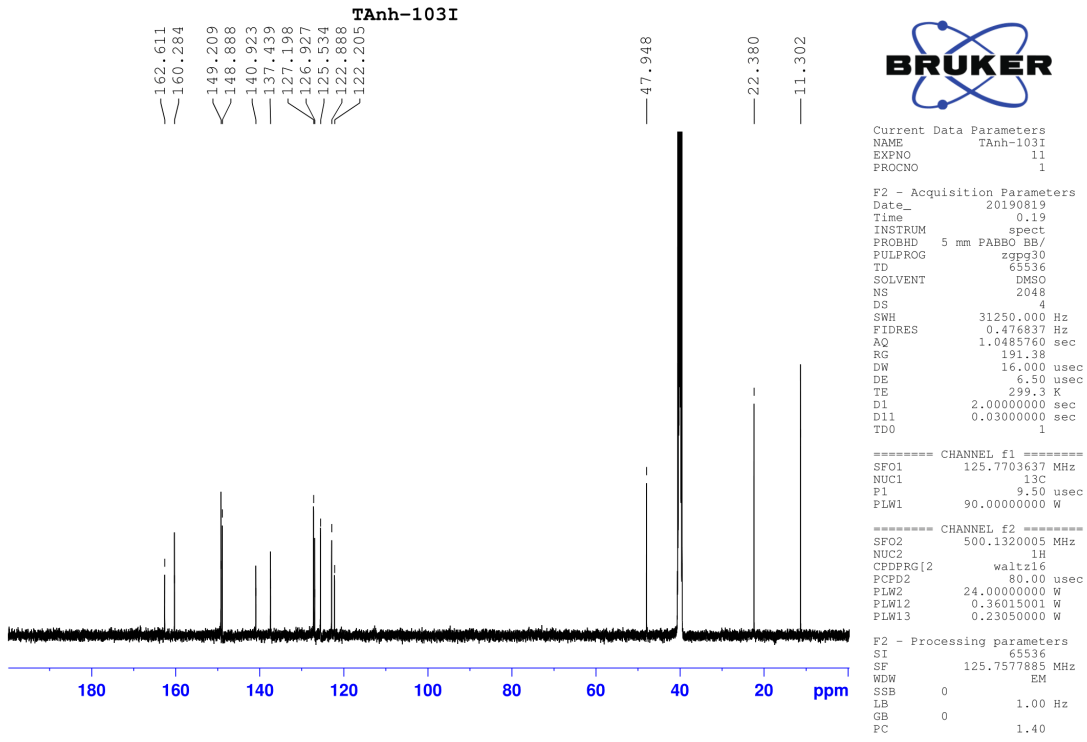


Figure S34. ^{13}C NMR of compound 10d



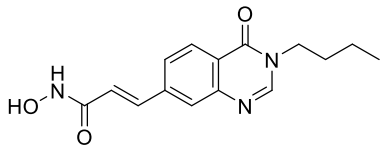


Figure S35. ^{1}H NMR of compound 10e

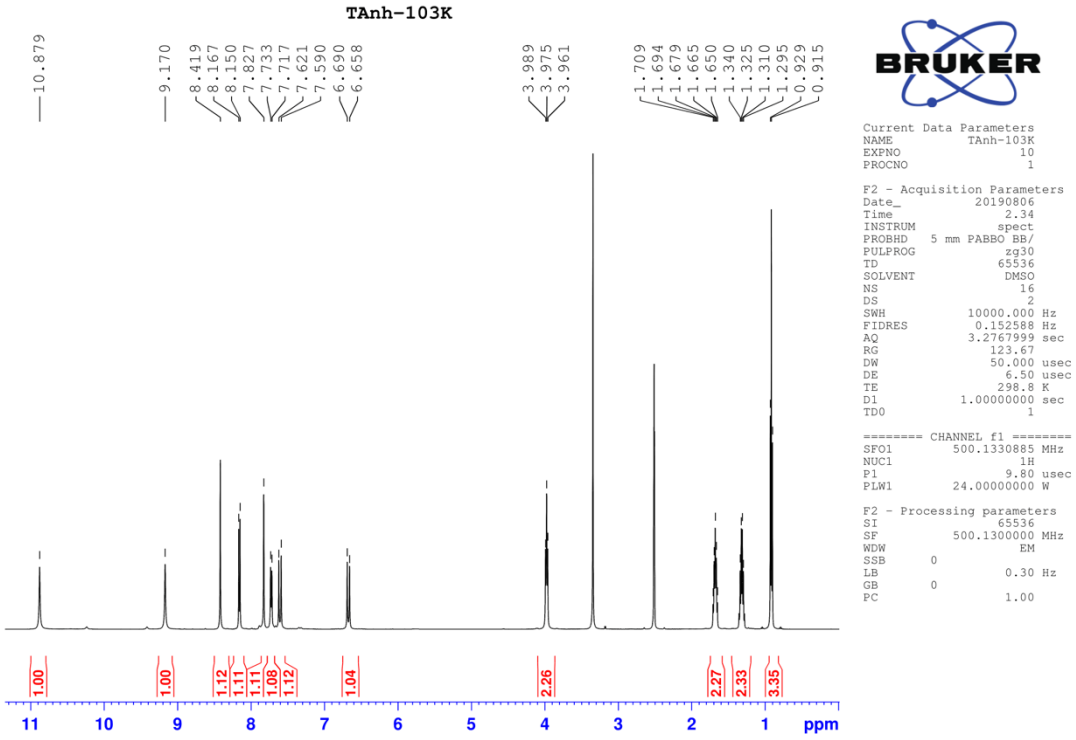
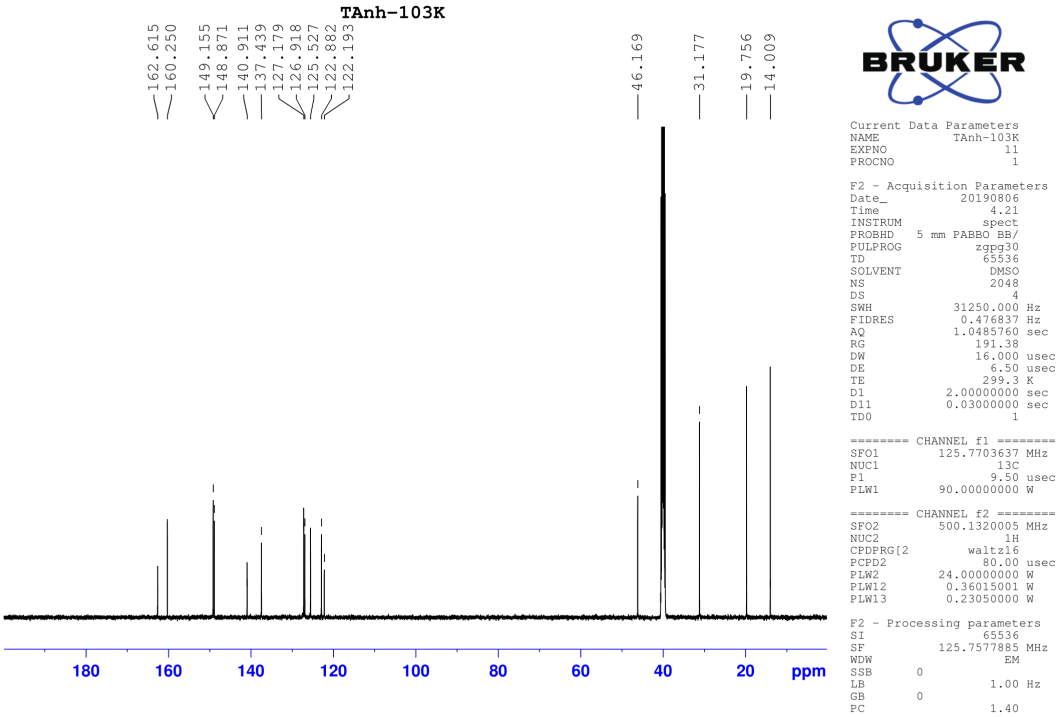


Figure S36. ^{13}C NMR of compound 10e



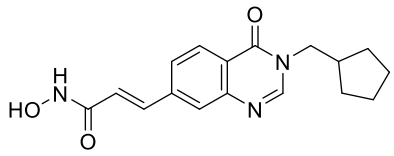


Figure S37. ^{1}H NMR of compound 10f

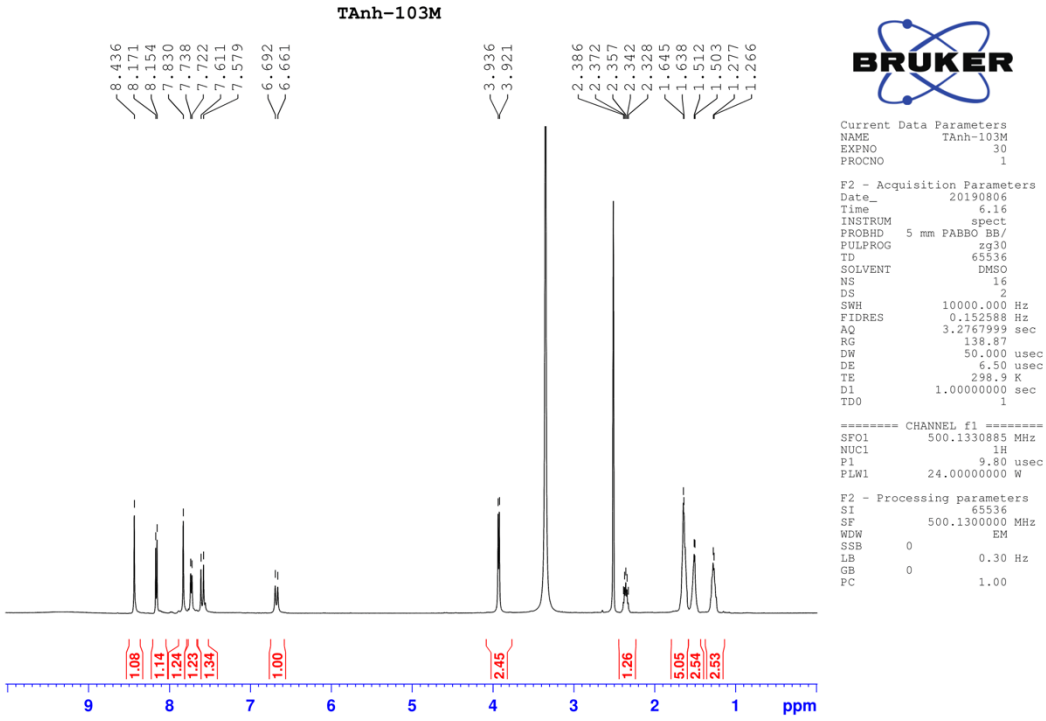
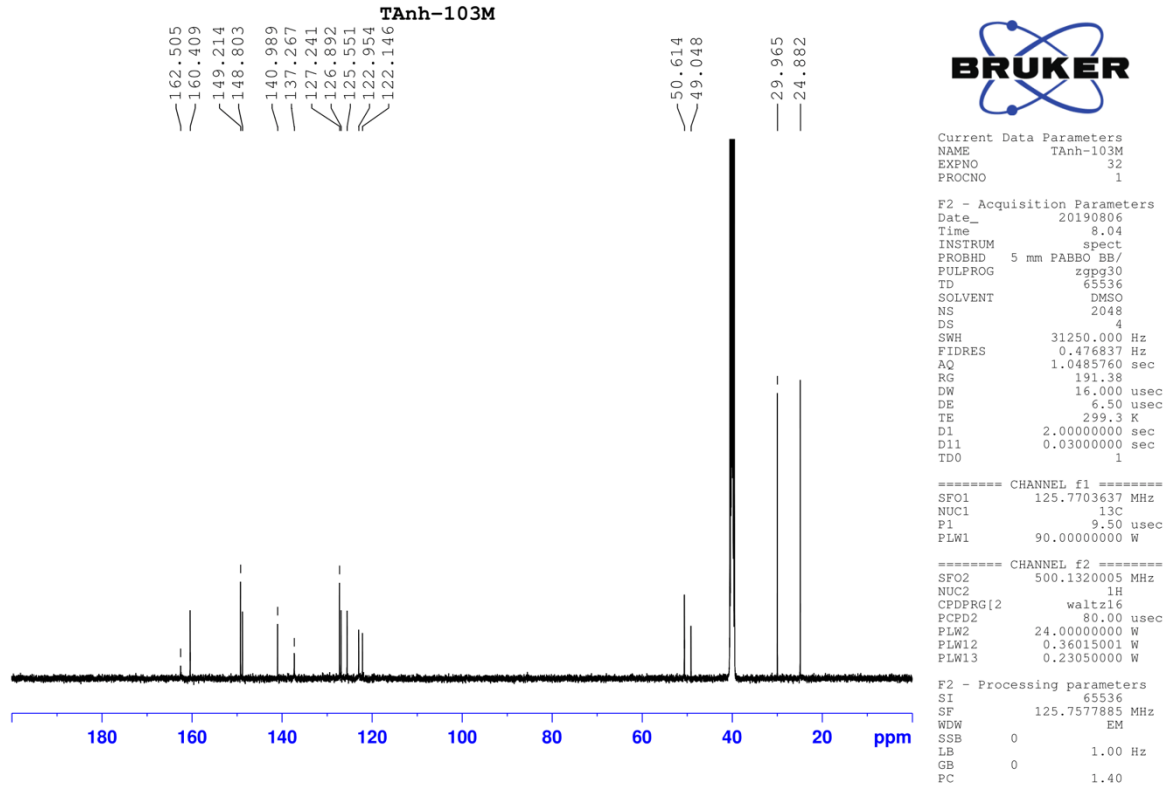


Figure S38. ^{13}C NMR of compound 10f



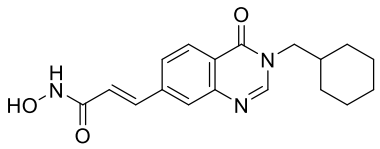


Figure S39. ^{1}H NMR of compound 10g

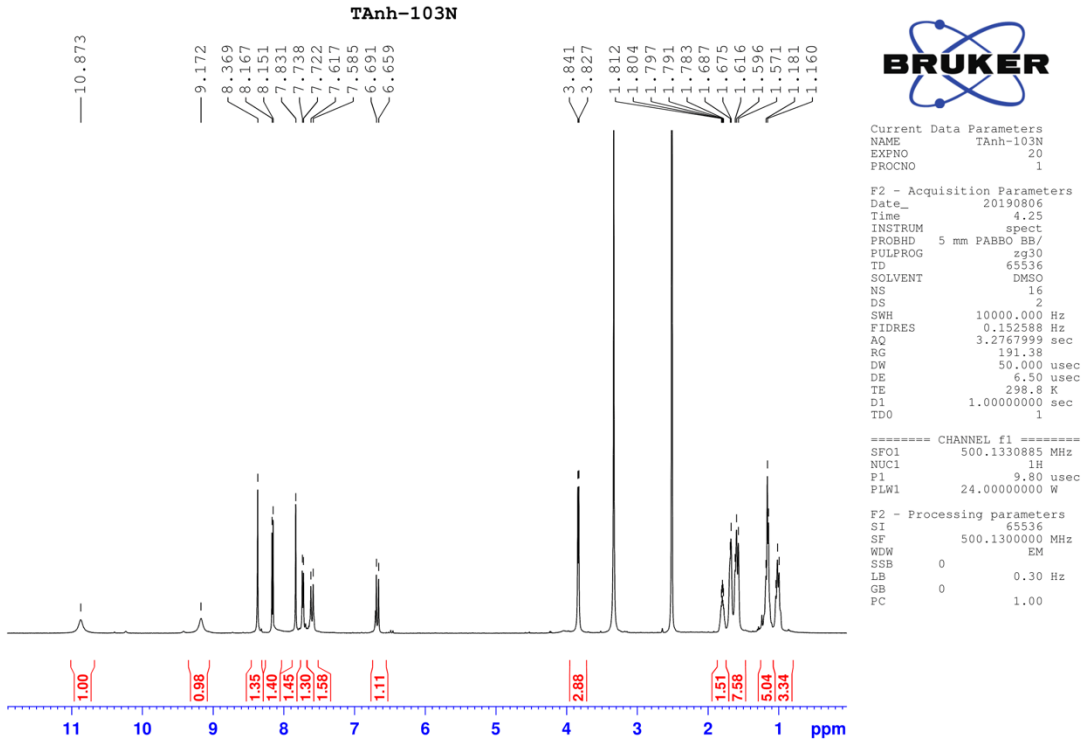
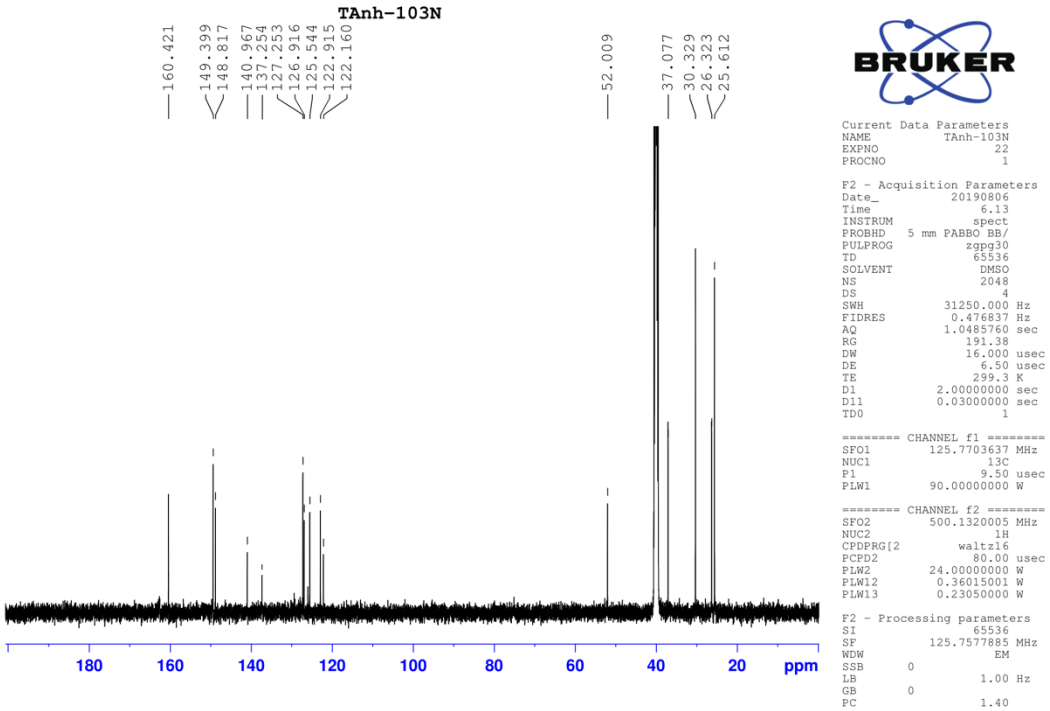


Figure S40. ^{13}C NMR of compound 10g



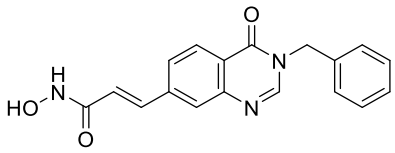


Figure S41. ^{1}H NMR of compound 10h

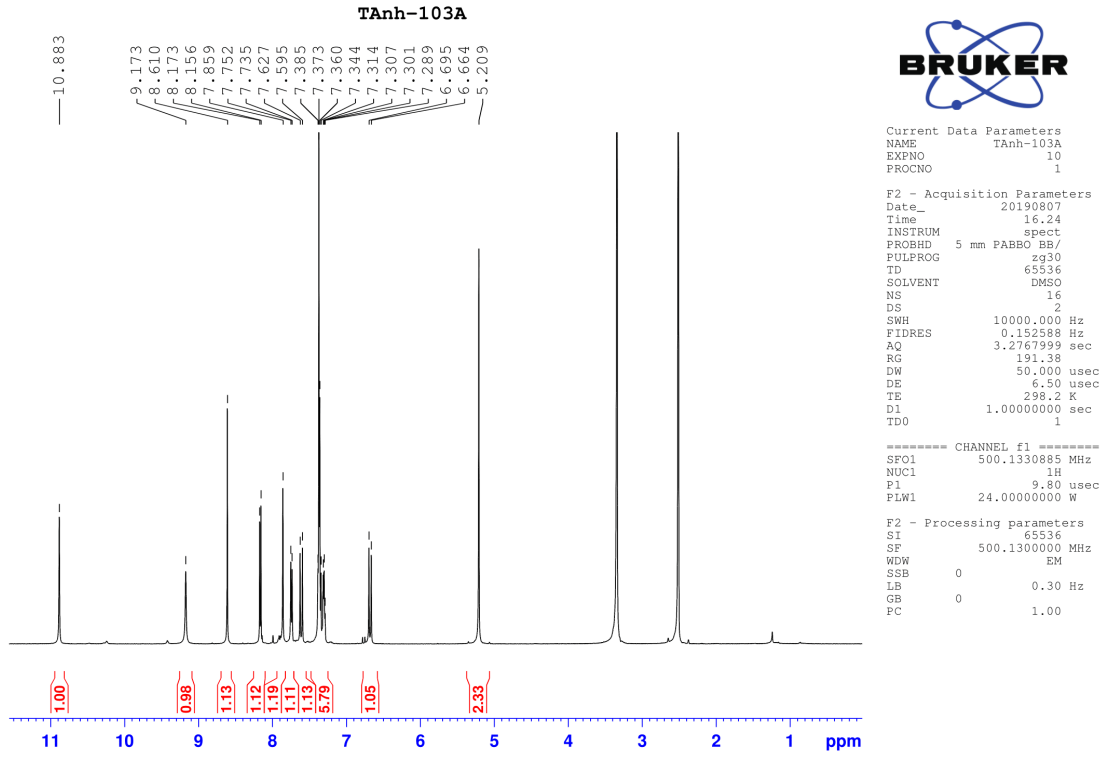
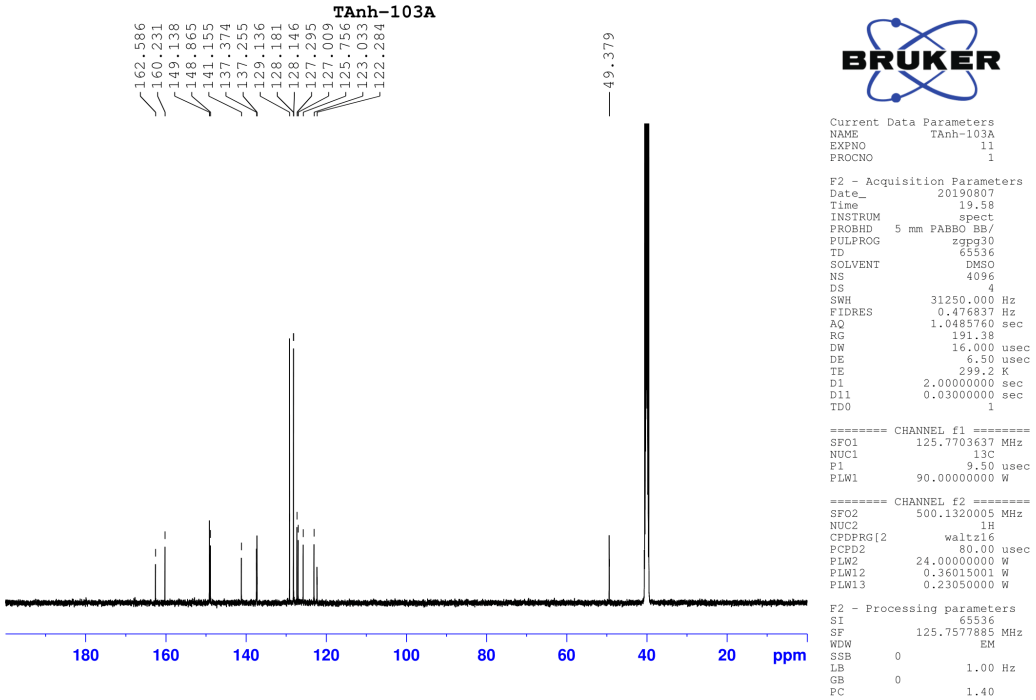


Figure S42. ^{13}C NMR of compound 10h



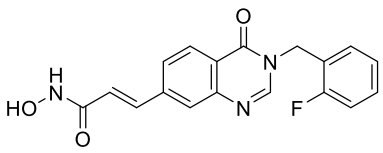
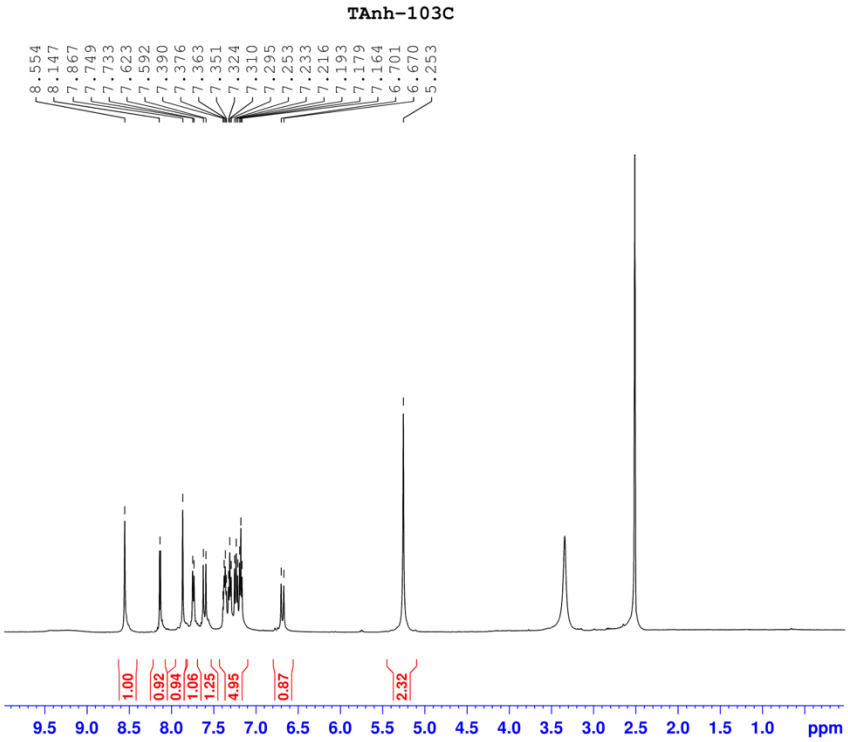


Figure S43. ^{1}H NMR of compound 10i



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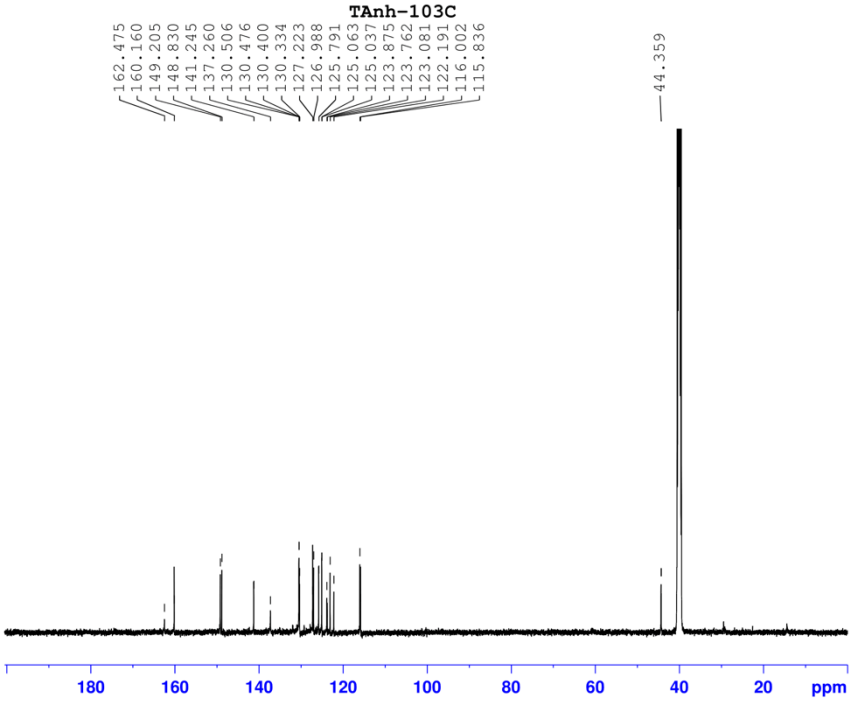
Current Data Parameters
NAME                 TAnh-103C
EXPNO                       20
PROCNO                       1

F2 - Acquisition Parameters
Date_               20190807
Time                   20.02
INSTRUM                spect
PROBHD    5 mm PABBO BB/
PULPROG                 zg30
TD                     65536
SOLVENT                 DMSO
NS                        16
DS                         2
SWH                10000.000 Hz
FIDRES                 0.152588 Hz
AQ                 3.2767999 sec
RG                     138.87
DW                     50.000 usec
DE                      6.50 usec
TE                     298.7 K
D1                 1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1            500.1330885 MHz
NUC1                       1H
P1                       9.80 usec
PLW1           24.00000000 W

F2 - Processing parameters
SI                         65536
SF              500.1300000 MHz
WDW                        EM
SSB          0
LB                       0.30 Hz
GB           0
PC                       1.00
```

Figure S44. ^{13}C NMR of compound 10i



```
Current Data Parameters
NAME                 TAnh-103C
EXPNO                       22
PROCNO                       1

F2 - Acquisition Parameters
Date_               20190807
Time                   23.36
INSTRUM                spect
PROBHD    5 mm PABBO BB/
PULPROG             zgpg30
TD                     65536
SOLVENT                 DMSO
NS                        4096
DS                           4
SWH                31250.000 Hz
FIDRES                 0.476837 Hz
AQ                 1.0485760 sec
RG                     191.38
DW                     16.000 usec
DE                      6.50 usec
TE                     299.2 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1            125.7703637 MHz
NUC1                       13C
P1                       9.50 usec
PLW1           90.00000000 W

======== CHANNEL f2 ========
SFO2            500.1320005 MHz
NUC2                       1H
CPDPRG[2             waltz16
PCPD2                   80.00 usec
PLW2           24.00000000 W
PLW12           0.36015001 W
PLW13           0.23050000 W

F2 - Processing parameters
SI                         65536
SF              125.7577885 MHz
WDW                        EM
SSB          0
LB                       1.00 Hz
GB           0
PC                       1.40
```

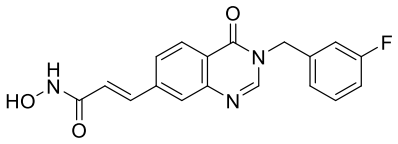
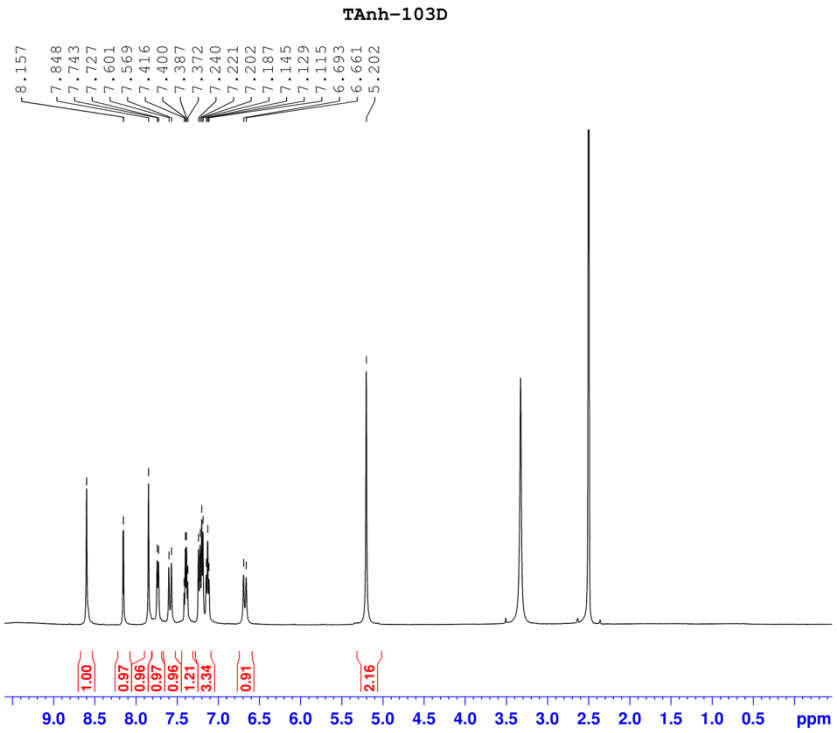


Figure S45. ^{1}H NMR of compound 10j



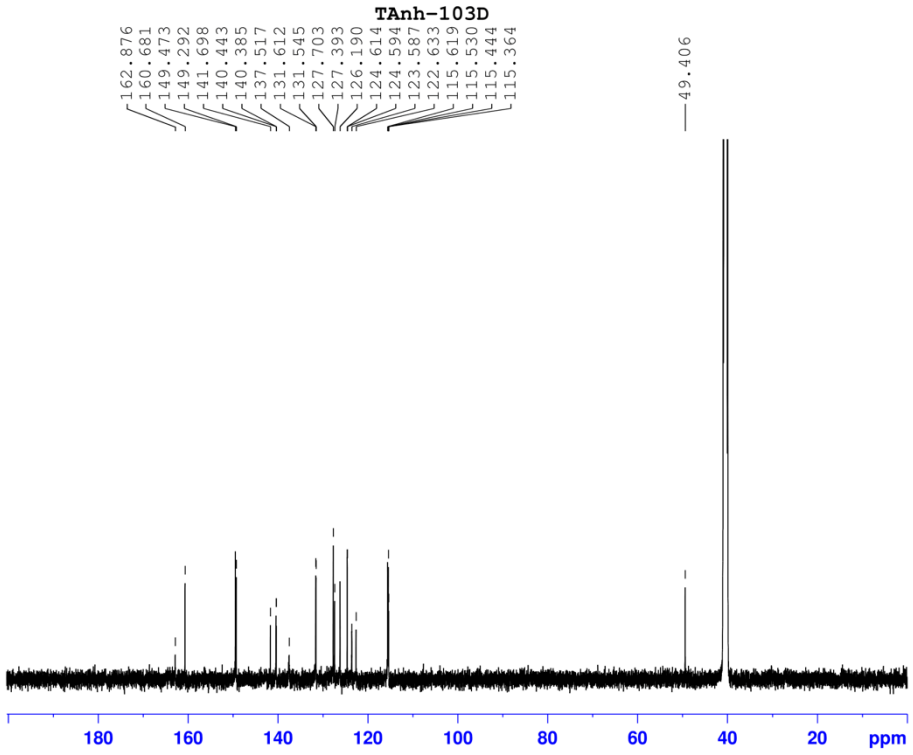

```
Current Data Parameters
NAME              TAnh-103D
EXPNO                    20
PROCNO                    1

F2 - Acquisition Parameters
Date_            20190730
Time                14.56
INSTRUM             spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT              DMF
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ              3.2767999 sec
RG                 191.38
DW              50.000 usec
DE               6.50 usec
TE               298.2 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1330885 MHz
NUC1                  1H
P1                 9.80 usec
PLW1         24.00000000 W

F2 - Processing parameters
SI                 65536
SF            500.1302158 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Figure S46. ^{13}C NMR of compound 10j




```
Current Data Parameters
NAME              TAnh-103D
EXPNO                    21
PROCNO                    1

F2 - Acquisition Parameters
Date_            20190731
Time                 0.29
INSTRUM             spect
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT              DMF
NS                  2048
DS                     4
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ              1.0485760 sec
RG                 191.38
DW              16.000 usec
DE               6.50 usec
TE               299.6 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          125.7703637 MHz
NUC1                 13C
P1                 9.50 usec
PLW1         90.00000000 W

======== CHANNEL f2 ========
SFO2          500.1320005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2         24.00000000 W
PLW12         0.36015001 W
PLW13         0.23050000 W

F2 - Processing parameters
SI                 65536
SF            125.7577885 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

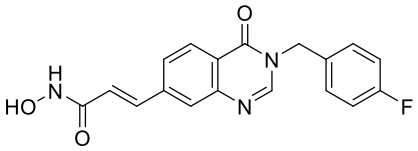


Figure S47. ^{1}H NMR of compound 10k

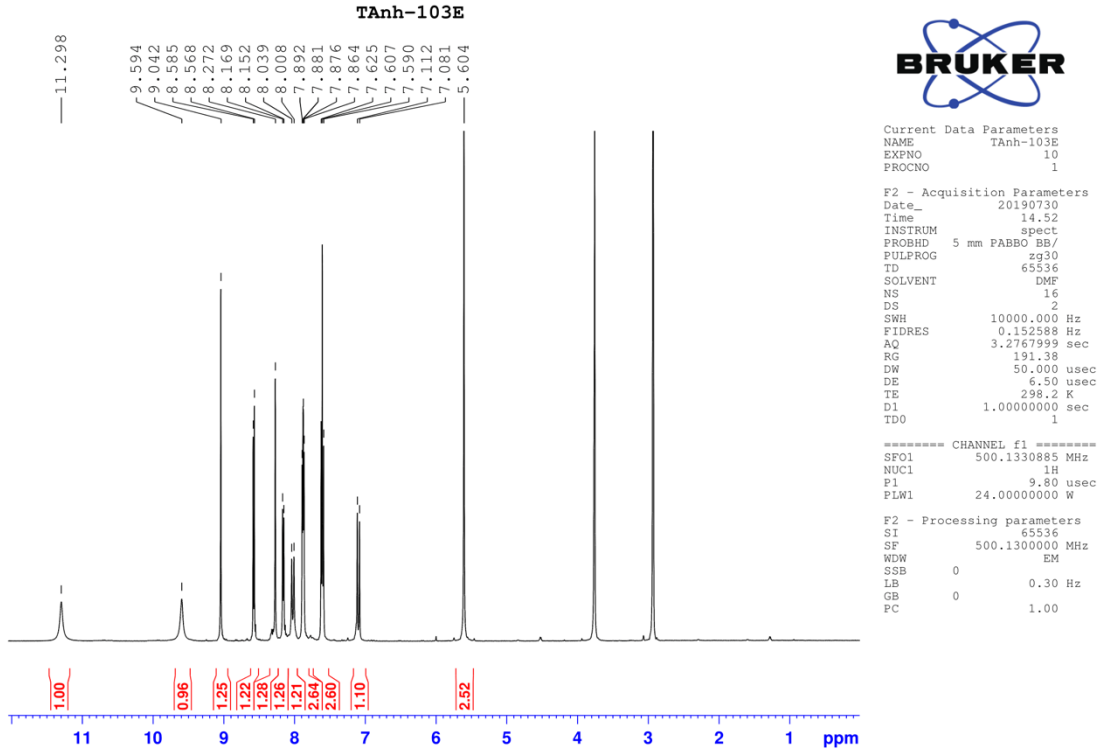
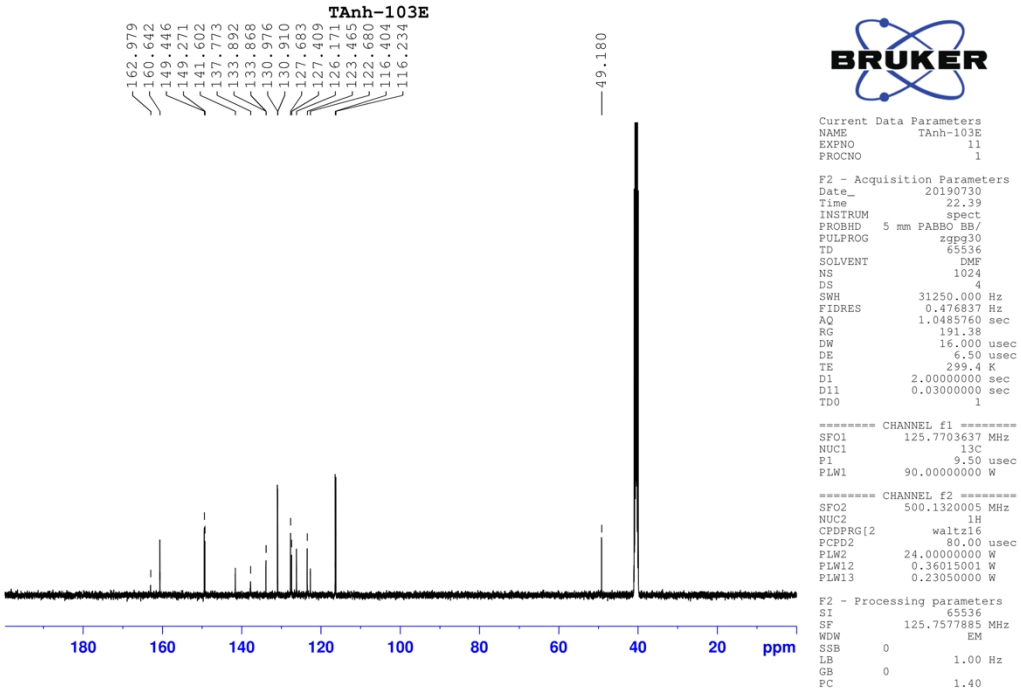


Figure S48. ^{13}C NMR of compound 10k



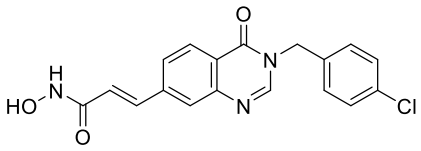


Figure S49. ^{1}H NMR of compound 10l

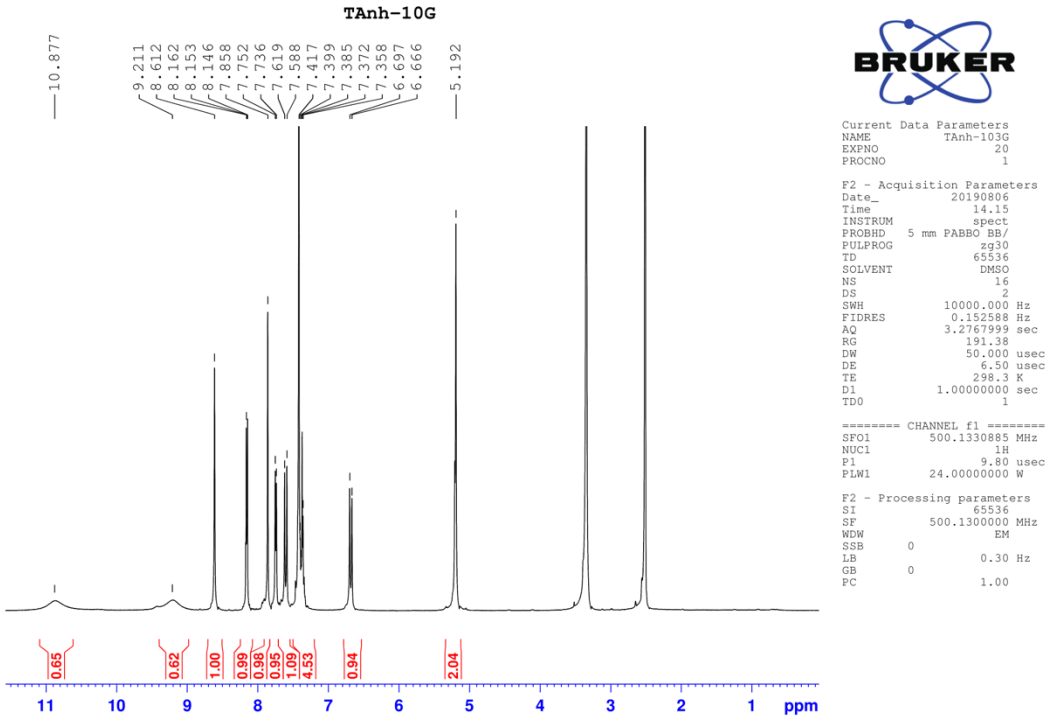
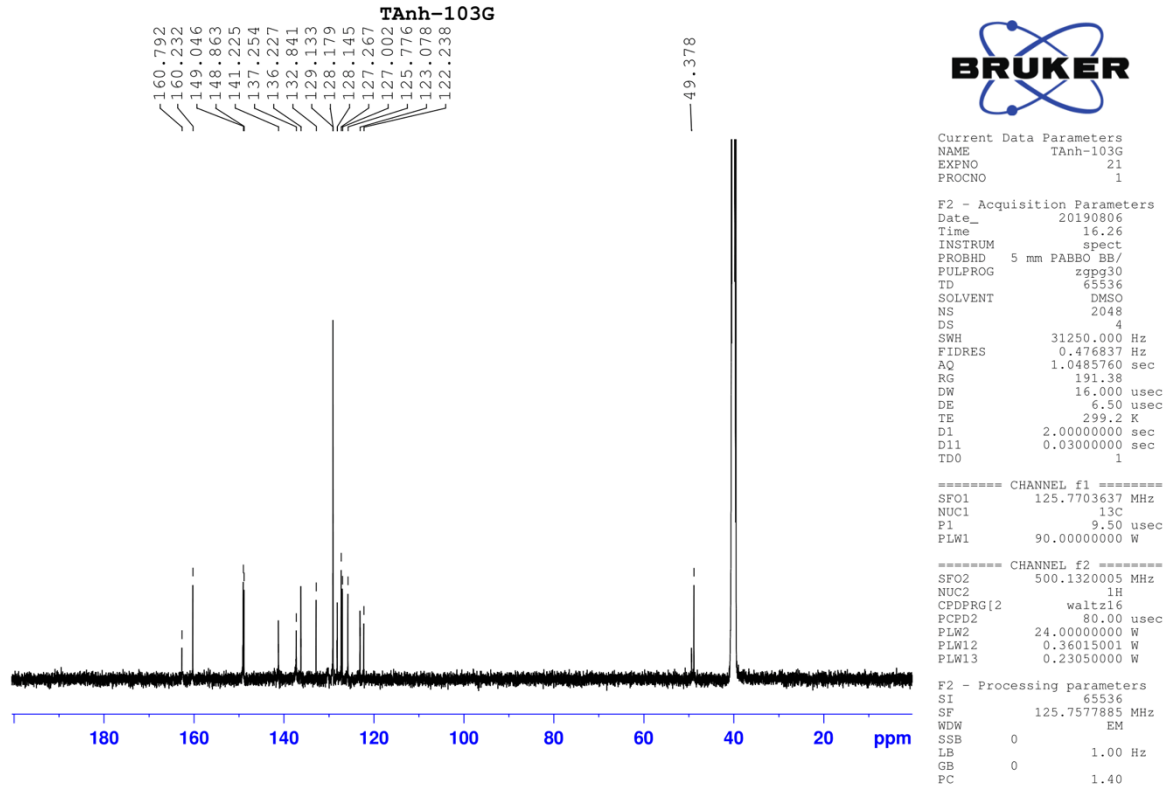


Figure S50. ^{13}C NMR of compound 10l



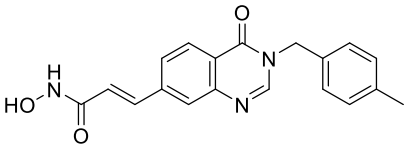


Figure S51. [1]H NMR of compound 10m

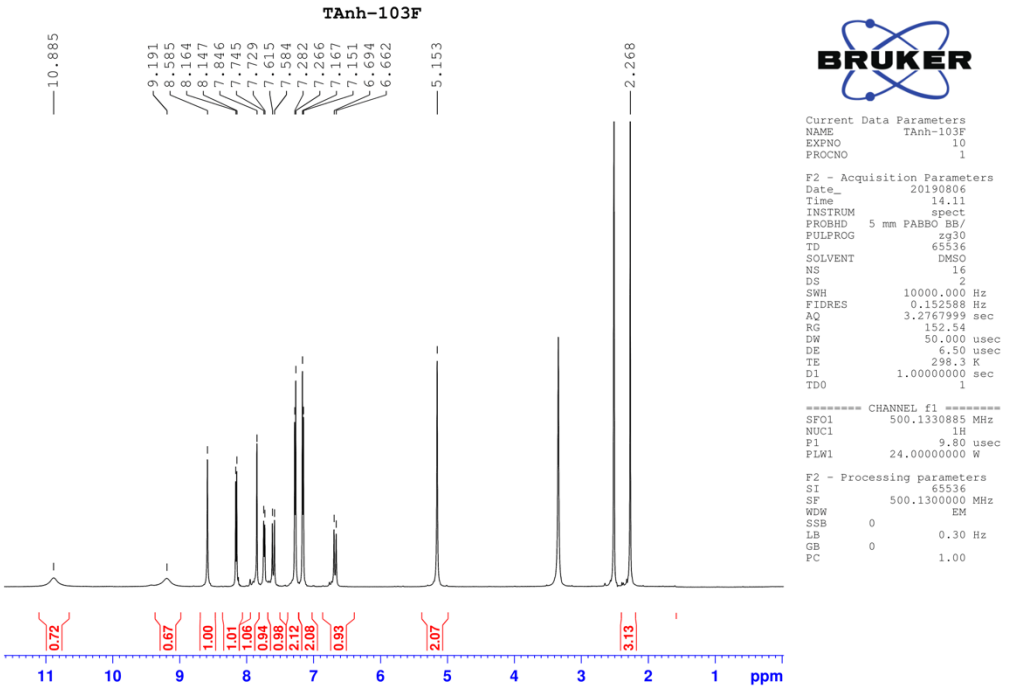


Figure S52. [13]C NMR of compound 10m