Electronic Supplementary Material (ESI) for ChemComm.

Discovery of a new class of highly potent necroptosis inhibitors targeting the Mixed Lineage Kinase Domain-Like protein

Bo Yan, Lei Liu, Shaoqiang Huang, Yan Ren, Huayi Wang, Zhenglin Yao, Lin Li, She Chen, Xiaodong Wang* and Zhiyuan Zhang*

Table of Contents

1. Supplementary figures and tables

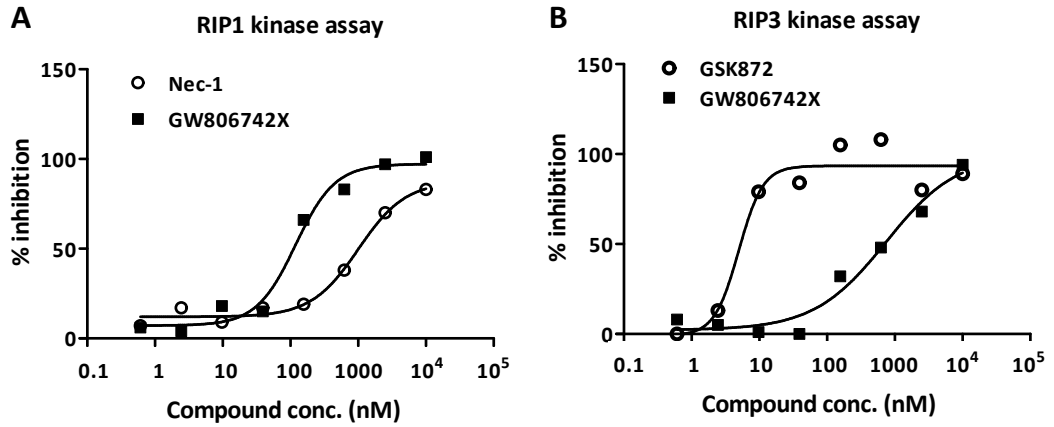


Figure S1. The reported MLKL inhibitor GW806742X showed inhibition to both human RIP1 and RIP3 kinase activity (IC_{50} = 119.4 nM and 741.9 nM respectively).

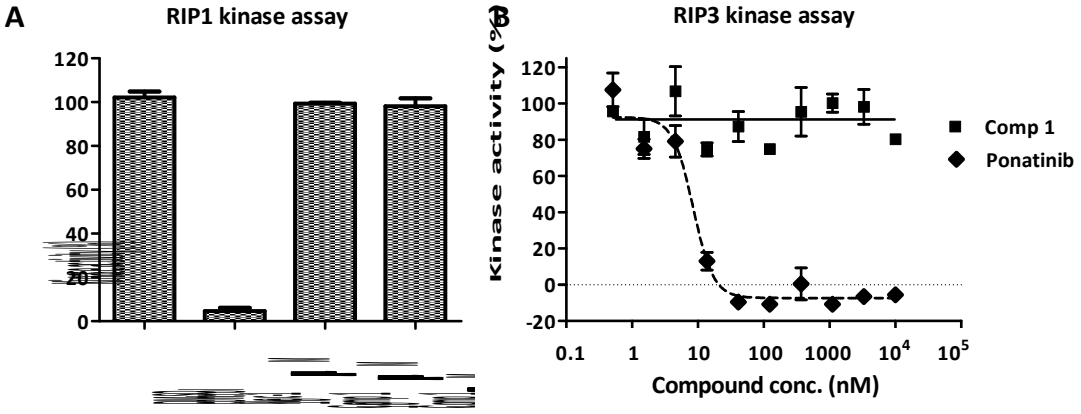
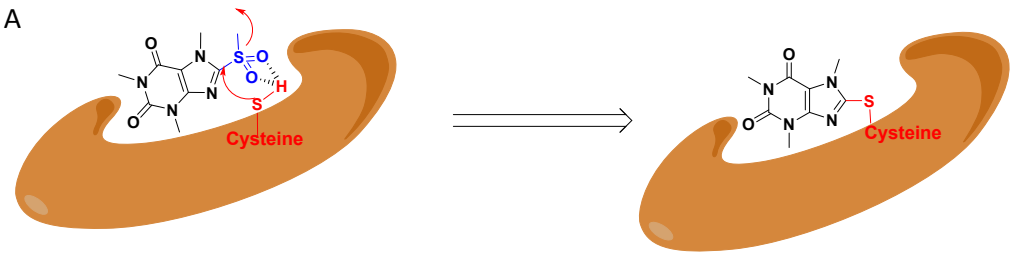


Figure S2. Compound **1** showed no inhibition to human RIP1 (A) and RIP3 kinase activity (B).



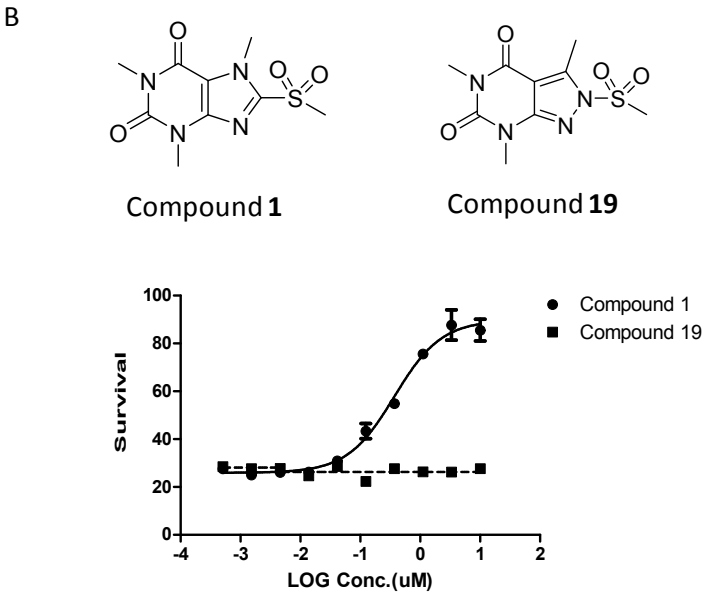


Figure S3. The leaving ability of sulfone was crucial for the potency of compound **1**. (A) Proposed covalent binding mode between compound **1** and target proteins. (B) The structure and anti-necroptosis potency of compound **19**.

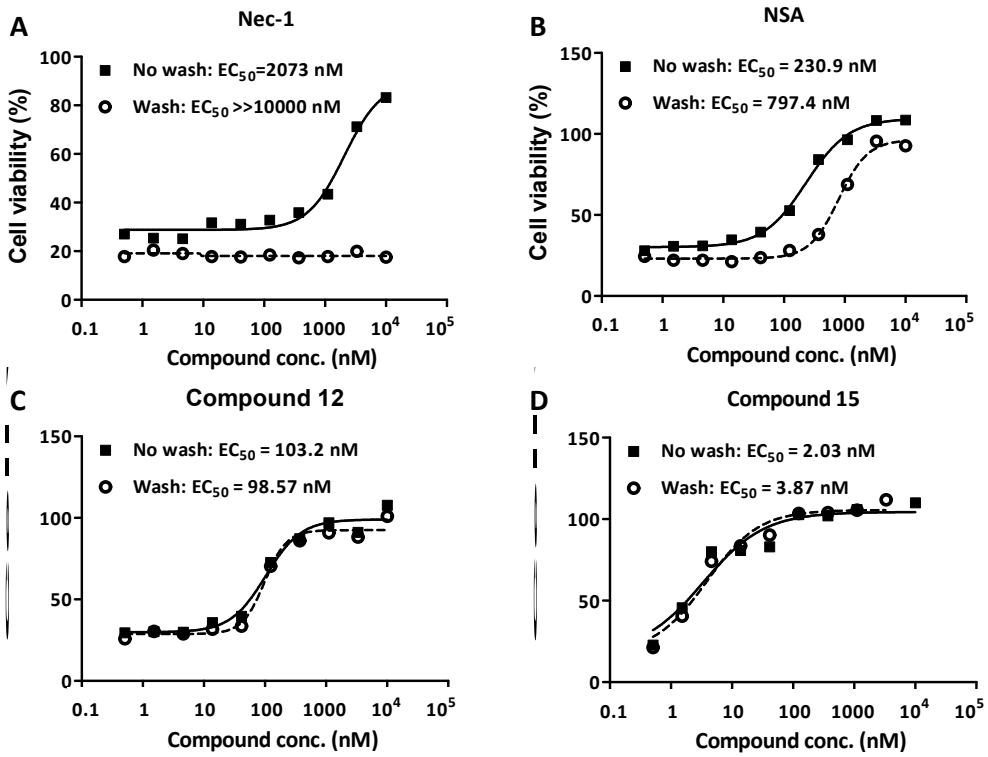


Figure S4. Compound **12** and **15** were covalent necroptosis inhibitors. We first incubated Nec-1, NSA, compound**12** and **15** with HT-29 cells for 2 hours, then washed away unbound

compounds with PBS buffer and replaced with fresh medium. TNF-α/Smac mimetic/z-VAD (TSZ) was added into medium to induce necroptosis. The cell viability was detected using the Cell Titer-Glo Assay kit (Promega). NSA was used as a positive control and Nec-1 as a negative control respectively.

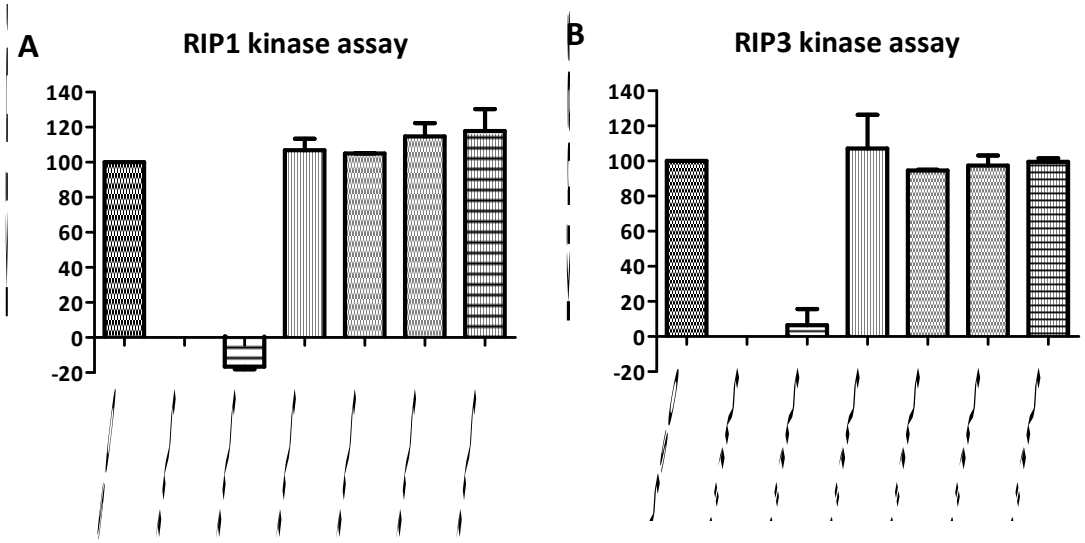


Figure S5. 10 μM compound **12** and compound **14**–**16** had no inhibition to human RIP1 (A) and RIP3 kinase activity (B).

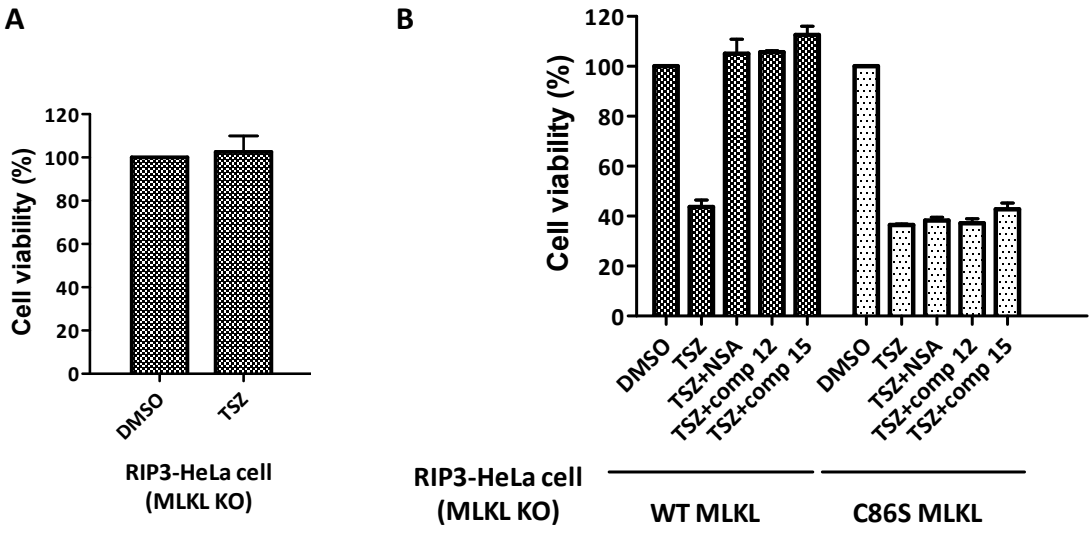


Figure S6. Compound **12** and **15** targeted cysteine86 of human MLKL. MLKL was knocked out (KO) in RIP3-HeLa cells and the RIP3-HeLa-MLKL KO cells resist necroptosis induced by TSZ (A). The RIP3-HeLa-MLKL KO cells were transfected with a C86S mutant or wild-type human MLKL-expressing construct. Similar to NSA (5 μM), compound **12** (1 μM) and **15** (200 nM) can protect RIP3-Hela-WT MLKL cells, but not RIP3-Hela-C86S MLKL cells from TSZ-induced necroptosis (B).

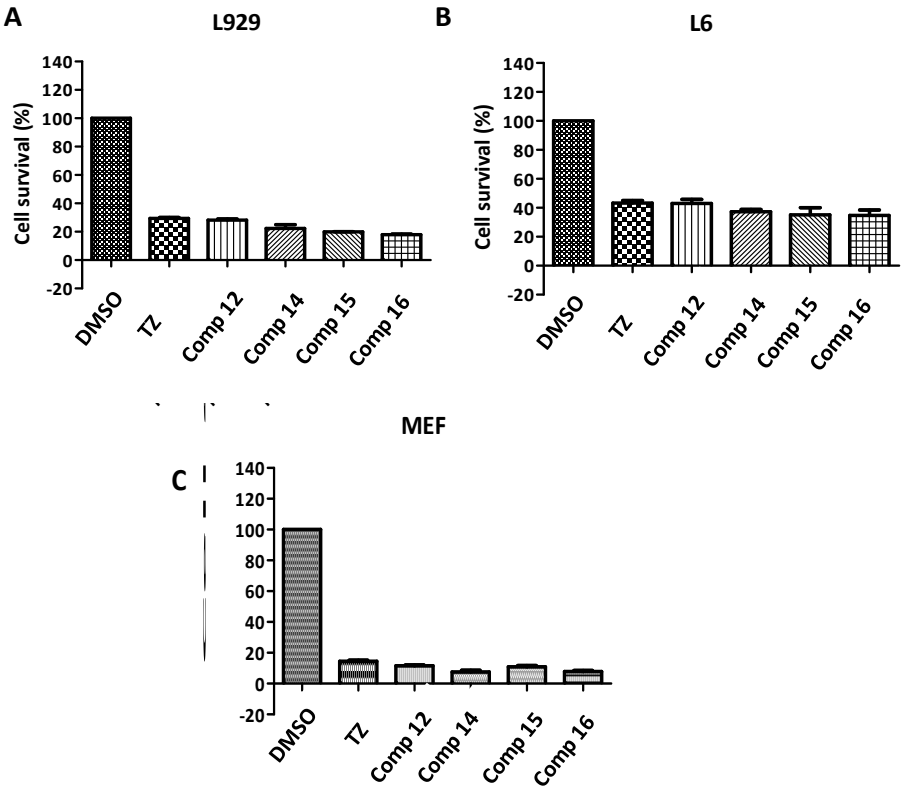


Figure S7. Compound **12** and compound **14–16** showed no anti-necroptosis activity in mouse MEF, L929 and rat L6 cells. We first incubate compound **12** (10 μM), **14** (5 μM), **15** (5 μM) or **16** (5 μM) with cells for 2 hours, then TNF-α/Smac mimetic/z-VAD (TSZ) or TNF-α/ z-VAD (TZ) was added into medium to induce necroptosis. The cell viability was detected using the Cell Titer-Glo Assay kit (Promega).

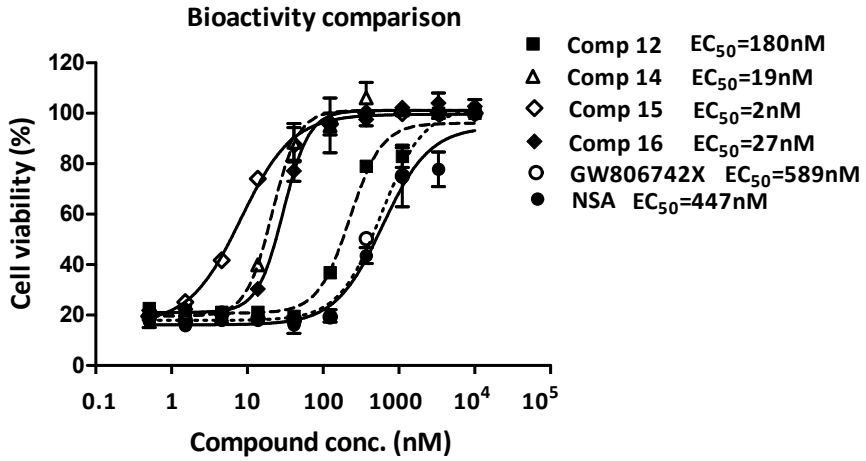
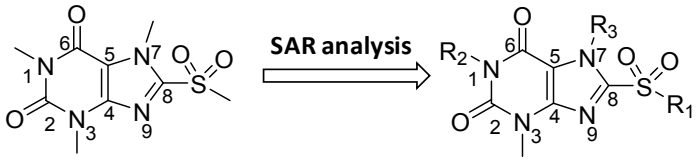
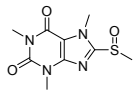
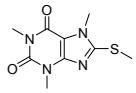
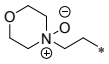


Figure S8. Compound **15** was the most potent MLKL inhibitor ever reported. Comparison of

the anti-necroptosis potency of NSA, GW806742X, compound **12**, and compound **14–16** in the TSZ-induced HT29 death assay.

Table S1. The SAR analysis of compound **1** on the 8- / 1- / 7- position.



Comp	R_1	R_2	R_3	EC_{50}(nM)	Comp	R_1	R_2	R_3	EC_{50}(nM)
20	Me	Me	Me	390 ± 8	**29**	Me		Me	1186 ± 28
21				3230 ± 426	**30**	Me		Me	2521 ± 260
22				>10000	**31**	Me		Me	>10000
23	Et	Me	Me	497 ± 50	**32**	Me	Ph	Me	>10000
24	n-Pr	Me	Me	738 ± 60	**33**	Me		Me	>10000
25	i-Pr	Me	Me	2500 ± 400	**34**	Me	Me	Et	261 ± 7
26	Benzyl	Me	Me	725 ± 100	**35**	Me	Me	n-Pr	1605 ± 80
27	Me	Et	Me	509 ± 118	**36**	Me	Me		>2500
28	Me	n-Pr	Me	973 ± 45	**37**	Me	Me		>2500

2 Materials and Instrumentation

The following antibodies were used: Anti-FLAG Rabbit mAb (Cell Signaling); GAPDH Rabbit mAb (Cell Signaling); β-actin Rabbit mAb (Cell Signaling); COX IV Rabbit mAb (Cell Signaling); Anti-Rabbit HRP (Cell Signaling); Anti-hMLKL Rabbit mAb (Abcam); Anti-RIP3 Rabbit mAb (Abcam); Anti-hMLKL (phospho 358) Rabbit mAb (Abcam). Hoechst 33342 was purchased in Thermo Fisher Scientific.

All chemical reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals. DCM, DMF, acetonitrile were distilled from calcium hydride; THF was distilled from sodium/benzophenone ketyl prior to use. ^{1}HNMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with $CDCl_3$ or DMSO as the solvent unless

otherwise stated. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (1H, δ 7.26; 13C, δ77.00) or DMSO (1H, δ 2.50; 13C, δ39.52). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constants. Infrared spectra were recorded on a Thermo Fisher FT-IR200 spectrophotometer. High-resolution mass spectra were obtained using a Bruker APEX Flash chromatography. The samples were analyzed by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA)Detector). The system was equipped with a Waters C_{18} 5μm SunFire separation column(150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade acetonitrile (solvent B) with a flow rate of 0.3 mL/min.

3 *In vitro* kinase assay

The RIP1 kinase assay was performed in white 384-well plate. The assay buffer contained 25mM HEPES (pH7.2), 20 mM $MgCl_2$, 12.5 mM $MnCl_2$, 5 mM EGTA, 2 mM EDTA, 12.5 mM β-glycerol phosphate and 2 mM DTT. RIPK1 was first incubated with compounds or DMSO control for 15 min, then ATP/MBP substrate mixture was added to initiate the reaction. The RIP3 kinase assay conditions were almost identical with that of RIP1 assay, except the assay buffer contained 5 mM $MgCl_2$ instead of 20 mM $MgCl_2$ and 12.5 mM $MnCl_2$. After 90 min reaction at room temperature, the ADP-Glo reagent and detection solution were added following the technical manual of ADP-Glo™ kinase assay kit (Promega). The luminescence was measured on PerkinElmer Enspire.

All the data were analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). The curves were fitted using a non-linear regression model with a sigmoidal dose response.

4 Cell culture and high-throughput screening

Cell culture: HT-29 cells were cultured in McCoy's 5A culture medium (Gibco). RIP3-HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco). MLKL-flag HT-29 cells were cultured in McCoy's 5A culture medium (Gibco) containing puromycin

(0.1%), hygromycin B (0.5%), blasticidin (0.2%). The medium contained 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotic-antimycotic solution. The cells were cultured at 37 °C in an atmosphere of 5% CO_2.

High-throughput screening: 2,000 HT-29 cells were split into each well of a 384-well assay plate. Necroptosis was induced by adding 20 ng/ml TNF-a (T), 100 nM Smac mimetic (S), and 20 mM z-VAD (Z) to the well. Identical concentrations of these necrosis-inducing agents were used in subsequent experiments unless otherwise stated. Individual compounds from a chemical library of ~200,000 compounds were delivered into each well at a final concentration of 10 mM. Cell viability in this and subsequent panels was determined by measuring ATP levels by Cell Titer-Glo assay after 24 hrs.

5 Cell survival assay

Cell survival assay was performed in 96-well cell culture plate. 3,000 cells were plated in each well and cultured at 37℃ overnight. For HT-29, cells were treated with TSZ and compounds for 24 hours. Then the cell survival ratio was determined using the Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega) according to the manufacturer's instructions. Luminescence was recorded with a PerkinElmer Enspire plate reader.

All the data were analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). The curves were fitted using a non-linear regression model with a sigmoidal dose response.

6 Western-Blot analysis

Cell pellet was collected and re-suspended with lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM Na_3VO4, 25 mM ß-glycerol-phosphate, 0.1mM PMSF, complete protease inhibitor cocktail phosphatase inhibitor cocktail(Roche)). The re-suspended cell pellet was incubated on ice for 30 min and centrifuged at 15,000 g for 10 min. The supernatants were collected for Western-blot analysis.

7 Immunofluorescence Staining

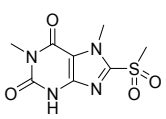
The MLKL-flag HT-29 cells were seeded in Lab-Tek eight-chambered slides (Thermo) for 12h. After treatment with different conditions as indicated for 6h, the cells were washed

with PBS buffer for three times and fixed in freshly prepared 4% paraformaldehyde for 30 min at room temperature followed by three washes in PBS and incubation in PBS containing 0.1% Triton X-100 for 15 min. The cells were then blocked for 30 min in blocking buffer (5% BSA in PBS). Primary antibodies were diluted in 5% BSA in PBS and incubated with the cells at 4 °C overnight. The next day, the cells were washed three times with PBS followed by incubation with a fluorescein-conjugated secondary antibody for one hour. The nuclei were stained with Hoechst 33342 for another 15 min. After three washes in PBS, the slides were covered and sealed and were examined. Similar results were obtained in at least three independent experiments. The colocalization was analyzed by the fluorescence intensity quantification using Zeiss LSM software.

8 Fractionation by Phase Separation

The pellets from treated cells were re-suspended in 5X volume of Triton X-114 lysis buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 2% Triton X-114, and complete protease inhibitor [Roche]) and incubated on ice for 30 min. The cell lysate was centrifuged at 15,000 × *g* at 4°C for 10 min, and then the supernatant was harvested as the detergent soluble fraction. After warming at 30°C for 3 min, the detergent soluble fraction was centrifuged at 1,500 × *g* for 5 min at room temperature. The aqueous layer was collected then re-centrifuged at 1,500 × *g* for 5 min to remove the contamination from the detergent enriched layer and saved as the aqueous faction (Aq). The detergent enriched layer was diluted with basal buffer (20 mM HEPES, pH 7.4, 150 mM NaCl) to the same volume of the detergent soluble fraction and re-centrifuged at 1,500 × *g* for 5 min. The washed detergent enriched layer was diluted with the basal buffer to the same volume as the aqueous faction and saved as the detergent fraction (Det).

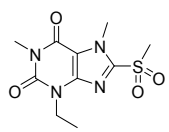
9 Chemical synthesis



TC013-19

1,7-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione (7) and **3-ethyl-1,7 -dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione (8)**

Step1: synthesis of 1,7-dimethyl-1H-purine-2,6(3H,7H)-dione **(4)**



TC013-20

To a solution of ethyl 4-amino-1-methyl-1H-imidazole-5- carboxylate (2.0g, 11.83mmol) and ethyl methylcarbamate (2.92g, 28.32mmol) in

anhydrous THF (2mL) was reacted at 75°C under nitrogen for 30min. Then to the mixture was added potassium 2-methylpropan-2-olate (1.98g, 17.64mmol) and reacted at 75°C for overnight. Then the solvent was removed and extracted with dichloromethane (3*50 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel (DCM:MeOH=20:1) to give a white solid (1.2g, yield 56.33%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.82 (s, 1H), 7.90 (s, 1H), 3.84 (s, 3H), 3.16 (s, 3H). Mass(m/z): 181.06$[M+H]^+$.

Step2: synthesis of 8-chloro-1,7-dimethyl-1H-purine-2,6(3H,7H)-dione**(5)**

To a solution of 1,7-dimethyl-1H-purine-2,6(3H,7H)-dione (0.53g, 2.94mmol) in anhydrous THF (10mL) was added NCS(0.59g, 4.36mmol) and stirred under nitrogen at RT for overnight. Then the solvent was removed and extracted with dichloromethane (3*20 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated to give a white solid (0.45g). ^{1}H-NMR (400MHz, DMSO-d_6): δ 12.03 (s, 1H), 3.81 (s, 3H), 3.16 (s, 3H). Mass(m/z): 215.03 $[M+H]^+$.

Step3: synthesis of 1,7-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione**(6)**

To a solution of 8-chloro-1,7-dimethyl-1H-purine-2,6(3H,7H)-dione (100mg,0.46mmol) in DMF (10mL)and H_2O (1mL) was added sodium methanethiolate (130.8mg, 1.86mmol) and reacted in the microwave on a Biotage Smith Synthesis at 120°C for 1h. Then the mixture was cooled to RT and acidified to PH=3. The reaction mixture was poured into water and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated to give a white solid (38.3mg).Mass(m/z): 227.05 $[M+H]^+$.

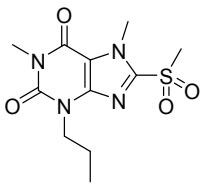
Step4: synthesis of 1,7-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione **(7)**

To a solution of 1,7-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione (15.0mg, 0.06mmol)in MeOH (3mL) was added oxone (163.2mg, 0.26mmol) in H_2O (5mL). Then the mixture was stirred at RT for 5h. Then the solvent was removed and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep. TLC to give **7** as a white solid (12.8mg,yield 74.85%). ^{1}H NMR (400 MHz, dmso) δ 12.19 (s, 1H), 4.16 (s, 3H), 3.49 (s, 3H), 3.20 (s, 3H). ^{13}C NMR (101 MHz, dmso) δ 156.16 (s), 151.21 (s), 145.80 (s), 145.17 (s), 109.65 (s), 43.46 (s), 34.10 (s), 27.53 (s). HRMS-ESI+: $[M + H]^+$ calcd for C8H10N4O4S, 259.0496; found, 259.0498.

Step5: synthesis of 3-ethyl-1,7-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione **(8)**

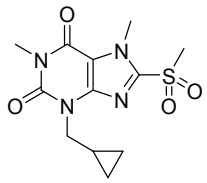
To a solution of 1,7-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione (15.0mg,0.06mmol) and K_2CO_3 (11.1mg, 0.08mmol) in anhydrous DMF (2mL) was added iodoethane (12.4mg, 0.08mmol) and stirred under nitrogen at RT for 2h. Then the reaction mixture was poured into water and extracted with dichloromethane (3*5 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep. TLC to give **8** as a white solid (10.5mg, yield 92.9%). ^{1}H-NMR (400 MHz,$CDCl_3$): δ 4.31 (s, 3H), 4.15 (q, *J*=6.4Hz, 2H), 3.44 (s, 3H), 3.41 (s, 3H), 1.33 (t, *J*=6.4Hz, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 155.41 (s), 150.68 (s), 146.09 (s), 145.50 (s), 109.73 (s), 42.70 (s), 38.78 (s), 34.05 (s), 28.15 (s), 13.25 (s). HRMS-ESI+: $[M + H]^+$ calcd for C10H14N4O4S, 287.0809; found, 287.0807.

1,7-dimethyl-8-(methylsulfonyl)-3-propyl-3,7-dihydro-1H-purine-2,6-dione (9)



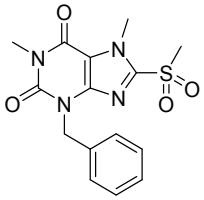
9 was prepared from ethyl 4-amino-1-methyl-1H-imidazole-5-carboxylate and ethyl methylcarbamate according to the similar procedure outlined for **8** (white solid, 28.3mg, yield 84.3%).^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.31 (s, 3H), 4.05 (t, *J*=7.6Hz, 2H), 3.44 (s, 3H), 3.41 (s, 3H), 1.80-1.74 (m, 2H), 0.96 (t, *J*=7.6Hz, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 155.41 (s), 150.88 (s), 146.02 (s), 145.83 (s), 109.67 (s), 45.15 (s), 42.68 (s), 34.03 (s), 28.18 (s), 21.21 (s), 11.13 (s). HRMS-ESI+: $[M + H]^+$ calcd for C11H16N4O4S, 301.0965; found, 301.0960.

3-(cyclopropylmethyl)-1,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (10)



10 was prepared from ethyl 4-amino-1-methyl-1H-imidazole-5-carboxylate and ethyl methylcarbamate according to the similar procedure outlined for **8** (white solid , 31.4mg, yield 94.1%).^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.32 (s, 3H), 3.95 (d, *J*=7.2Hz, 2H), 3.43 (s, 3H), 3.42 (s, 3H), 1.33-1.30 (m, 1H), 0.52-0.49 (m, 2H), 0.46-0.44 (m, 2H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 151.54 (s), 147.16 (s), 142.03 (s), 141.96 (s), 105.85 (s), 44.30 (s), 38.81 (s), 30.16 (s), 24.34 (s), 6.06 (s). HRMS-ESI+: $[M + H]^+$ calcd for C12H16N4O4S, 313.0965; found, 313.0962.

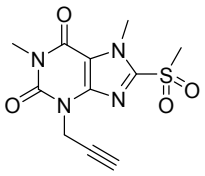
3-benzyl-1,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (11)



11 was prepared from ethyl 4-amino-1-methyl-1H-imidazole-5-carboxylate and ethyl methylcarbamate according to the similar

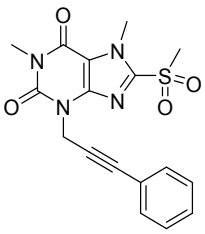
procedure outlined for **8** (white solid, 12mg, yield 51%).1H NMR (400 MHz, cdcl3) δ 7.48-7.46 (m, 2H), 7.33-7.25 (m, 3H), 5.24 (s, 2H), 4.30 (s, 3H), 3.46 (s, 3H), 3.41 (s, 3H). 13C NMR (101 MHz, cdcl3) δ 155.26 (s), 150.92 (s), 146.00 (s), 145.55 (s), 135.81 (s), 128.82 (s), 128.59 (s), 128.12 (s), 109.74 (s), 46.74 (s), 42.62 (s), 34.06 (s), 28.29 (s).

1,7-dimethyl-8-(methylsulfonyl)-3-(prop-2-yn-1-yl)-3,7-dihydro-1H-purine-2,6-dione (12)



12 was prepared from ethyl 4-amino-1-methyl-1H-imidazole-5-carboxylate and ethyl methylcarbamate according to the similar procedure outlined for **8** (white solid, 10.3mg, yield 44.89%).[1]H-NMR (400 MHz, $CDCl_3$):δ 4.82 (d, *J*=2.4Hz, 2H), 4.31 (s, 3H), 3.46 (s, 3H), 3.42 (s, 3H), 2.25 (t, *J*=2.4Hz, 1H). [13]C NMR (101 MHz, $cdcl_3$) δ 155.15 (s), 150.34 (s), 146.23 (s), 144.67 (s), 109.82 (s), 77.10 (s), 72.16 (s), 42.68 (s), 34.13 (s), 32.68 (s), 28.30 (s). HRMS-ESI+: $[M + H]^+$ calcd for C11H12N4O4S, 297.0652; found, 297.0659.

1,7-dimethyl-8-(methylsulfonyl)-3-(3-phenylprop-2-yn-1-yl)-3,7-dihydro-1H-purine-2,6-dione (14)



Step1: synthesis of 1,7-dimethyl-8-(methylthio)-3-(prop-2-yn-1-yl)-1H–purine-2,6 (3H,7H)-dione**(13)**

To a solution of 1,7-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione (400mg) and K_2CO_3 (12.8mg) in anhydrous DMF (5mL) was added 3-bromoprop-1-yne (219.2mg) and stirred under nitrogen at RT for 2h. Then the reaction mixture was poured into water and extracted with dichloromethane (3*5 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep. TLC (PE:EA 1:1) to obtain 206mg (yield 44.89%) as a white solid. [1]H NMR (400 MHz, $cdcl_3$) δ 4.86 (d, *J* = 2.0 Hz, 2H), 3.83 (s, 3H), 3.40 (s, 3H), 2.72 (s, 3H), 2.24 (t, *J* = 2.0 Hz, 1H). [13]C NMR (101 MHz, $cdcl_3$) δ 154.44 (s), 152.24 (s), 150.64 (s), 147.17 (s), 108.74 (s), 77.86 (s), 71.49 (s), 32.41 (s), 32.04 (s), 27.86 (s), 14.77 (s).

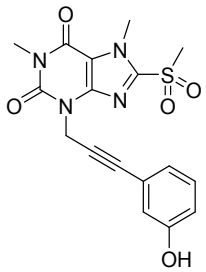
Step2: synthesis of 1,7-dimethyl-8-(methylsulfonyl)-3-(3-phenylprop-2-yn-1-yl)-1H-purine-2,6 (3H,7H)-dione **(14)**

To the solution of 1,7-dimethyl-8-(methylthio)-3-(prop-2-yn-1-yl)-1H-purine-2,6

(3H,7H)-dione (**13**) in DMF(10ml) was added iodobenzene, copper(I) iodide (catalytic), $(Ph_3P)_4Pd$ (catalytic) and TEA . Then the mixture was heated to 60℃ under nitrogen protection for 3 hours. The resulting reaction was cooled to room temperature, filtrated and extracted with EA (3*5ml) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (PE:EA 2:1) to obtain a yellow oil.

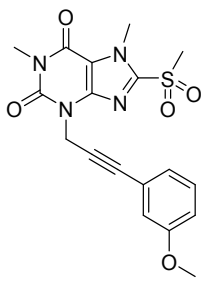
Dissolved the yellow oil in THF (2mL) and mixed with oxone in H_2O (2mL). Then the mixture was stirred at RT for 5h. Then the solvent was removed and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep.TLC to give **14** as a white solid(8.3mg, yield 57.7%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39-7.38 (m, 2H), 7.29-7.25 (m, 3H), 5.06 (s, 2H), 4.32 (s, 3H), 3.46 (s, 3H), 3.44 (s, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 155.27 (s), 150.42 (s), 146.20 (s), 144.91 (s), 131.89 (s), 128.65 (s), 128.23 (s), 122.10 (s), 109.91 (s), 83.69 (s), 82.48 (s), 42.76 (s), 34.16 (s), 33.59 (s), 28.38 (s). HRMS-ESI+: $[M + H]^+$ calcd for C17H16N4O4S, 373.0965; found, 373.0965.

3-(3-(3-hydroxyphenyl)prop-2-yn-1-yl)-1,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (15)



15 was prepared from 1,7-dimethyl-8-(methylthio)-1H- purine-2,6(3H,7H)-dione according to the similar procedure outlined for **14** (white solid, 13mg, yield 56.8%). ^{1}H-NMR (400 MHz, DMSO): δ 9.64 (s, 1H), 7.14 (s, 1H), 6.83-6.75 (m,3H), 4.98 (s, 2H), 4.21 (s, 3H), 3.54 (s, 3H), 3.28 (s, 3H). ^{13}C NMR (101 MHz, dmso) δ 157.67 (s), 155.29 (s), 150.42 (s), 145.71 (s), 144.84 (s), 130.23 (s), 122.79 (d, *J* = 10.1 Hz), 118.36 (s), 116.82 (s), 110.08 (s), 83.88 (s), 83.23 (s), 60.17 (s), 43.65 (s), 34.50 (s), 33.60 (s), 28.43 (s). HRMS-ESI+: $[M + H]^+$ calcd for C17H16N4O5S, 389.0914; found, 389.0914.

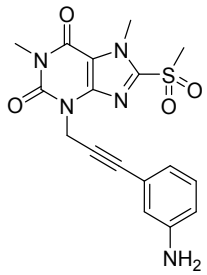
3-(3-(3-methoxyphenyl)prop-2-yn-1-yl)-1,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (16)



16 was prepared from 1,7-dimethyl-8-(methylthio)-1H- purine-2,6(3H,7H)-dione according to the similar procedure outlined for **14** (white solid, 11mg, yield 70%). ^{1}H NMR (400 MHz, $cdcl_3$) δ 7.18 (t, *J* = 8.0

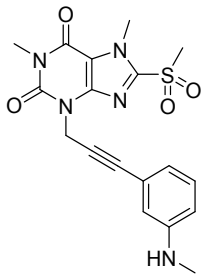
Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.92 (s, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 5.06 (s, 2H), 4.33 (s, 3H), 3.77 (s, 3H), 3.47 (s, 3H), 3.45 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 159.16 (s), 155.24 (s), 150.39 (s), 146.17 (s), 144.87 (s), 129.28 (s), 124.40 (s), 123.05 (s), 116.65 (s), 115.26 (s), 109.88 (s), 83.58 (s), 82.25 (s), 55.25 (s), 42.74 (s), 34.14 (s), 33.54 (s), 28.36 (s). HRMS-ESI+: [M + H]$^+$ calcd for C18H18N4O5S, 403.1071; found, 403.1071.

3-(3-(3-aminophenyl)prop-2-yn-1-yl)-1,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (17)



17 was prepared from 1,7-dimethyl-8-(methylthio)-1H- purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **14** (yellow solid, 8mg, yield 70%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.07 (t, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 5.04 (s, 2H), 4.32 (s, 3H), 3.47 (s, 3H), 3.44 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.25 (s), 150.38 (s), 146.16 (s), 145.93 (s), 144.89 (s), 129.16 (s), 122.76 (s), 122.32 (s), 118.20 (s), 115.72 (s), 109.89 (s), 83.90 (s), 81.84 (s), 42.74 (s), 33.58 (s), 29.67 (s), 28.35 (s). HRMS-ESI+: [M + H]$^+$ calcd for C17H17N5O4S, 388.1074; found, 388.1074.

1,7-dimethyl-3-(3-(3-(methylamino)phenyl)prop-2-yn-1-yl)-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione （18）



18 was prepared from 1,7-dimethyl-8-(methylthio)-1H- purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **14** (yellow solid, 10mg, yield 72%). ^{1}H NMR (400 MHz, dmso) δ 7.04 (t, *J* = 8.0 Hz, 1H), 6.55-6.49 (m, 3H), 5.79 (d, *J* = 5.2 Hz, 1H), 4.97 (s, 2H), 4.21 (s, 3H), 3.55 (s, 3H), 3.28 (s, 3H), 2.62 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR (101 MHz, dmso) δ 155.29 (s), 150.42 (s), 150.20 (s), 145.68 (s), 144.85 (s), 129.51 (s), 122.30 (s), 119.08 (s), 114.19 (s), 113.09 (s), 110.06 (s), 84.09 (s), 83.03 (s), 43.65 (s), 34.50 (s), 33.63 (s), 29.88 (s), 28.44 (s). HRMS-ESI+: [M + H]$^+$ calcd for C18H19N5O4S, 402.1231; found, 402.1231.

3,5,7-trimethyl-2-(methylsulfonyl)-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (19)

Step1： Synthesis of 6-hydrazinyl-1,3-dimethylpyrimidine-2,4(1H,3H)-dione

To a solution of 6-chloro-1,3-dimethylpyrimidine-2,4(1H,3H)-dione(2.0g, 11.45mmol) in isopropanol (6mL) was added hydrazine hydrate (6mL) and stirred at RT for overnight.

Then the precipitated white solid was filtered off and washed with water (3*15mL), dried well to obtain a white solid (1.67g, 85.6%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.06 (s, 1H), 5.09 (s, 1H), 4.37 (s, 2H), 3.21 (s, 3H), 3.08 (s, 3H). Mass(m/z): 171.08 [M+H]+.

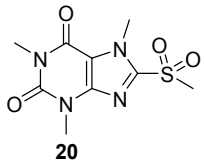
Step2: Synthesis of 3,5,7-trimethyl-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H, 7H)-dione

A mixture of 6-hydrazinyl-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (1.6g, 9.40mmol) and acetic anhydride (10mL) in dry pyride (12mL) were refluxed for 3h. Then the reaction was cooled to 0^{o}C and acidified with 1N HCl (30 mL). The solid obtained was collected by filtration, washed with 1N HCl (2*5 mL), water(2*10 mL) and dried to obtain a white solid (1.1g, 60.5%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.64 (br, 1H), 3.19 (s, 3H), 2.84 (s, 3H), 2.65 (s, 3H). Mass(m/z): 195.08 [M+H]+.

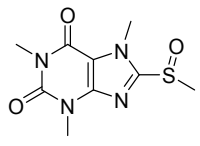
Step3: Synthesis of 3,5,7-trimethyl-2-(methylsulfonyl)-2H-pyrazolo[3,4-d] pyri-midine-4,6 (5H,7H)-dione **(19)**

To a solution of 3,5,7-trimethyl-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (50.0mg, 0.26mmol) in NaOH(aq) (10mL) was added methanesulfonyl chloride (44.2mg, 0.38mmol). Then the mixture was stirred at RT for overnight. The solid was precipitate out. The crude product was purified by Prep.TLC to obtain a white solid (31.2mg, 44.6%). ^{1}H-NMR (400 MHz,$CDCl_3$): δ 3.51(s, 3H), 3.42 (s, 3H), 3.37 (s, 3H), 2.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.92 (s), 151.52 (s), 151.23 (s), 148.52 (s), 102.26 (s), 41.91 (s), 29.98 (s), 27.96 (s), 12.37 (s). HRMS-ESI+: $[M + H]^+$ calcd for C9H12N4O4S, 273.0613; found, 273.0673.

1,3,7-trimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione (20) and 1,3,7- trimethyl-8-(methylsulfinyl)-3,7-dihydro-1H-purine-2,6-dione (21)



20



21

Step1: synthesis of 8-chloro-1,3,7-trimethyl-1H-purine-2,6(3H,7H)- dione

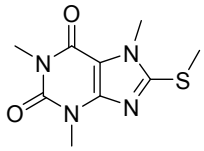
To a solution of 8-chloro-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (200.0mg, 0.93mmol) and K_2CO_3 (154.8mg, 1.12mmol) in anhydrous DMF (2mL) was added iodomethane (159.1mg, 1.12mmol) and stirred under nitrogen at RT for 4h. The reaction mixture was poured into water and extracted with dichloromethane (3*5mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by

chromatography on silica gel (PE:EA=2:1) to give a white solid (151.0mg, yield 70.4%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 3.96 (s, 3H), 3.56 (s, 3H), 3.41 (s, 3H). Mass(m/z): 229.04[M+H]$^+$.

Step2: synthesis of 8-mercapto-1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione

To a solution of 8-chloro-1,3,7-trimethyl-1H-purine-2,6(3H,7H)- dione (151.0mg, 0.66mmol) in anhydrous DMF (2mL) was added NaHS (111.3mg, 1.98mmol) and heated to 105^{o}C for 7h. Then the mixture was cooled to RT and acidified to pH=3. The mixture was poured into water and extracted with dichloromethane (3*5 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel (PE:EA=1:4) to give a white solid (128.0mg, yield 86.7%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.68 (s, 1H), 3.68 (s, 3H), 3.37 (s, 3H), 3.19 (s, 3H). Mass(m/z): 227.05 [M+H]$^+$.

Step3: synthesis of 1,3,7-trimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione **(22)**



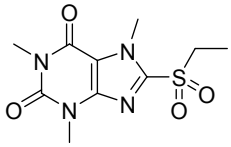
To a solution of 8-mercapto-1,3,7-trimethyl-1H-purine-2,6(3H,7H) -dione (20.0mg, 0.08mmol) and K_2CO_3 (14.7mg, 0.11mmol) in anhydrous DMF (2mL) was added iodomethane (15.1mg, 0.11mmol) and stirred under nitrogen at RT for 2h. The reaction mixture was poured into water and extracted with dichloromethane (3*3 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep.TLC to give a white solid **22** (19.2mg, yield 95.05%). 1H-NMR (400 MHz, CDCl3): δ^{1}H NMR (400 MHz, cdcl$_3$) δ 3.85 (s, 3H), 3.58 (s, 3H), 3.40 (s, 3H), 2.73 (s, 3H). HRMS-ESI+: [M + H]$^+$ calcd for C9H12N4O2S, 241.0754; found, 241.0753.

Step4: Synthesis of 1,3,7-trimethyl-8-(methylsulfinyl)-1H-purine-2,6(3H,7H)-dione **(21)** and 1,3,7- trimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione **(20)**

To a solution of **22** (18.0mg, 0.07mmol) in MeOH (2mL) was added oxone (69.2mg, 0.11mmol) in H_2O (2mL). Then the mixture was stirred at RT for 5h. Then the solvent was removed and extracted with dichloromethane (3*5 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep.TLC to give a white solid **21** (3.4mg, yield 20.5%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.28 (s, 3H), 3.58 (s, 3H), 3.41 (s, 3H), 3.18 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.20 (s), 151.32 (s), 150.46 (s), 147.04 (s), 110.17 (s), 38.93 (s), 33.24 (s), 29.85 (s), 28.11 (s). HRMS-ESI+: [M + H]$^+$ calcd for C9H12N4O3S, 257.0703; found, 257.0703 ; **20** white solid (6.3mg, yield 30.8%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.35 (s, 3H), 3.59 (s, 3H), 3.46 (s, 3H), 3.45 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ156.71 (s),

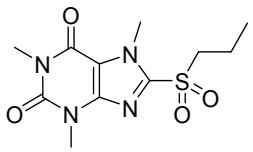
155.32 (s), 151.22 (s), 146.02 (s), 109.67 (s), 42.74 (s), 34.09 (s), 29.90 (s), 28.24 (s). HRMS-ESI+: $[M + H]^+$ calcd for C9H12N4O4S, 273.0652; found, 273.0652.

8-(ethylsulfonyl)-1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (23)



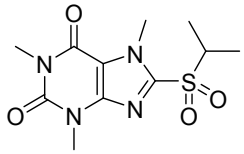
23 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **20** (white solid, 12.3mg, yield 42.7%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.32 (s, 3H), 3.62 – 3.48 (m, 5H), 3.40 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.31 (s), 151.20 (s), 146.24 (s), 144.95 (s), 109.69 (s), 49.56 (s), 34.17 (s), 29.91 (s), 28.23 (s), 6.79 (s). HRMS-ESI+: $[M + H]^+$ calcd for C10H14N4O4S, 287.0809; found, 287.0809.

1,3,7-trimethyl-8-(propylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (24)



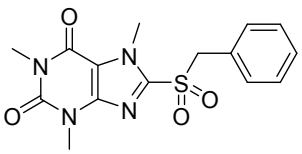
24 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **20** (white solid, 15.2mg, yield 39.1%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.32 (s, 3H), 3.57 (s, 3H), 3.50 (t, *J* = 7.2 Hz, 2H), 3.41 (s, 3H), 1.92 (m, 2H), 1.11 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.34 (s), 151.22 (s), 146.22 (s), 145.36 (s), 109.66 (s), 56.52 (s), 34.18 (s), 29.93 (s), 28.24 (s), 15.86 (s), 12.89 (s). HRMS-ESI+: $[M + H]^+$ calcd for C11H16N4O4S, 301.0965; found, 301.0965.

8-(isopropylsulfonyl)-1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (25)



25 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **20** (white solid, 31.5mg, yield 93.75%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.34 (s, 3H), 3.82 – 3.70 (m, 1H), 3.58 (s, 3H), 3.42 (s, 3H), 1.44 (s, 3H), 1.43 (s, 3H). HRMS-ESI+: $[M + H]^+$ calcd for C11H16N4O4S, 301.0965; found, 301.0953.

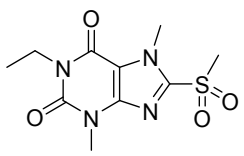
8-(benzylsulfonyl)-1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (26)



26 was prepared from 8-chloro-1,3-dimethyl-1H-purine- 2,6 (3H,7H)-dione according to the similar procedure outlined for **20** (white solid, 28.4mg, yield 51.6%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.39-7.37 (m, 1H), 7.36 – 7.32 (m, 2H), 7.17-7.15 (m, 2H), 4.66 (s, 2H), 3.71 (s, 3H), 3.64 (s, 3H), 3.40 (s, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.13 (s), 151.06 (s), 146.34 (s), 143.80 (s), 130.94 (s), 129.73 (s), 129.06 (s), 126.01 (s), 109.48 (s), 61.94 (s), 33.63 (s), 30.08 (s), 28.28

(s). HRMS-ESI+: $[M + H]^+$ calcd for C15H16N4O4S, 349.0965; found, 349.0961.

1-ethyl-3,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (27)



Step1: synthesis of 8-chloro-3,7-dimethyl-1H-purine-2,6(3H,7H)–dione

To a solution of 3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (4.0g, 22.19mmol) in anhydrous THF (20mL) was added NCS(4.5g, 33.71mmol) and stirred under nitrogen at RT for overnight. Then the solvent was removed and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and recrystallized from EtOH:MeOH=2:1 to give a white solid (2.5g, yield 70%).^{1}H NMR (400 MHz, DMSO-d_6): δ11.27(s, 1H), 3.81(s, 3H), 3.30(s, 3H). Mass(m/z): 215.03 $[M+H]^+$.

Step2: synthesis of 8-chloro-1-ethyl-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione

To a solution of 8-chloro-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (500mg, 2.33mmol) and K_2CO_3 (460.3mg, 2.79mmol) in anhydrous DMF (5mL) was added iodoethane (519.6mg, 2.79mmol) and stirred under nitrogen at RT for 4h. Then the reaction mixture was poured into water and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel (PE:EA=2:1) to give a white solid (141.2mg, yield 16.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.08 (q, *J*=7.2Hz, 2H), 3.95 (s, 3H), 3.54 (s, 3H), 1.24 (t, *J*=7.2Hz, 3H). Mass(m/z): 243.06 $[M+H]^+$.

Step3: synthesis of 1-ethyl-3,7-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione

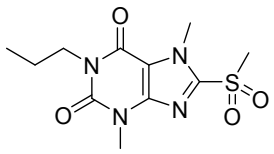
To a solution of 8-chloro-1-ethyl-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (40.0mg, 0.16mmol) in anhydrous DMF (2mL) was added NaSMe (0.48mmol) and heated to 100^{o}C for 3h. Then the mixture was cooled to RT and acidified to pH=3. Then the reaction mixture was poured into water and extracted with dichloromethane (3*5mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated to give a white solid. (12.0mg, yield 43.5%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.07 (q, *J*=6.8Hz, 2H), 3.84 (s, 3H), 3.56 (s, 3H), 2.71 (s, 3H), 1.24 (t, *J*=6.8Hz, 3H). Mass(m/z): 255.08 $[M+H]^+$.

Step4: synthesis of 1-ethyl-3,7-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione **(27)**

To a solution of 1-ethyl-3,7-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione (10mg,

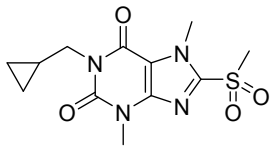
0.03mmol) in MeOH (1mL) was added oxone (120.8mg, 0.19mmol) in H_2O (1mL). Then the mixture was stirred at RT for 5h. Then the solvent was removed and extracted with dichloromethane (3*5mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep.TLC to give a white solid (8.2mg, yield 77.4%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.32 (s, 3H), 4.09 (q, *J* = 6.8 Hz, 2H), 3.56 (s, 3H), 3.44 (s, 3H), 1.25 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.10 (s), 150.88 (s), 146.04 (s), 146.95 (s), 109.82 (s), 42.79 (s), 36.94 (s), 34.11 (s), 29.84 (s), 13.16 (s). HRMS-ESI+: $[M + H]^+$ calcd for C10H14N4O4S, 287.0809; found, 287.0807.

3,7-dimethyl-8-(methylsulfonyl)-1-propyl-3,7-dihydro-1H-purine-2,6-dione (28)



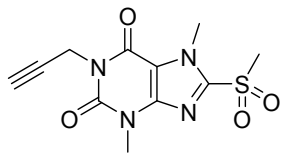
28 was prepared from 3,7-dimethyl-1H-purine-2,6(3H,7H) -dione according to the similar procedure outlined for **27** (white solid，8.7mg, yield 61.4%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.31 (s, 3H), 3.97 (t, *J* = 7.6 Hz, 2H), 3.55 (s, 3H), 3.43 (s, 3H), 1.71-1.62 (m, 2H), 0.96 (t, *J* = 7.6 Hz, 3H). HRMS-ESI+: $[M + H]^+$ calcd for C11H16N4O4S, 301.0965; found, 301.0965.

1-(cyclopropylmethyl)-3,7-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (29)



29 was prepared from 3,7-dimethyl-1H-purine-2,6(3H,7H) -dione according to the similar procedure outlined for **27** (white solid , 15mg, yield 53.8%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.32 (s, 3H), 3.91 (d, *J* = 7.2 Hz, 2H), 3.57 (s, 3H), 3.43 (s, 3H), 1.31 – 1.21 (m, 1H), 0.50 – 0.44 (m, 2H), 0.44 – 0.38 (m, 2H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 155.36 (s), 151.18 (s), 146.00 (s), 145.84 (s), 109.74 (s), 45.95 (s), 42.73 (s), 34.02 (s), 29.79 (s), 9.90 (s), 3.79 (s). HRMS-ESI+: $[M + H]^+$ calcd for C12H16N4O4S, 313.0965; found, 313.0962.

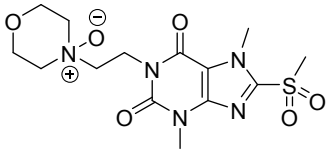
3,7-dimethyl-8-(methylsulfonyl)-1-(prop-2-yn-1-yl)-3,7-dihydro-1H-purine-2,6-dione (30)



30 was prepared from 3,7-dimethyl-1H-purine-2,6(3H,7H) -dione according to the similar procedure outlined for **27** (white solid, 4.8mg, yield 45.7%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.80 (d, *J* = 2.0 Hz, 2H), 4.33 (s, 3H), 3.59 (s, 3H), 3.44 (s, 3H), 2.20 (t, *J* = 2.4 Hz, 1H). HRMS-ESI+: $[M + H]^+$ calcd for C11H12N4O4S, 297.0652; found, 297.0646.

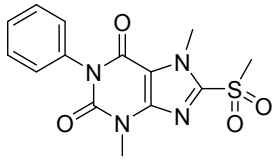
4-(2-(3,7-dimethyl-8-(methylsulfonyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-

yl)ethyl)morpholine 4-oxide (31)



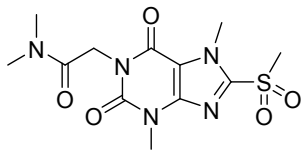
31 was prepared from 3,7-dimethyl-1H-purine-2,6(3H,7H)-dione according to the similar procedure outlined for **27** (white solid, 4.1mg, yield 39.7%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.62-4.59 (m, 2H), 4.30-4.15 (m, 7H), 4.15-3.97 (m, 2H), 3.97-3.55 (m, 2H), 3.41 (s, 3H) 3.39-3.37 (m, 5H). HRMS-ESI+: $[M + H]^+$ calcd for C14H21N5O6S, 388.1285; found, 388.1282.

3,7-dimethyl-8-(methylsulfonyl)-1-phenyl-3,7-dihydro-1H-purine-2,6-dione (32)



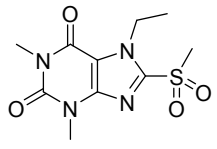
32 was prepared from 3,7-dimethyl-1H-purine-2,6(3H,7H) -dione according to the similar procedure outlined for **27** (white solid, 14.3mg, yield 64.2%). ^{1}H NMR (400 MHz, $cdcl_3$): δ 7.54-7.44 (m, 3H), 7.26 – 7.19 (m, 2H), 4.31 (s, 3H), 3.60 (s, 3H), 3.47 (s, 3H). HRMS-ESI+: $[M + H]^+$ calcd for C14H14N4O4S, 335.0809; found, 335.0809.

2-(3,7-dimethyl-8-(methylsulfonyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)-N,N-dimethylacetamide (33)



33 was prepared from 3,7-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **27** (white solid , 6.8mg, yield 63.1%). ^{1}H NMR (400 MHz, $cdcl_3$) δ 4.83 (s, 2H), 4.28 (s, 3H), 3.55 (s, 3H), 3.41 (s, 3H), 3.11 (s, 3H), 2.98 (s, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 165.91 (s), 155.09 (s), 151.05 (s), 146.50 (s), 146.11 (s), 109.71 (s), 42.89 (s), 42.30 (s), 36.23 (s), 35.81 (s), 34.15 (s), 29.99 (s). HRMS-ESI+: $[M + H]^+$ calcd for C12H17N5O5S, 344.1023; found, 344.1017.

7-ethyl-1,3-dimethyl-8-(methylsulfonyl)-3,7-dihydro-1H-purine-2,6-dione (34)



Step1: synthesis of 8-chloro-7-ethyl-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione

To a solution of 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione (3.0g, 14.01mmol) and K_2CO_3 (2.32g, 16.81mmol) in anhydrous DMF (30mL) was added iodoethane and stirred under nitrogen at RT for 4h. The reaction mixture was poured into water and extracted with EA (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel (PE:EA=4:1) to

give a white solid 213mg (yield 94.7%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.40 (q, *J* = 7.2 Hz, 2H), 3.55 (s, 3H), 3.41 (s, 3H), 1.44 (t, *J* = 7.2 Hz, 3H).Mass(m/z): 243.06 $[M+H]^+$.

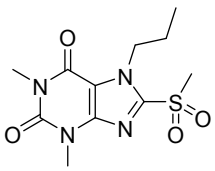
Step2: synthesis of 7-ethyl-1,3-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione

To a solution of 8-chloro-7-ethyl-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (213mg, 0.88mmol) in anhydrous DMF (2mL) was added sodium methanethiolate (1.5mL, 4.3mmol) and heated to 105^{o}C for 8h. Then the reaction mixture was poured into water and extracted with dichloromethane (3*25 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel (PE:EA=2:1) to give a white solid. (31.2mg, yield 19.2%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.28 (q, *J* = 7.2 Hz, 2H), 3.57 (s, 3H), 3.40 (s, 3H), 2.72 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H). Mass (m/z): 255.08 $[M+H]^+$.

Step3: synthesis of 7-ethyl-1,3-dimethyl-8-(methylsulfonyl)-1H-purine-2,6(3H,7H)-dione **(34)**

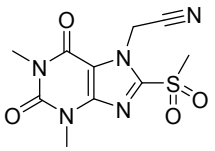
To a solution of 7-ethyl-1,3-dimethyl-8-(methylthio)-1H-purine-2,6(3H,7H)-dione (24.7mg, 0.09mmol) in MeOH (2mL) was added oxone (237.3mg, 0.38mmol) in H_2O (2mL). Then the mixture was stirred at RT for 5h. Then the solvent was removed and extracted with dichloromethane (3*10 mL) and the organic layer was separated, dried over Na_2SO_4, filtered, concentrated and purified by Prep.TLC to give **34** a white solid (20.3mg, yield 73.1%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.78 (q, *J* = 7.2 Hz, 2H), 3.57 (s, 3H), 3.45 (s, 3H), 3.42 (s, 3H), 1.54 (t, *J* = 7.2 Hz, 3H). HRMS-ESI+: $[M + H]^+$ calcd for C10H14N4O4S, 287.0809; found, 287.0809.

1,3-dimethyl-8-(methylsulfonyl)-7-propyl-3,7-dihydro-1H-purine-2,6-dione (35)



35 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **34** (white solid, 41mg, yield 93%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 4.68 (t, *J* = 7.6 Hz, 2H), 3.57 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H), 2.00 – 1.91 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 154.93 (s), 151.21 (s), 146.18 (s), 145.78 (s), 109.20 (s), 49.02 (s), 42.98 (s), 29.91 (s), 28.26 (s), 24.83 (s), 10.71 (s). HRMS-ESI+: $[M + H]^+$ calcd for C11H16N4O4S, 301.0965; found, 301.0965.

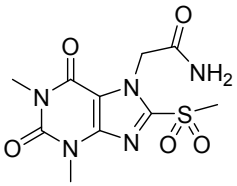
2-(1,3-dimethyl-8-(methylsulfonyl)-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)acetonitrile (36)



36 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H) -dione according to the similar procedure outlined for **34** (white solid,

8.2mg, yield 46.86%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 5.81 (s, 2H), 3.60 (s, 3H), 3.46 (s, 3H), 3.44 (s, 3H).

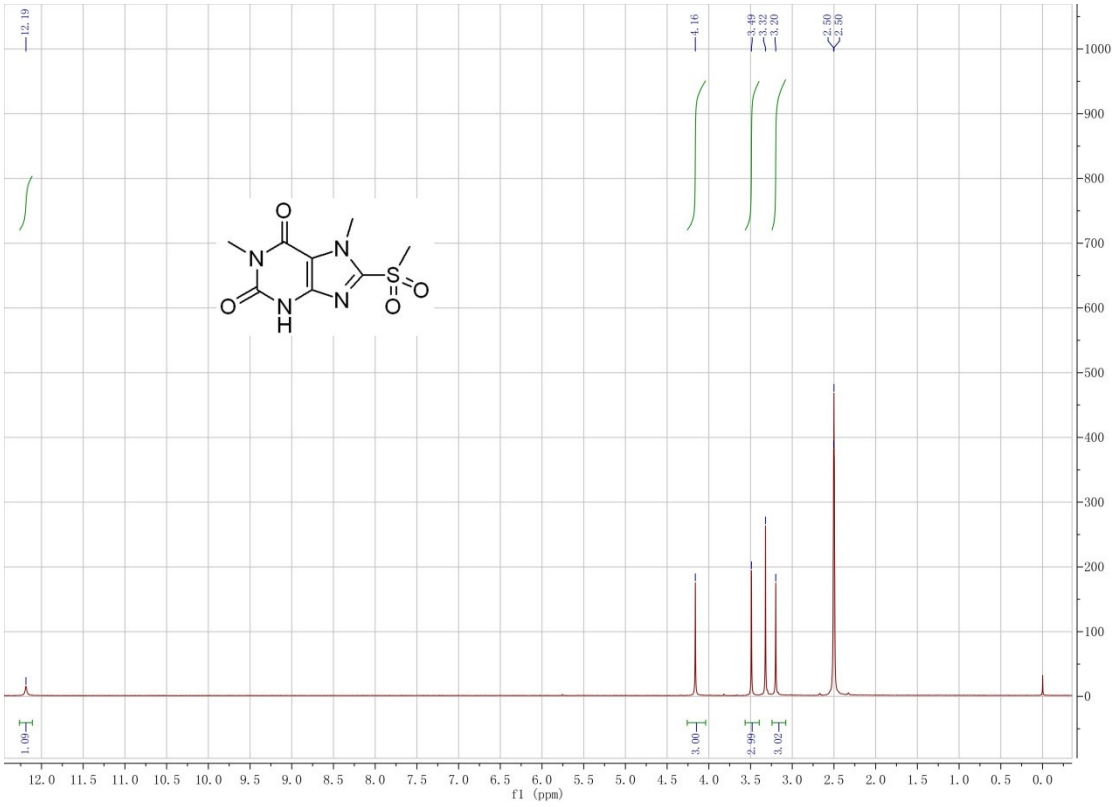
2-(1,3-dimethyl-8-(methylsulfonyl)-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)acetamide (37)

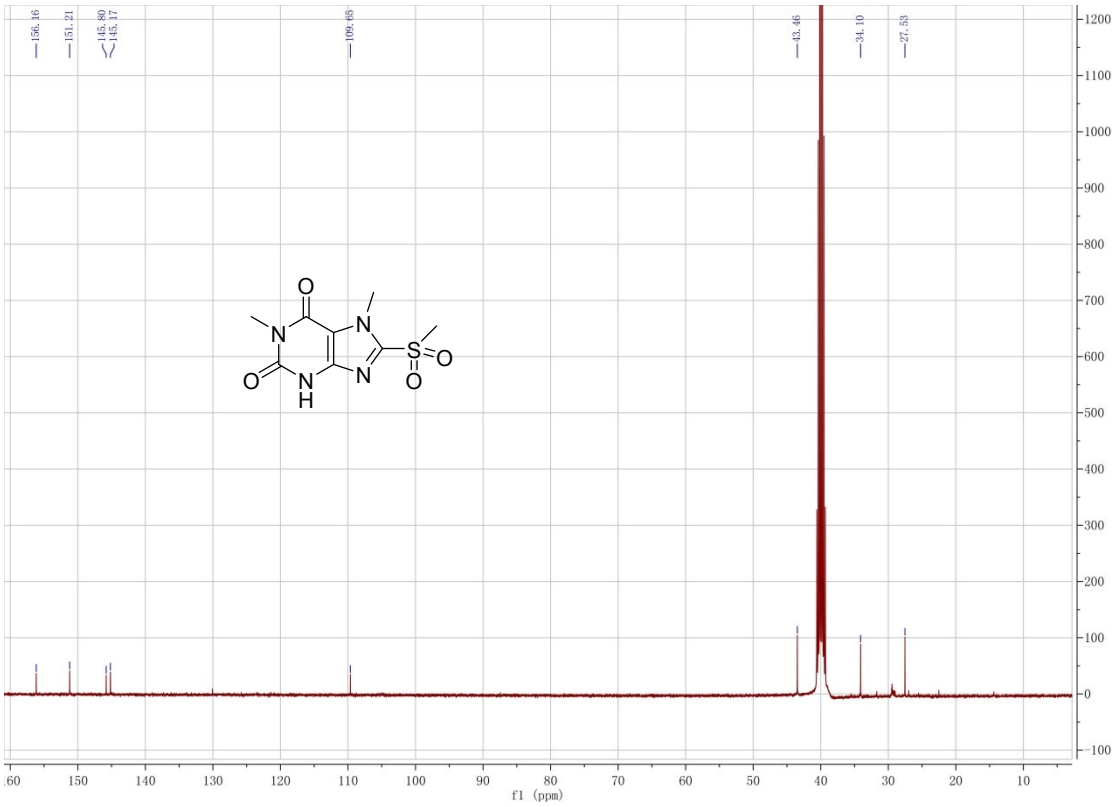


37 was prepared from 8-chloro-1,3-dimethyl-1H-purine-2,6 (3H,7H)-dione according to the similar procedure outlined for **34** (white solid , 15.4mg, yield 73.65%). ^{1}H NMR (400 MHz, dmso) δ 7.81 (s, 1H), 7.42 (s, 1H), 5.37 (s, 2H), 3.45 (s, 3H), 3.43 (s, 3H), 3.24 (s, 3H). HRMS-ESI+: [M + H]$^{+}$ calcd for C10H13N5O5S, 316.0710; found, 316.0710.

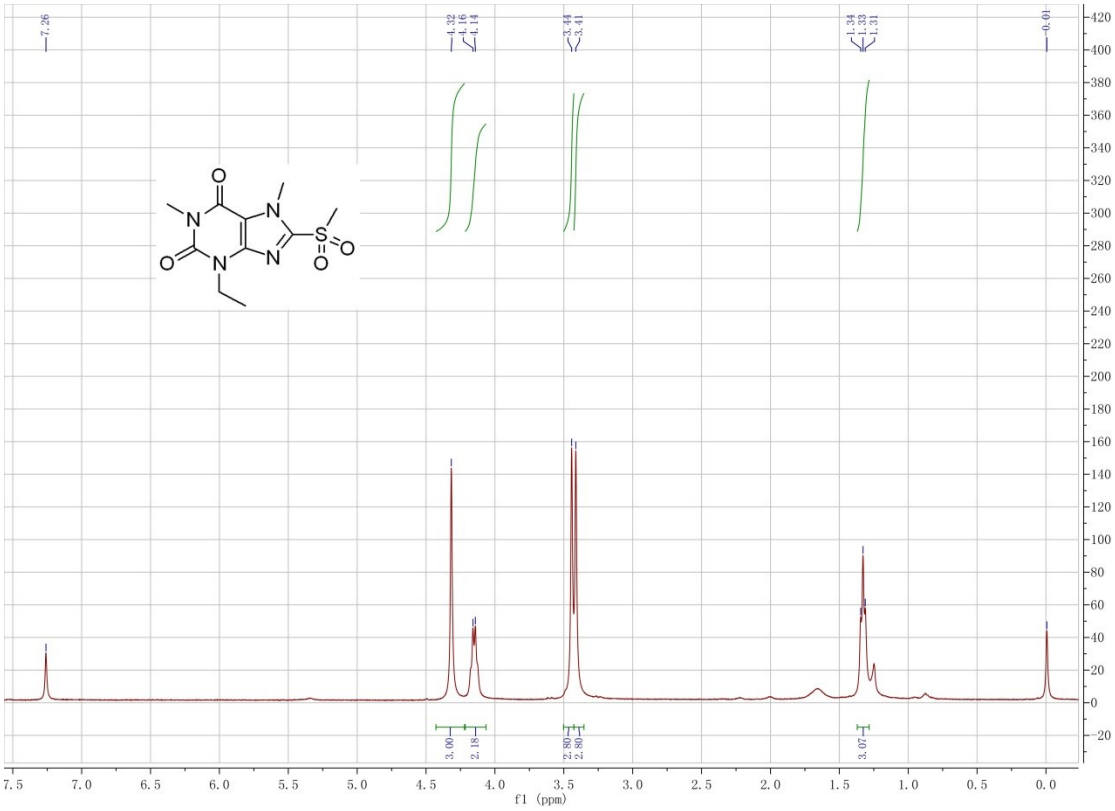
^{1}H and ^{13}C NMR spectra

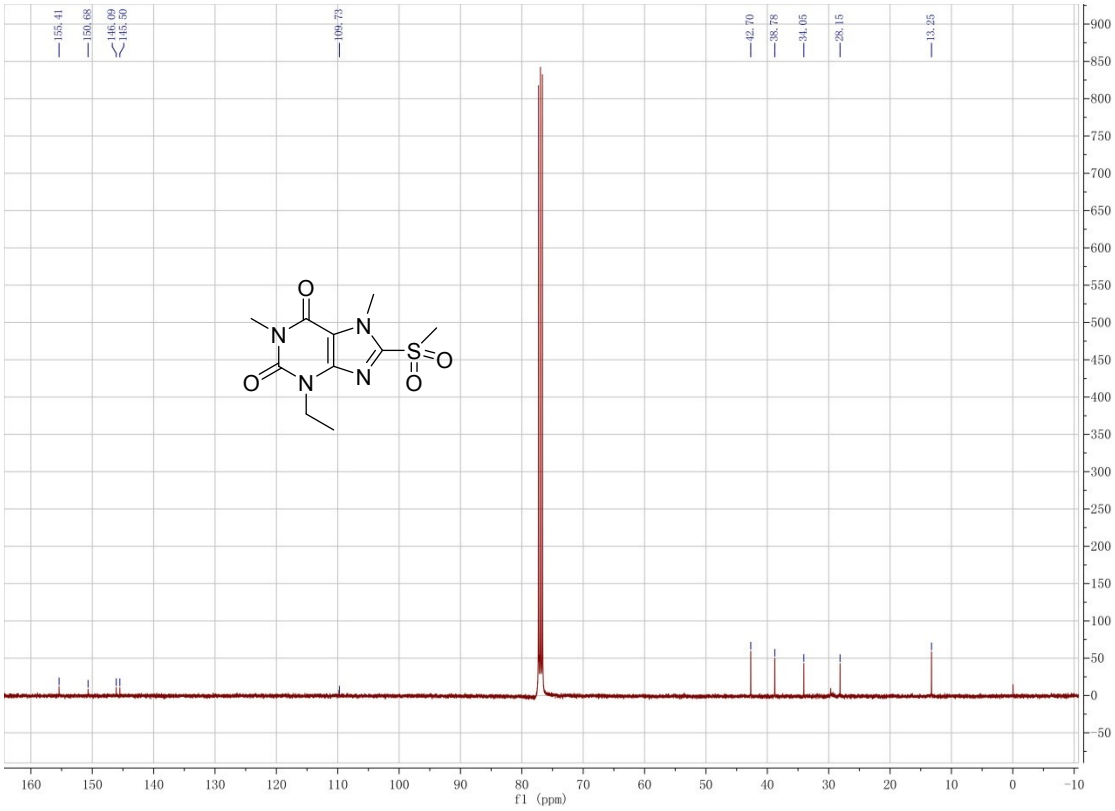
Compound 7



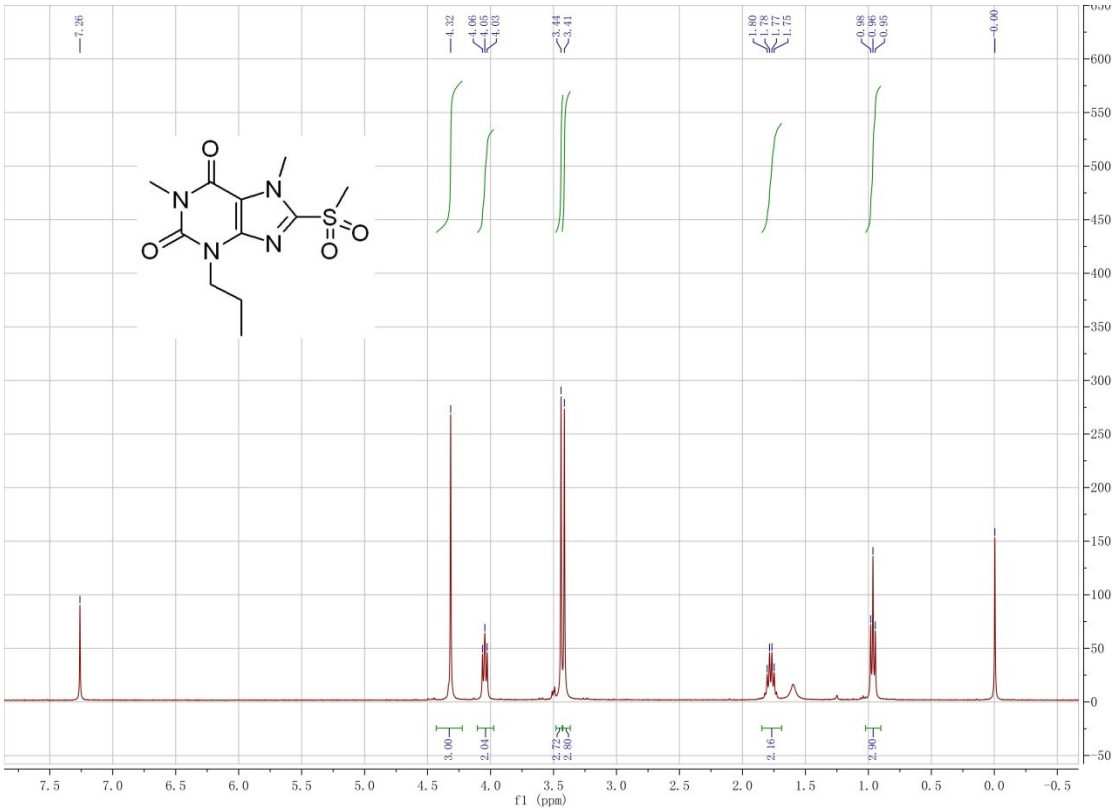

156.16
151.21
145.89
143.17
109.65
43.46
34.10
27.53
f1 (ppm)

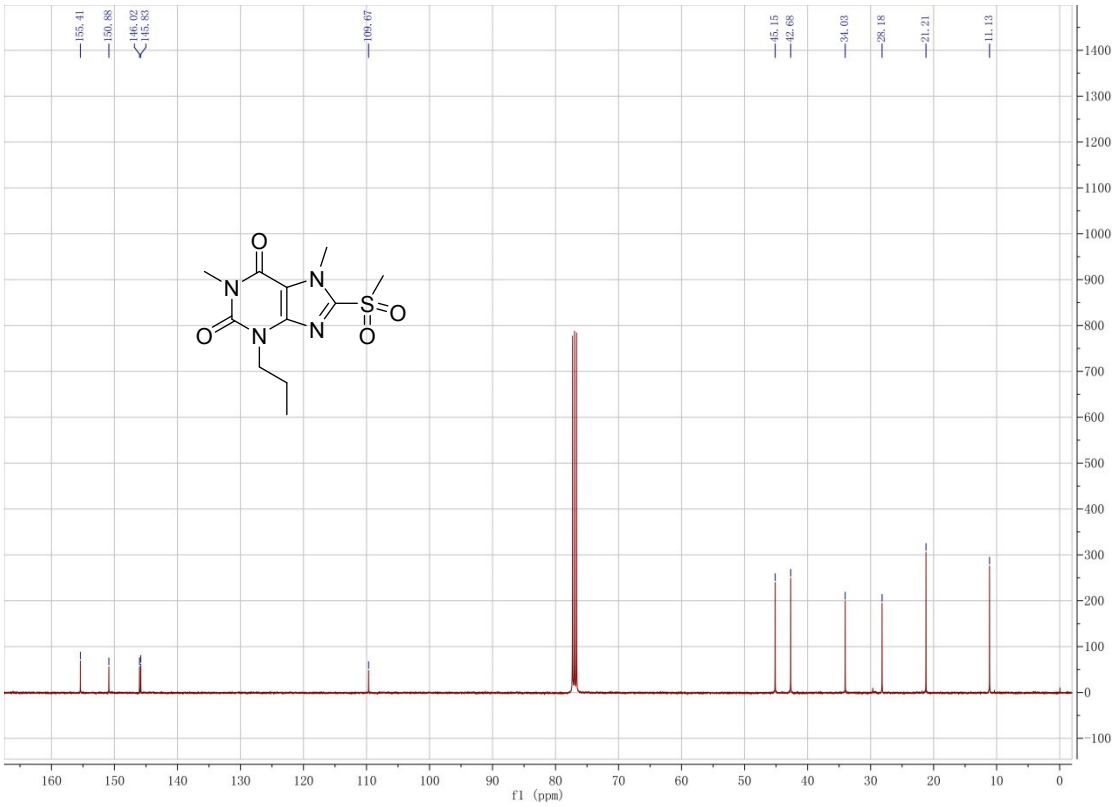
Compound 8


7.26
4.32
4.16
4.14
3.44
3.41
1.34
1.33
1.31
0.01
3.00
2.18
2.80
2.80
3.07
f1 (ppm)

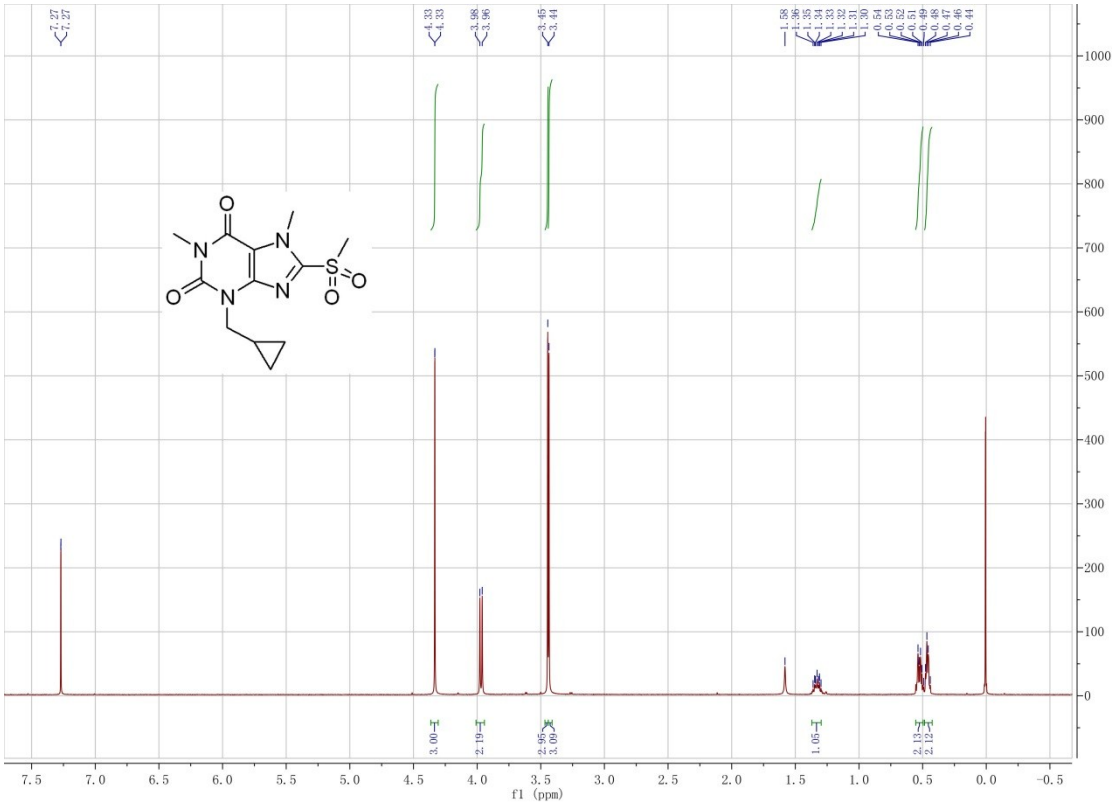

155.41
150.68
146.09
145.50
109.73
42.70
38.78
34.05
28.15
13.25
f1 (ppm)

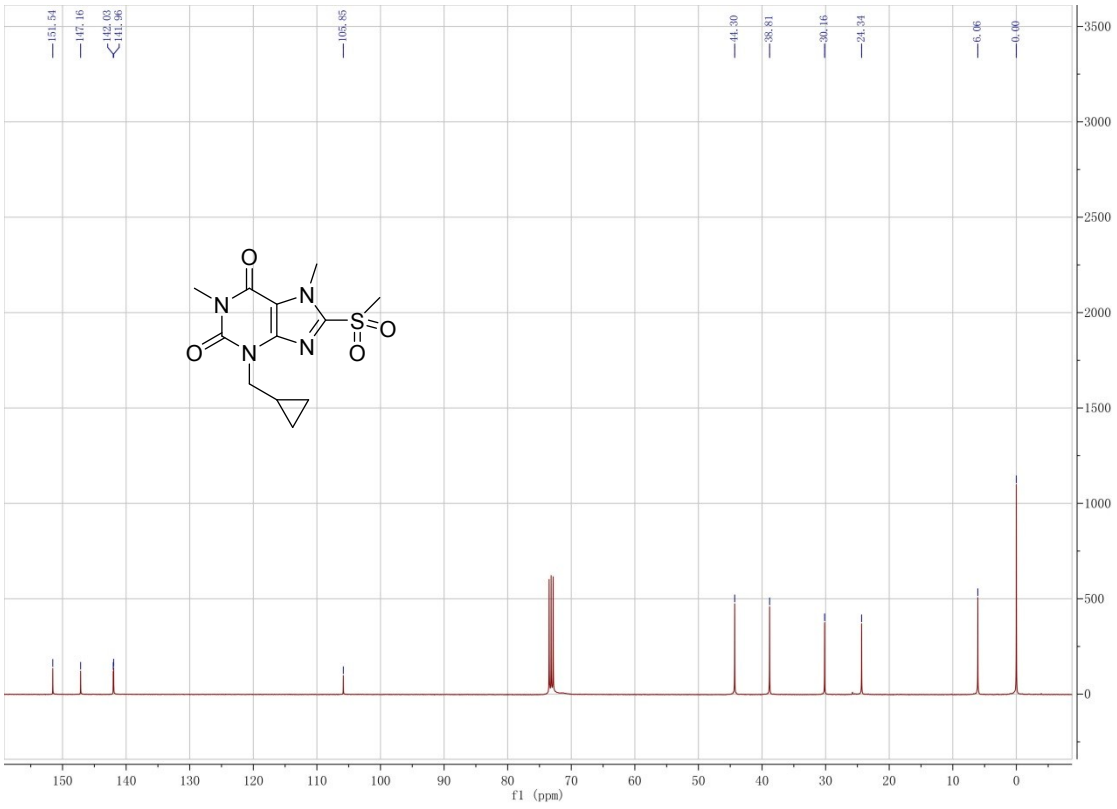
Compound 9


7.26
4.32
4.06
4.05
4.03
3.44
3.41
1.80
1.78
1.77
1.75
0.98
0.96
0.95
0.00
3.00
2.04
2.72
2.80
2.16
2.90
f1 (ppm)

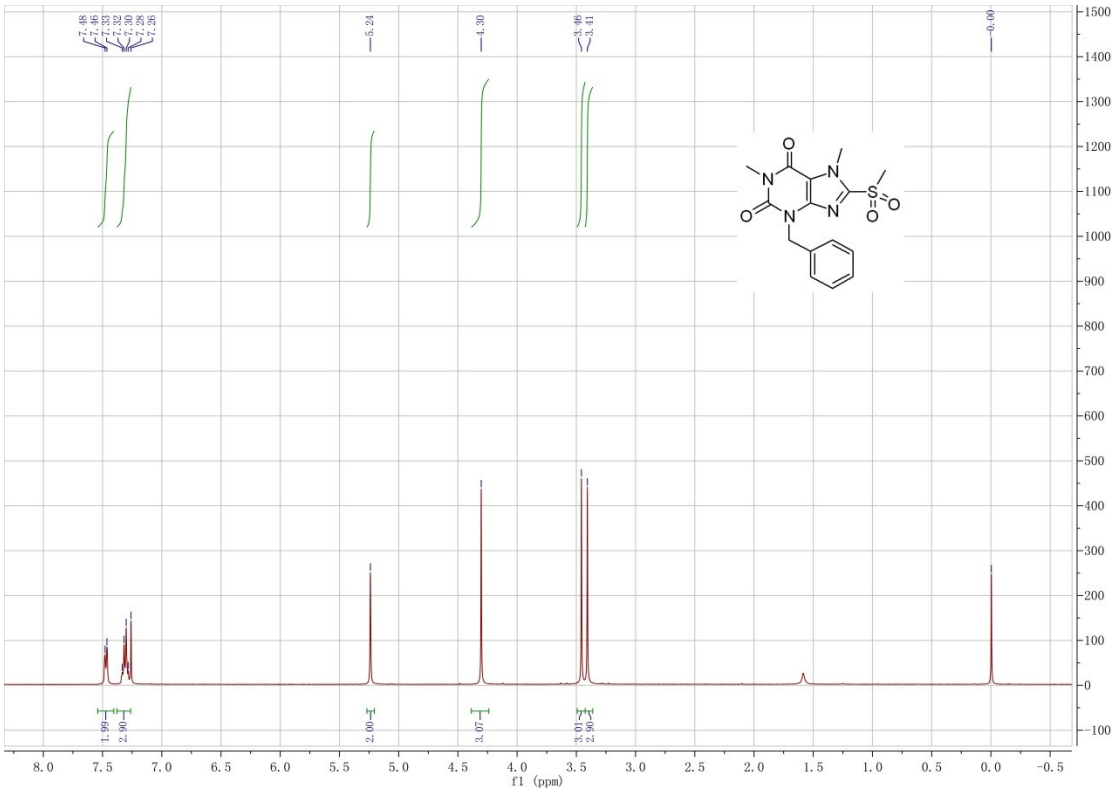

155.41
150.88
146.02
145.83
109.67
45.15
42.68
34.03
28.18
21.21
11.13
f1 (ppm)

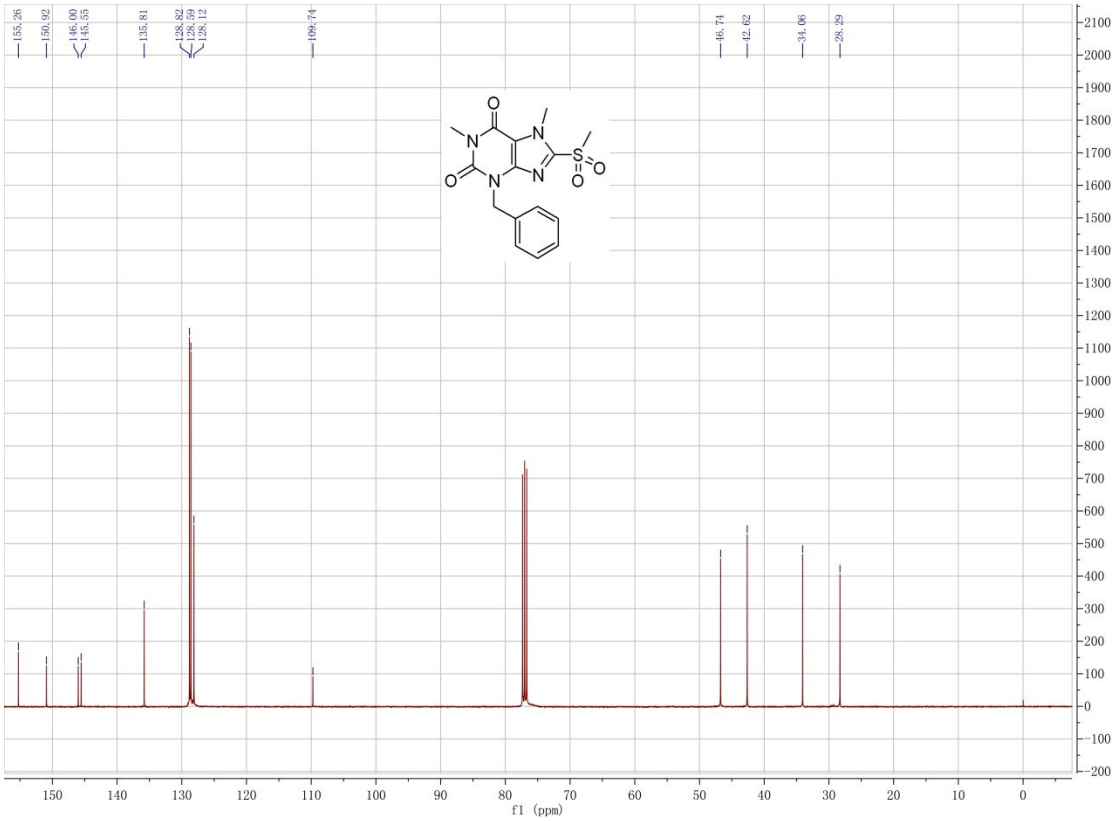
Compound 10


7.27
7.27
4.33
4.33
3.98
3.96
3.45
3.44
1.58
1.36
1.35
1.34
1.33
1.32
1.31
1.30
0.54
0.53
0.52
0.51
0.49
0.48
0.47
0.46
0.44
3.00
2.19
2.95
3.09
1.05
2.13
2.12
f1 (ppm)

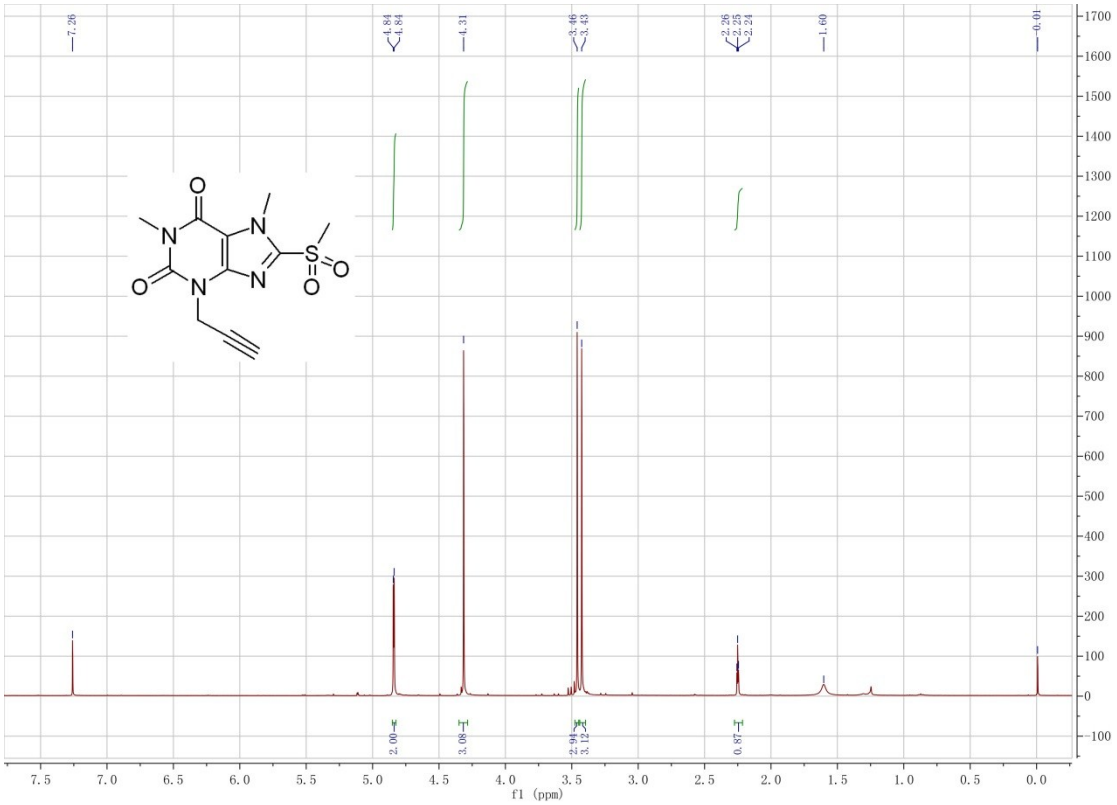

151.54
147.16
142.03
141.96
105.85
44.30
38.81
30.16
24.34
6.06
0.00
f1 (ppm)

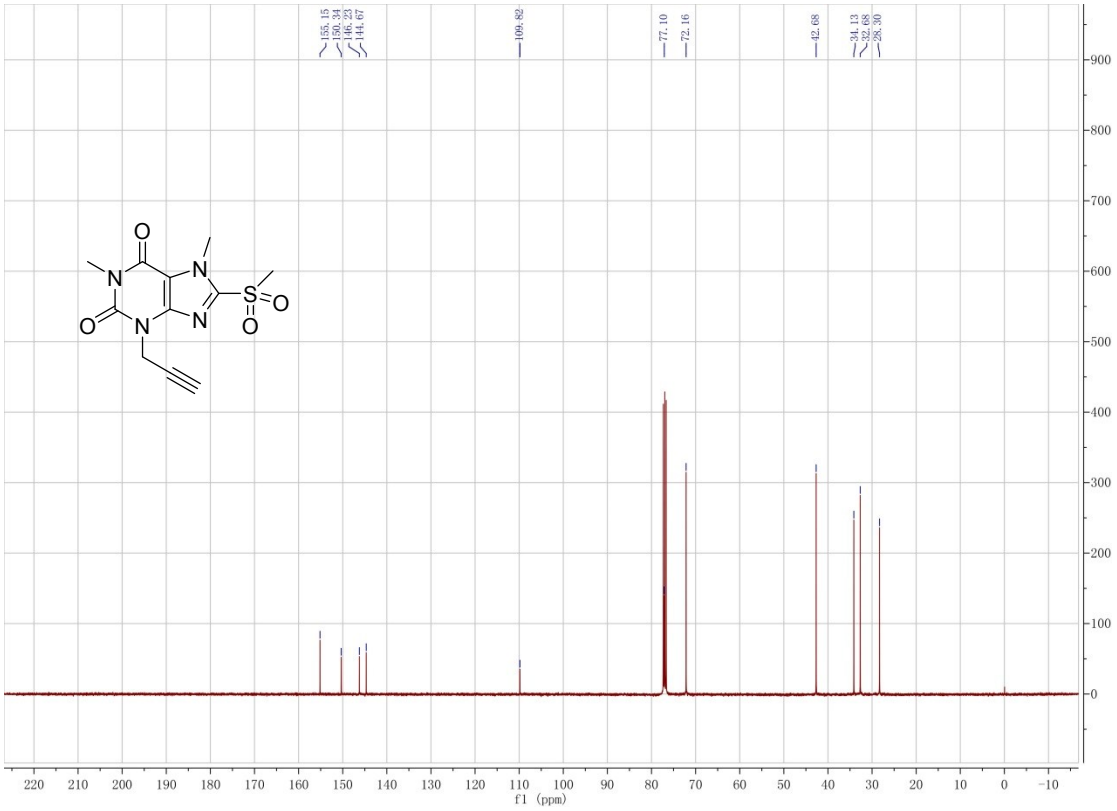
Compound 11


7.48
7.46
7.33
7.32
7.30
7.28
7.26
5.24
4.30
3.46
3.41
0.00
1.99
2.90
2.00
3.07
3.01
2.80
f1 (ppm)

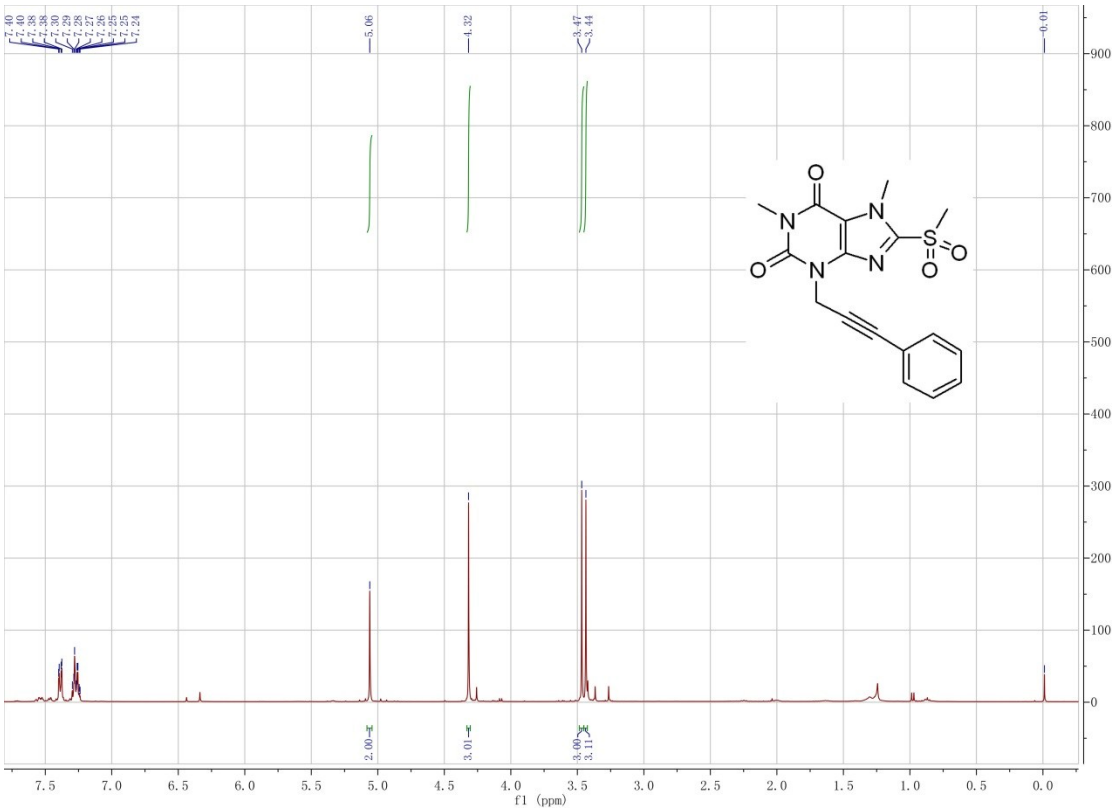

155.26
150.92
146.00
145.55
135.81
128.82
128.59
128.12
109.74
46.74
42.62
34.06
28.29
f1 (ppm)

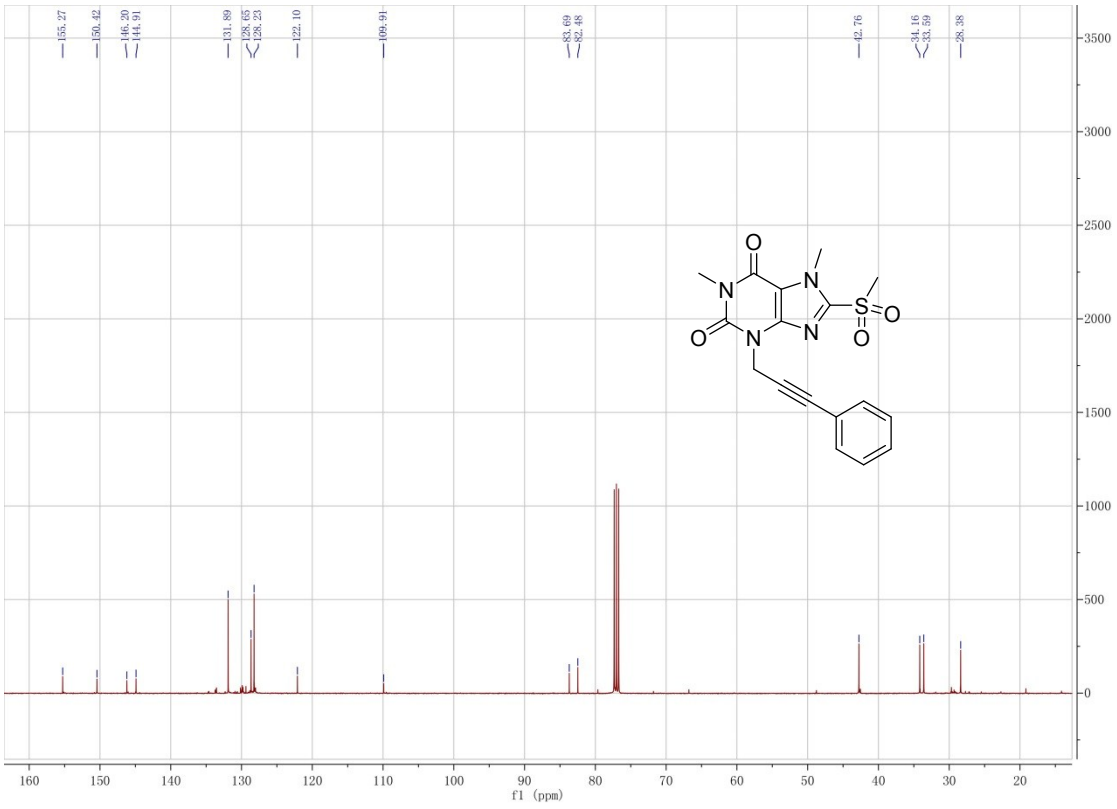
Compound 12


7.26
4.84
4.84
4.31
3.46
3.43
2.26
2.25
2.24
1.60
0.01
2.00
3.08
2.94
3.12
0.87
f1 (ppm)

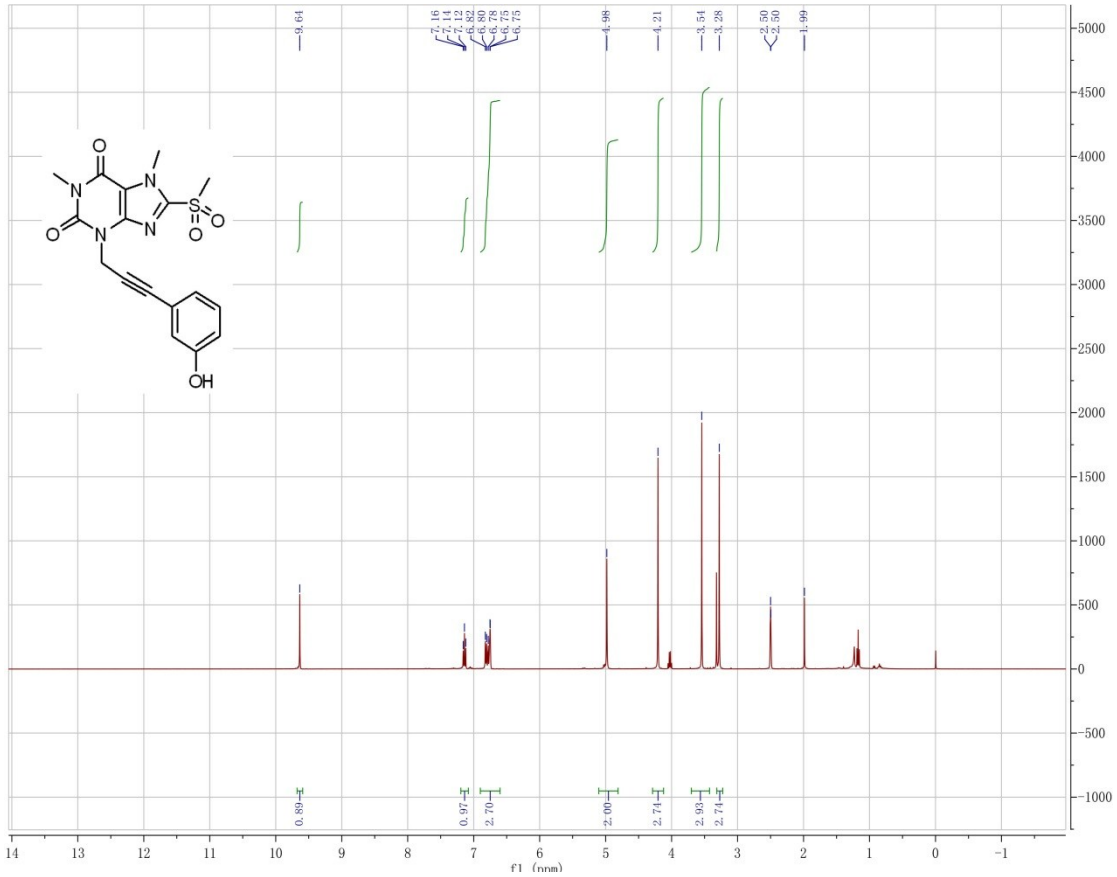

155.15
150.34
146.23
144.67
109.82
77.10
72.16
42.68
34.13
32.68
28.30
f1 (ppm)

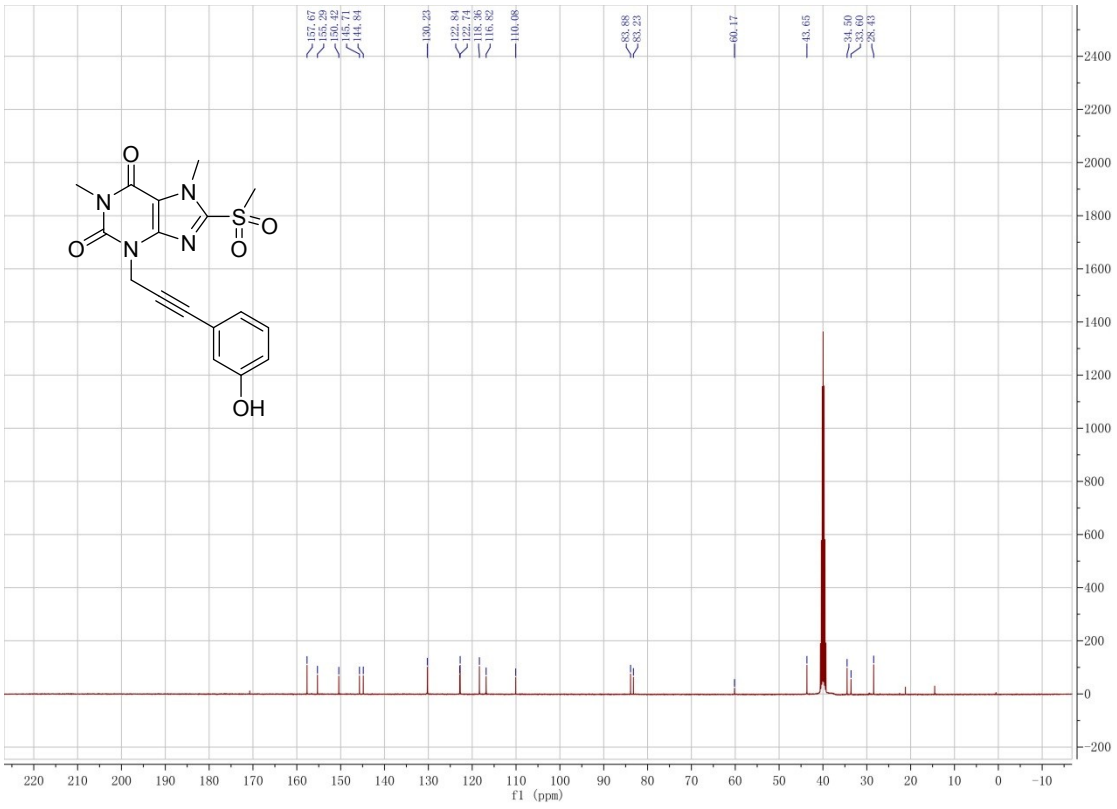
Compound 14


7.40
7.40
7.38
7.38
7.30
7.29
7.28
7.27
7.26
7.25
7.25
7.24
5.06
4.32
3.47
3.44
0.01
2.00
3.01
3.09
3.11
f1 (ppm)

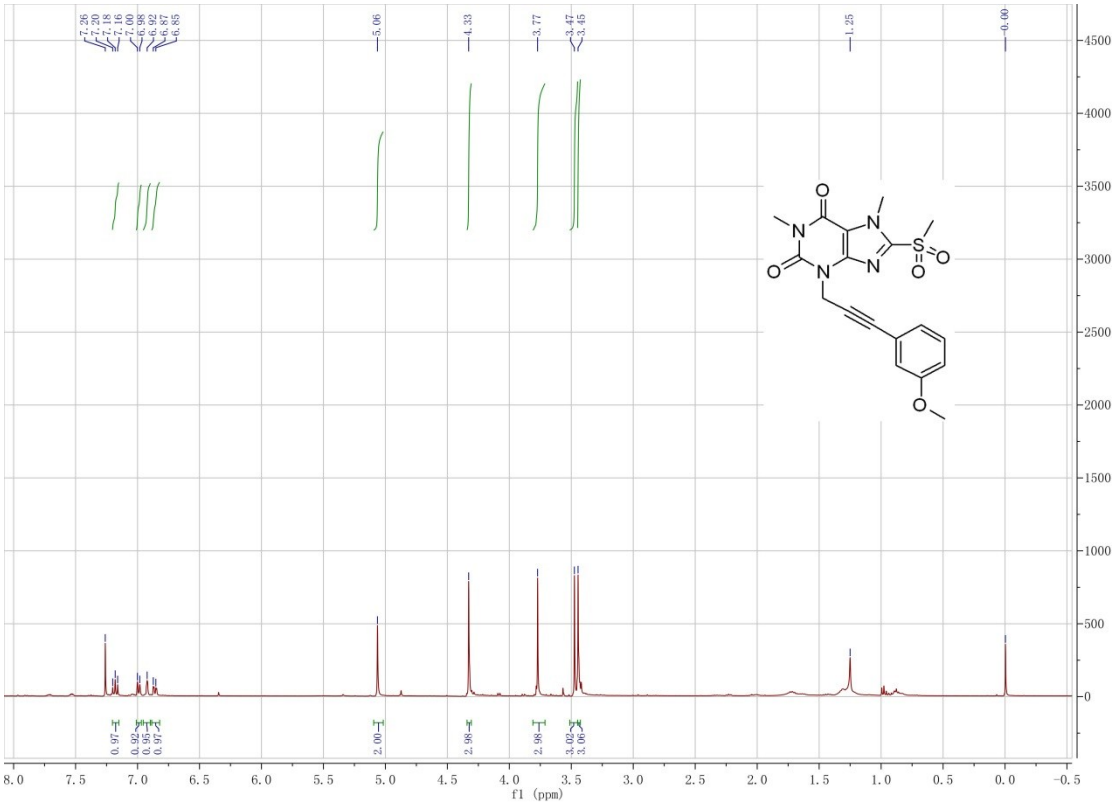

155.27
150.42
146.20
144.91
131.89
128.65
128.23
122.10
109.91
83.69
82.48
42.76
34.16
33.59
28.38
f1 (ppm)

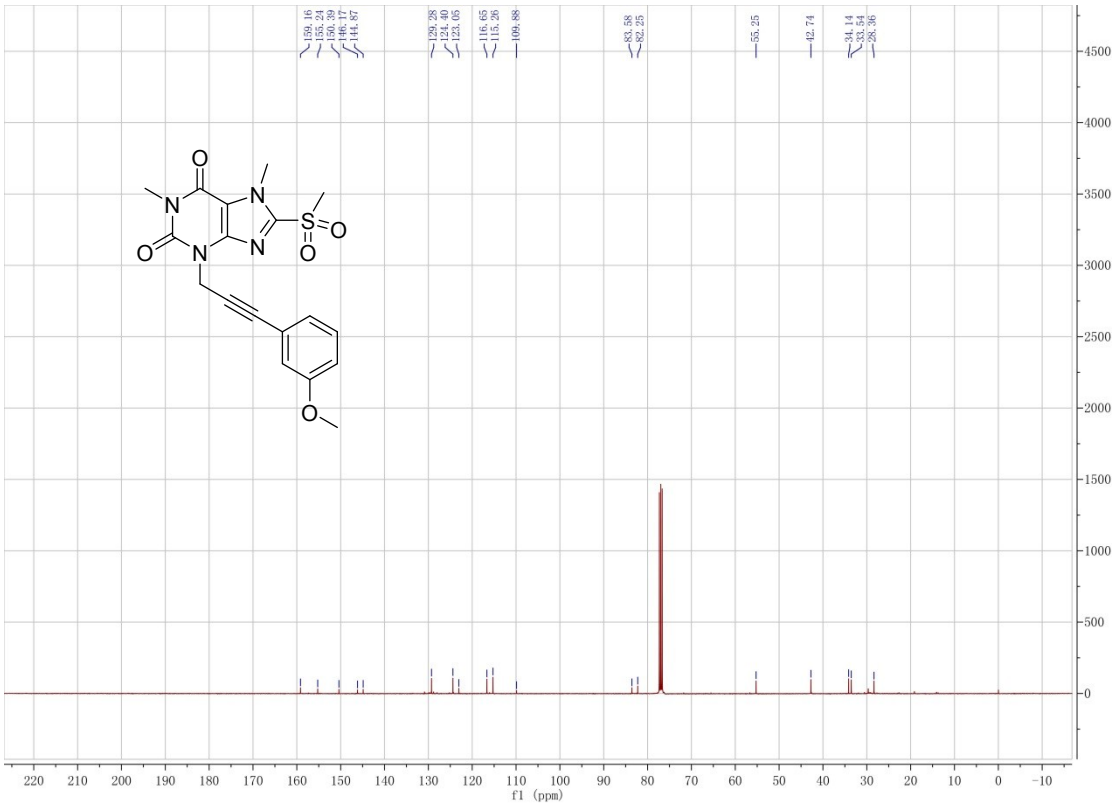
Compound 15


9.64
7.16
7.14
7.12
6.82
6.80
6.78
6.77
6.75
4.98
4.21
3.54
3.28
2.50
2.50
1.99
0.89
0.97
2.70
2.00
2.74
2.93
2.74
f1 (ppm)

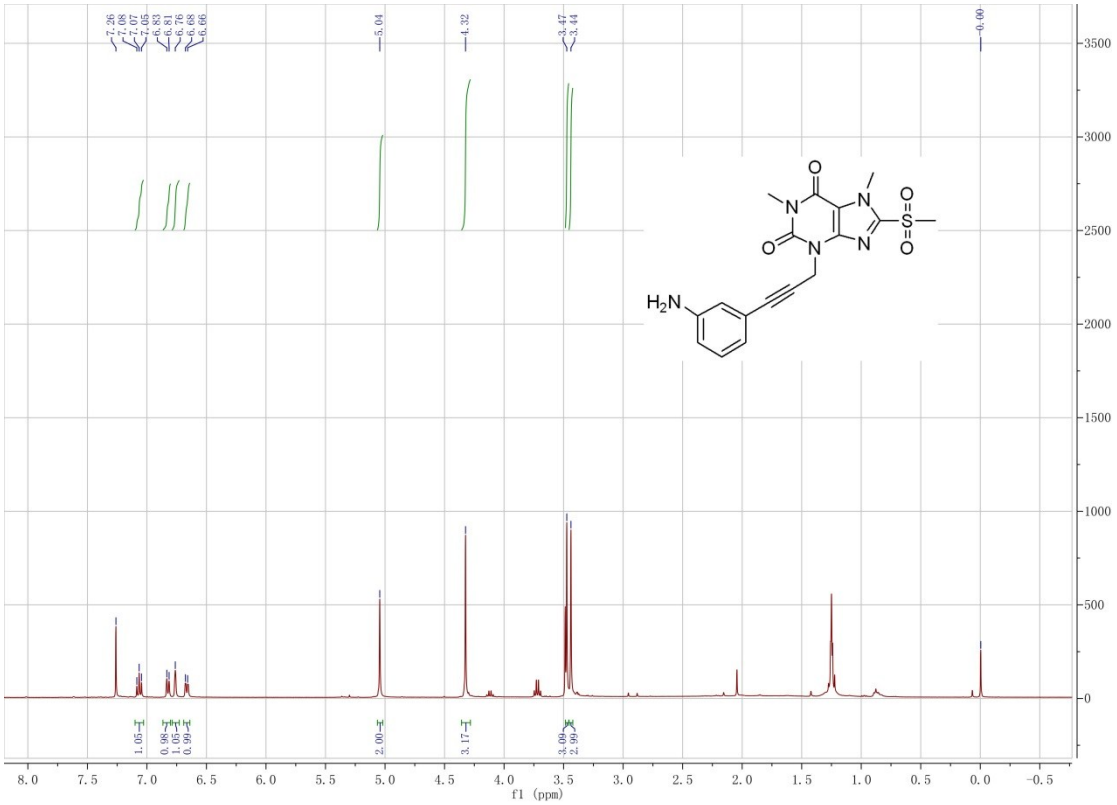

157.67
155.29
150.42
145.71
144.84
130.23
122.84
122.74
118.36
116.82
110.08
83.88
83.23
60.17
43.65
34.50
33.60
28.43
OH
f1 (ppm)

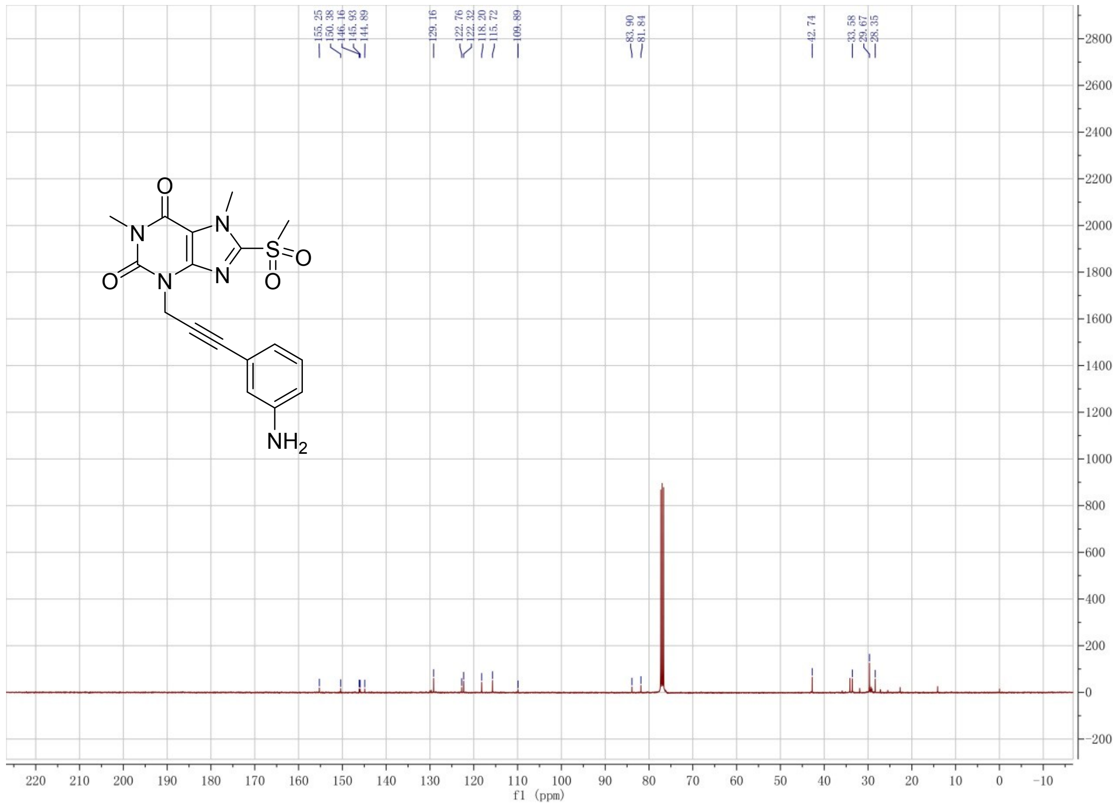
Compound 16


7.26
7.20
7.18
7.16
7.00
6.98
6.92
6.87
6.85
5.06
4.33
3.77
3.47
3.45
1.25
0.00
0.97
0.92
0.95
0.97
2.00
2.98
2.98
3.02
3.06
f1 (ppm)

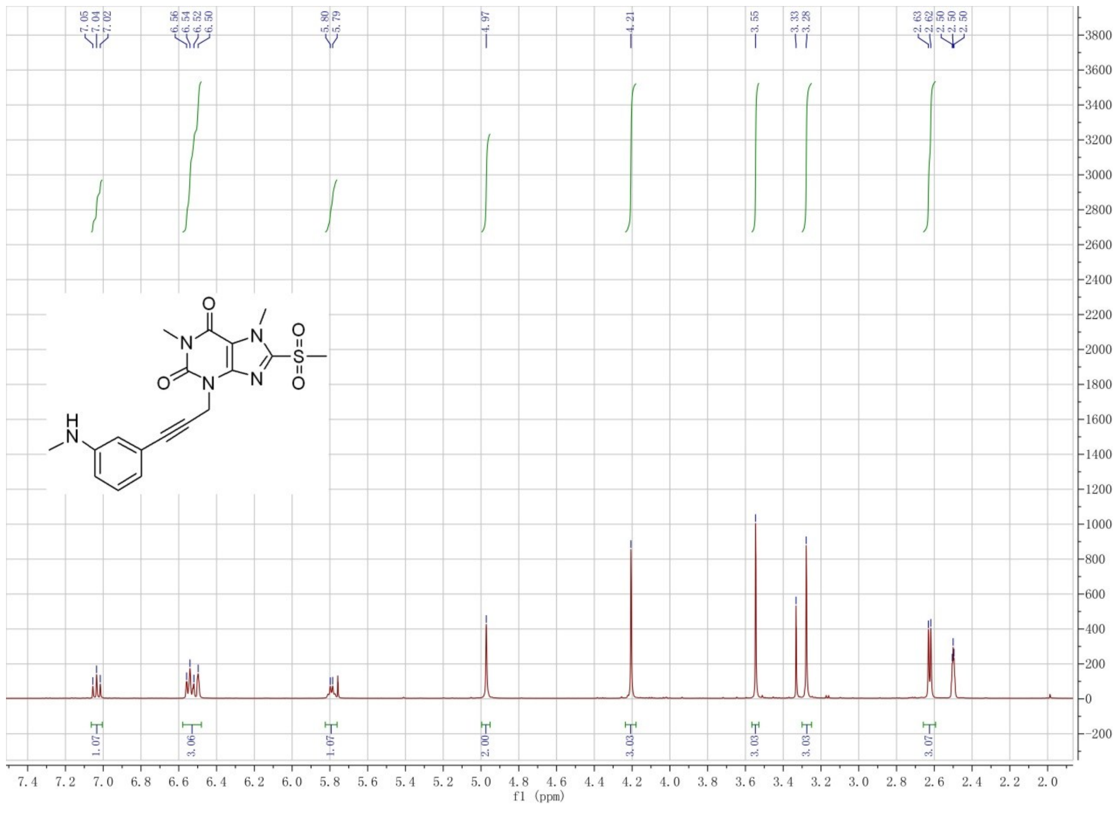

159.16
155.24
150.39
146.17
144.87
129.28
124.40
123.05
116.65
115.26
109.88
83.58
82.25
55.25
42.74
34.14
33.54
26.36
f1 (ppm)

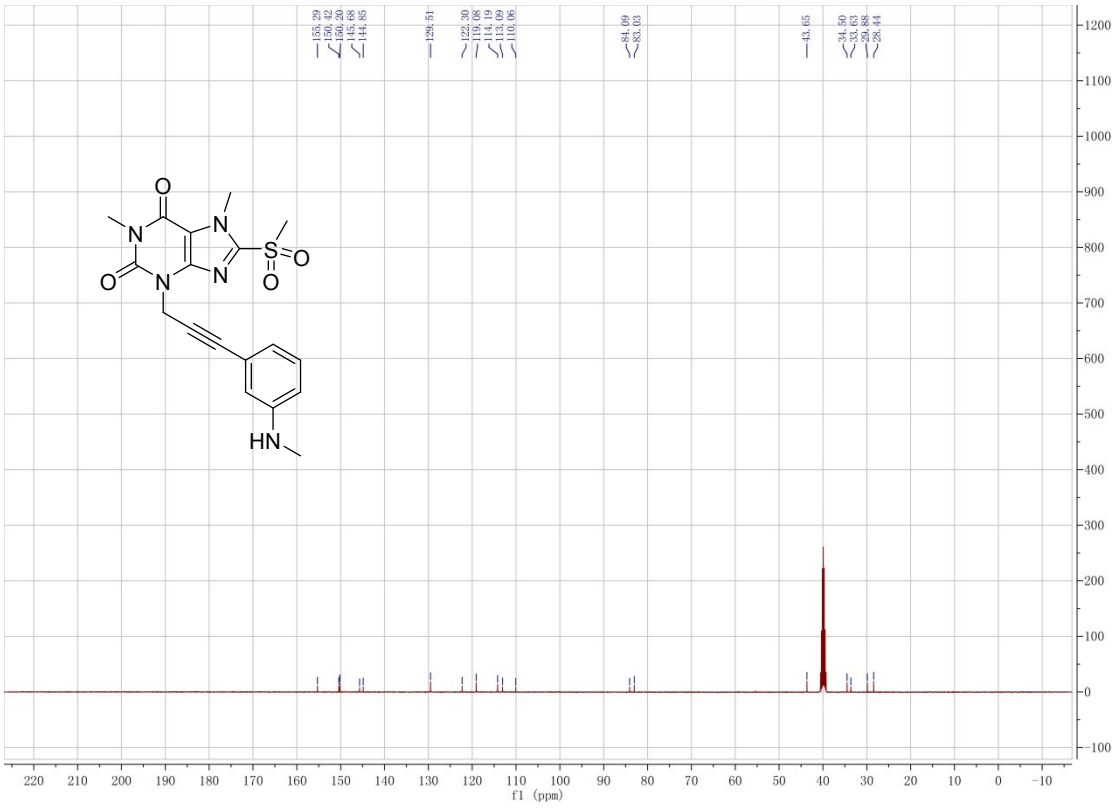
Compound 17


7.26
7.08
7.07
7.05
6.83
6.81
6.76
6.68
6.66
5.04
4.32
3.47
3.44
0.00
1.05
0.98
1.05
0.99
2.00
3.17
3.09
2.99
f1 (ppm)

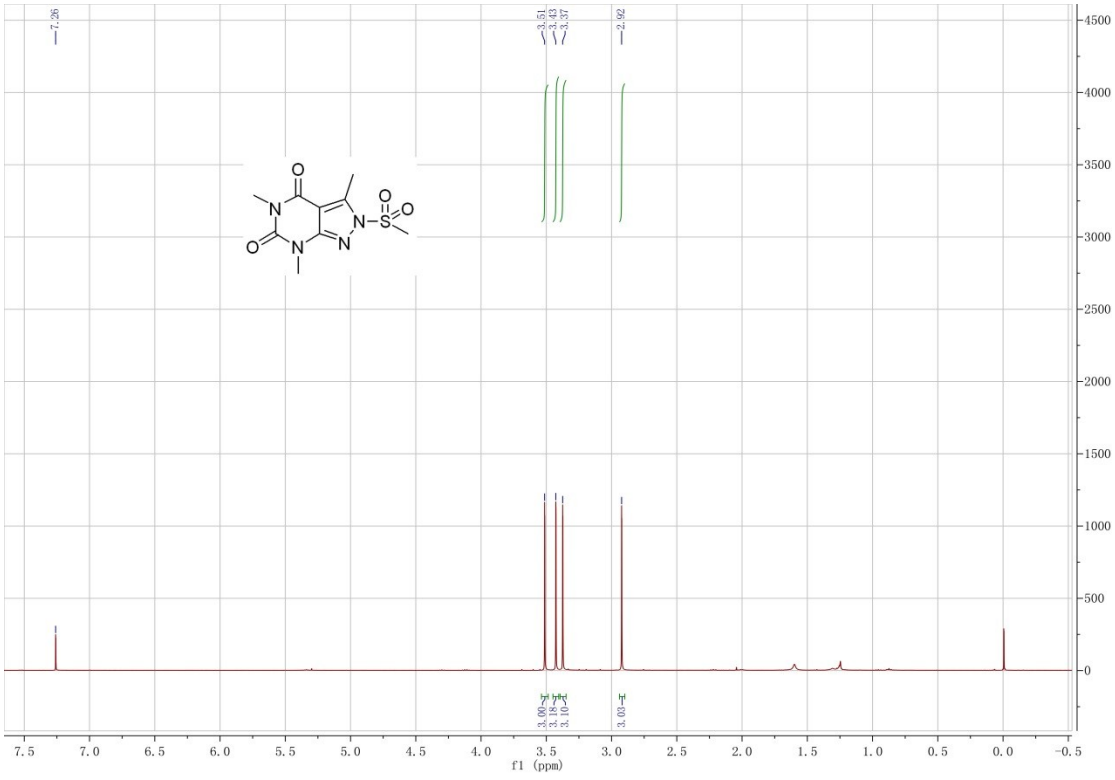


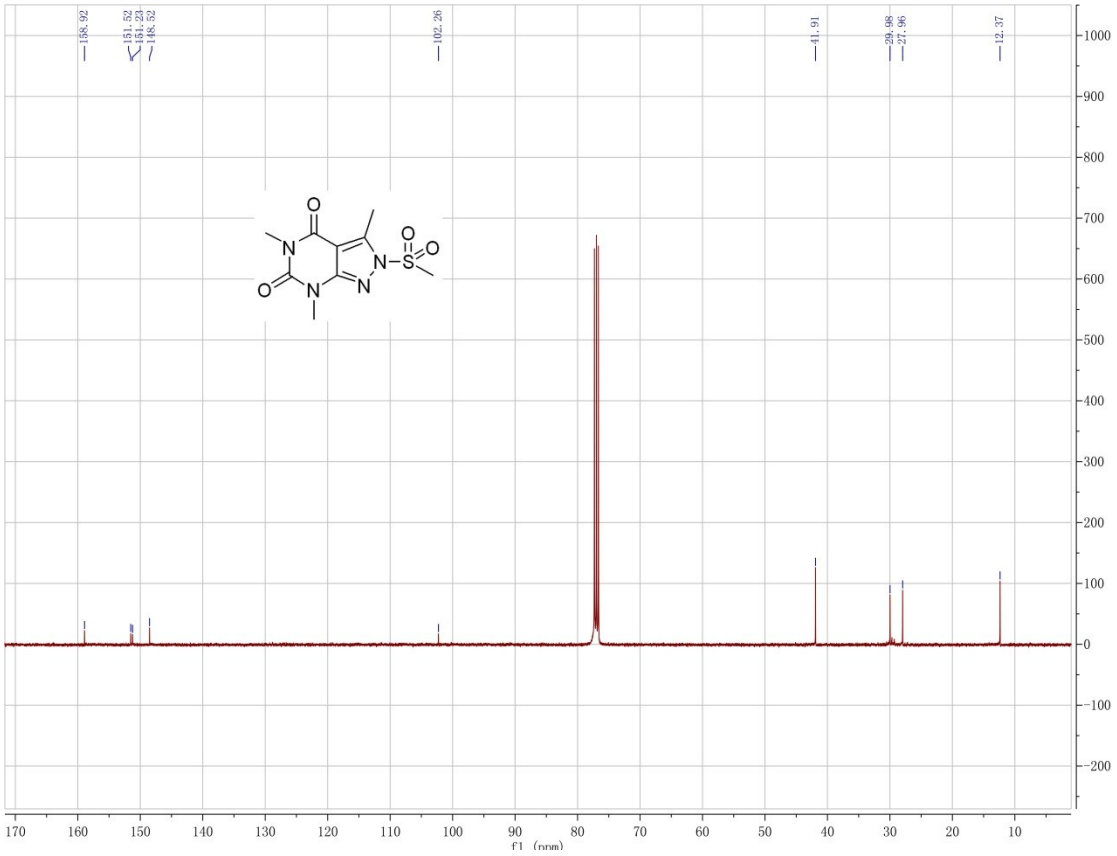
Compound 18



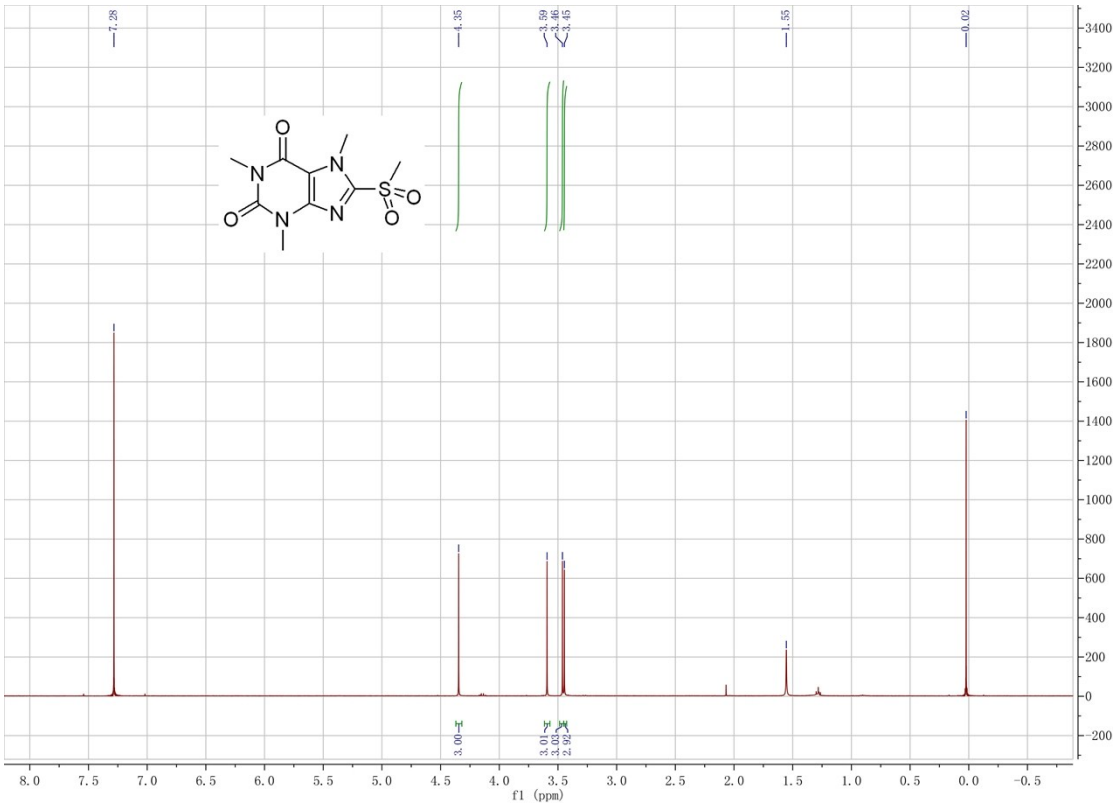

155.29
150.42
146.20
145.98
144.85
129.51
122.30
119.98
114.19
113.09
110.06
84.09
83.03
43.65
34.50
33.63
29.88
28.44
f1 (ppm)

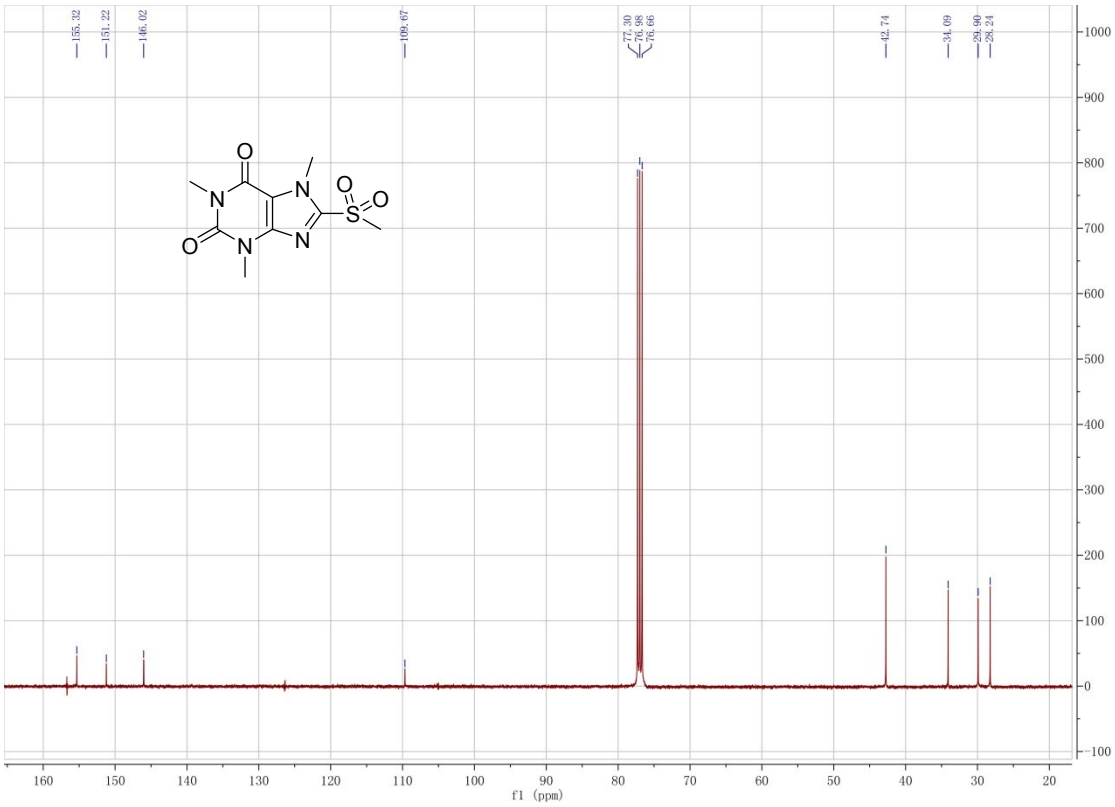
Compound 19


7.26
3.51
3.43
3.37
2.92
3.00
3.18
3.10
3.03
f1 (ppm)

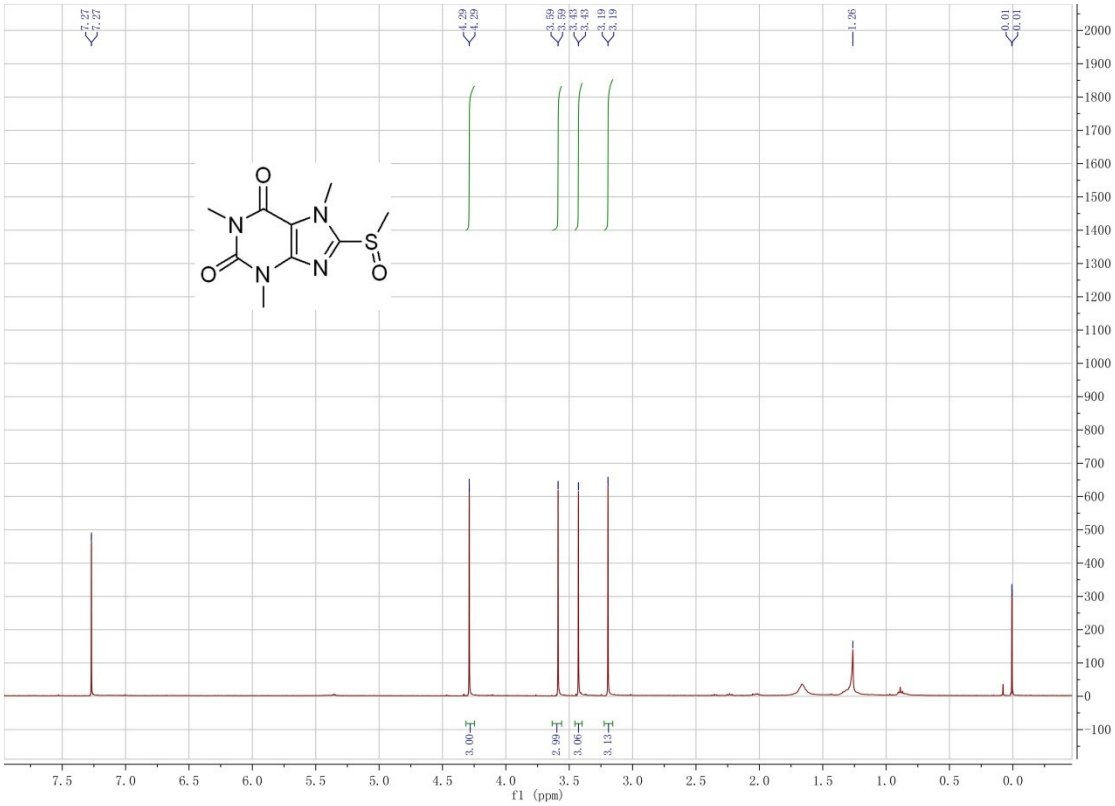

158.92
151.52
151.23
148.52
102.26
41.91
29.98
27.96
12.37
f1 (ppm)

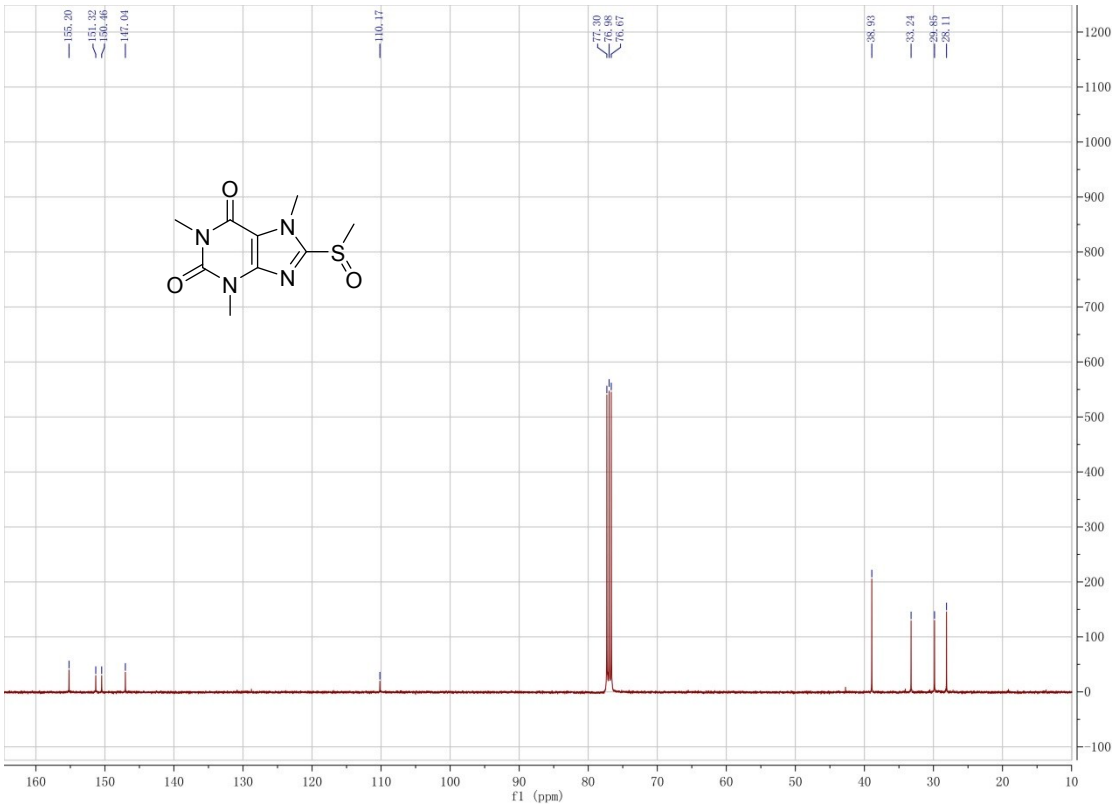
Compound 20


7.28
4.35
3.59
3.46
3.45
1.55
0.02
3.00
3.01
3.03
2.92
f1 (ppm)

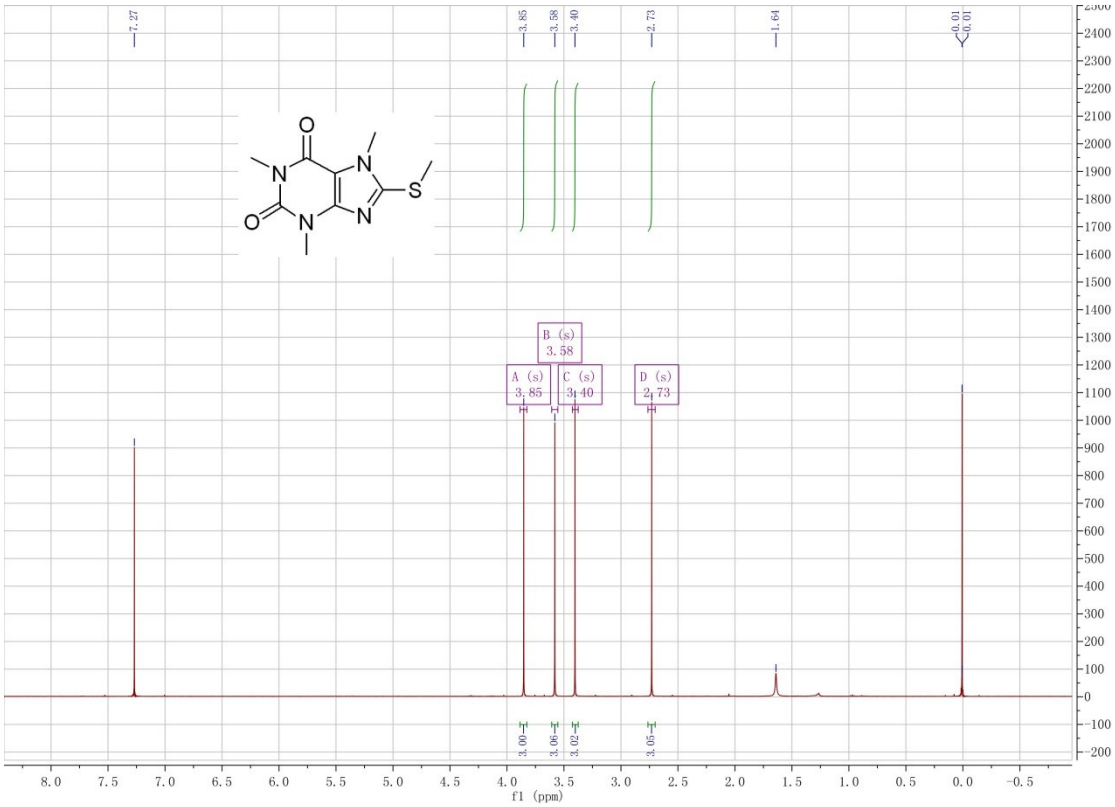

155.32
151.22
146.02
109.67
77.30
76.98
76.66
42.74
34.09
29.90
28.24
f1 (ppm)

Compound 21

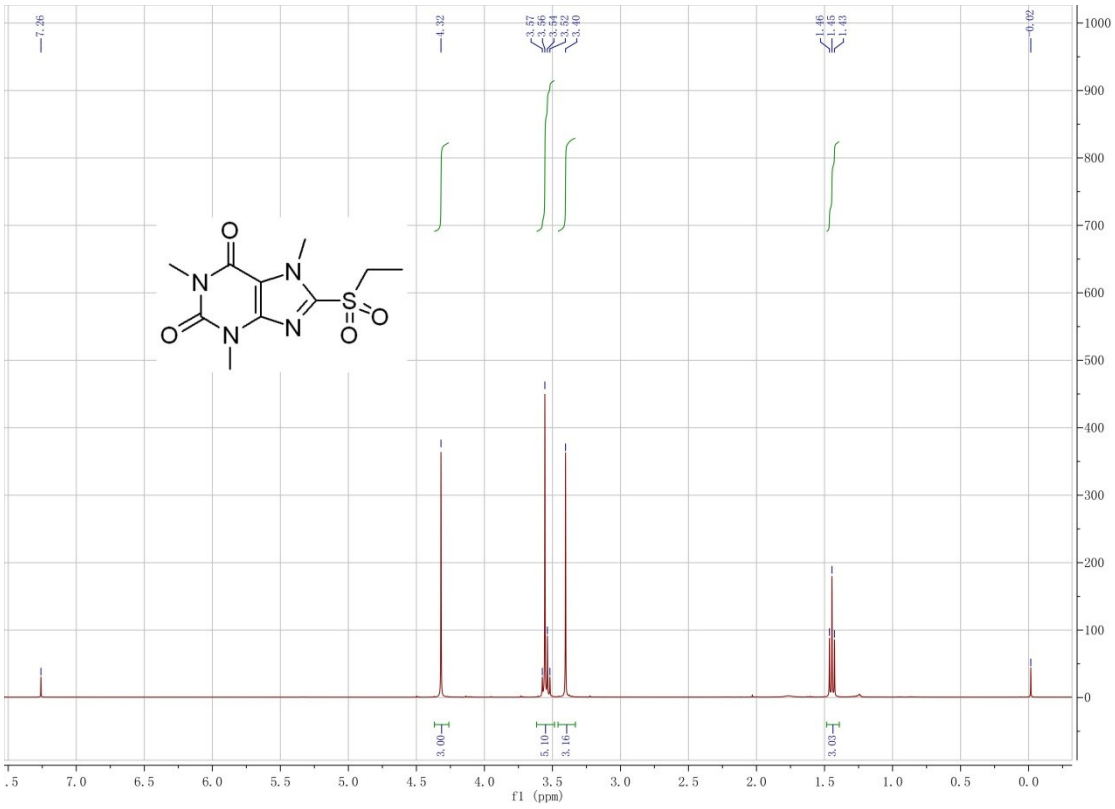

7.27
7.27
4.29
4.29
3.59
3.58
3.43
3.43
3.19
3.19
1.26
0.01
0.01
3.00
2.99
3.06
3.13
f1 (ppm)

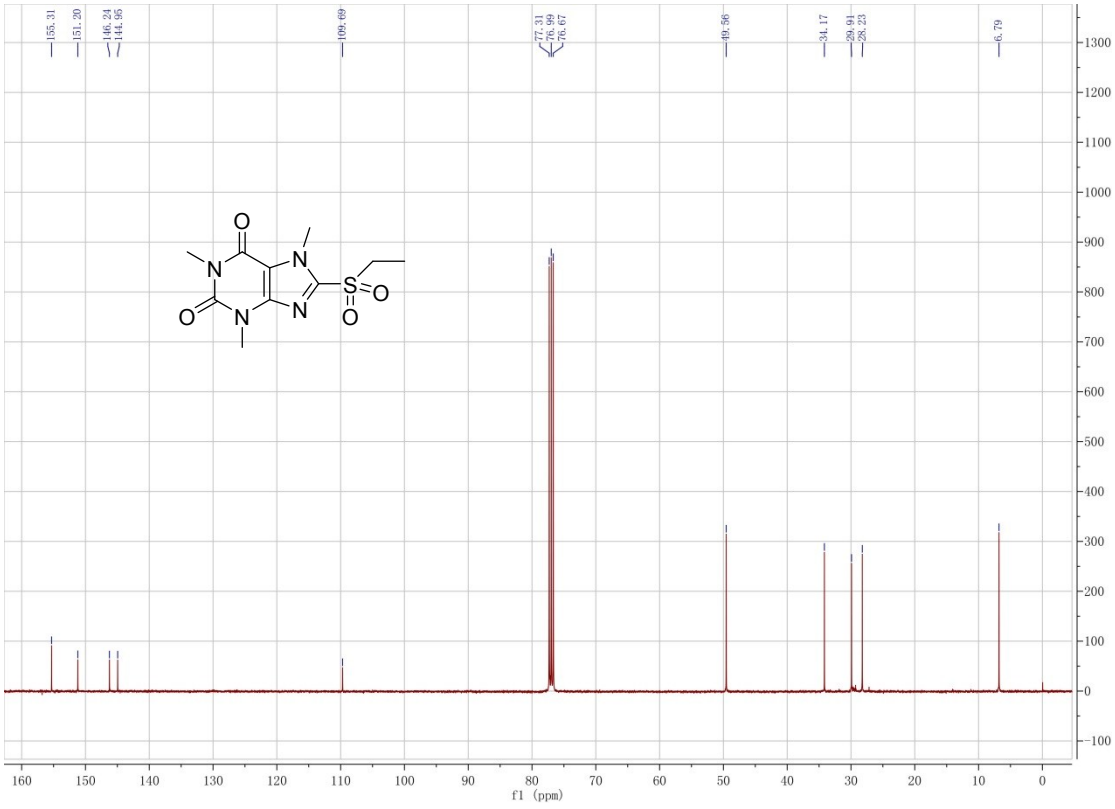


Compound 22

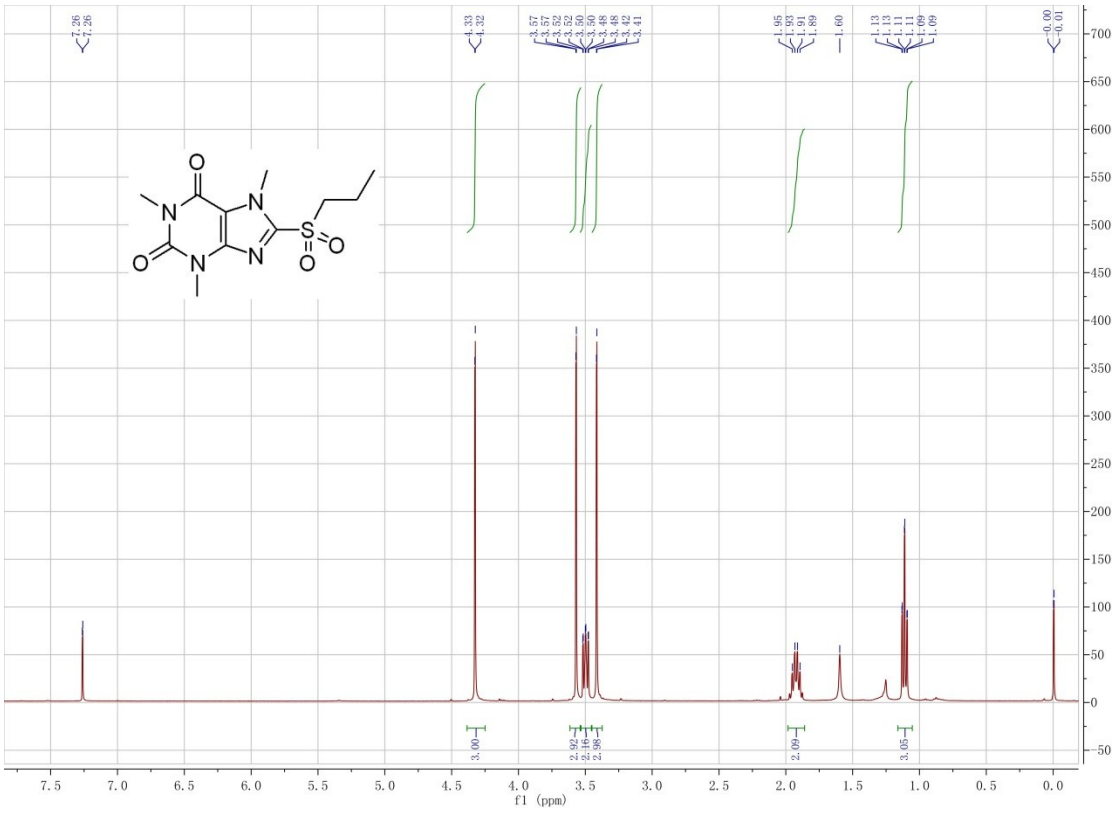


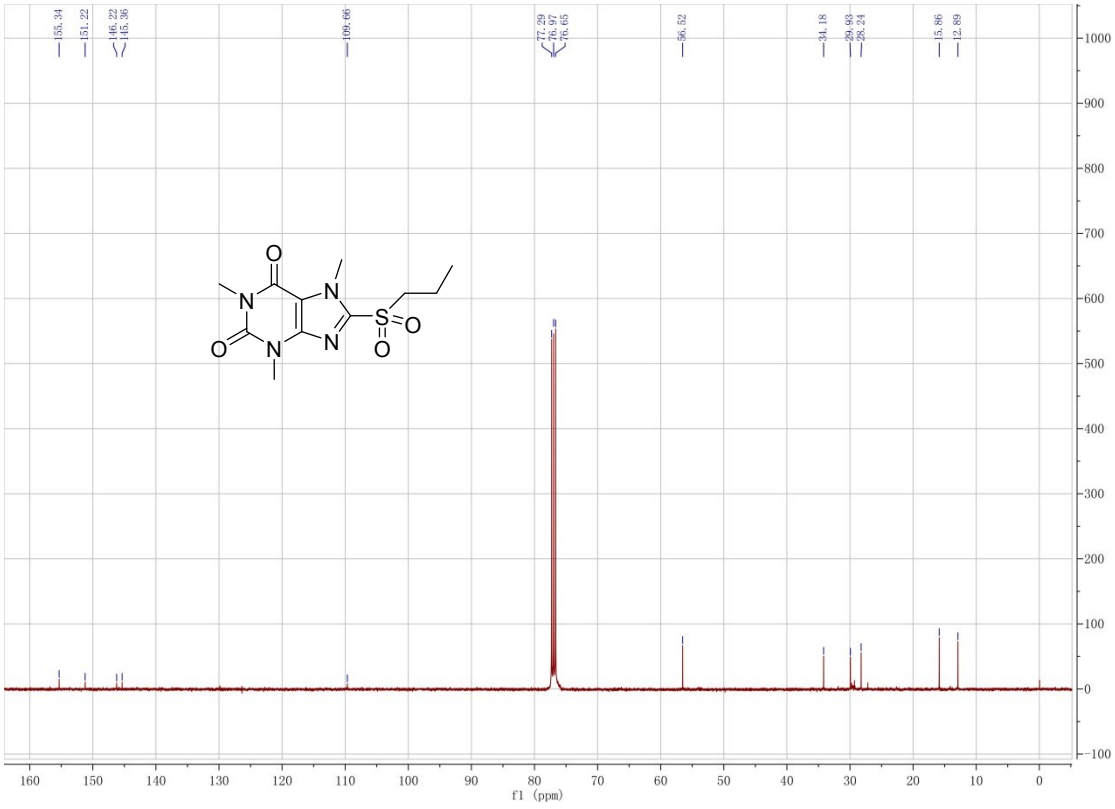
Compound 23


7.26
4.32
3.57
3.56
3.54
3.52
3.40
1.46
1.45
1.43
0.02
3.00
5.10
3.16
3.03
f1 (ppm)

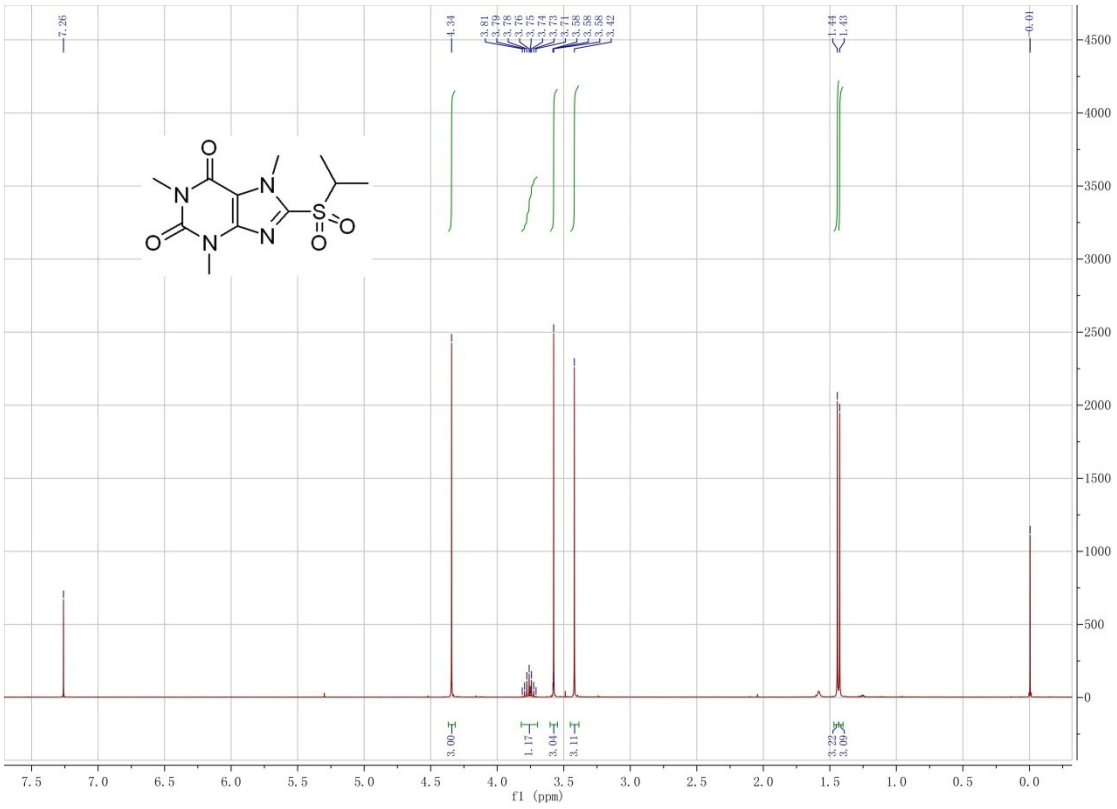

155.31
151.20
146.24
144.95
109.69
77.31
76.99
76.67
49.56
34.17
29.91
28.23
6.79
f1 (ppm)

Compound 24

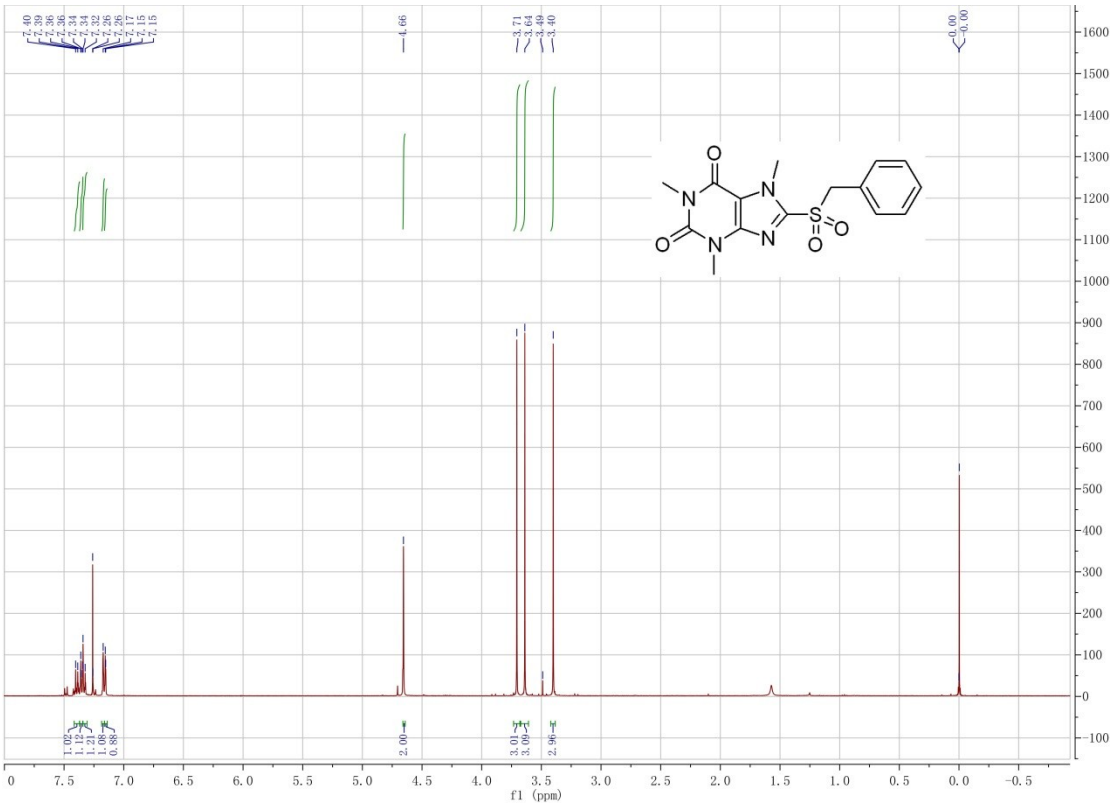

7.26
7.26
4.33
4.32
3.57
3.57
3.52
3.52
3.50
3.50
3.48
3.48
3.42
3.41
1.95
1.93
1.91
1.89
1.60
1.13
1.13
1.11
1.11
1.09
1.09
0.00
-0.01
3.00
2.92
2.16
2.98
2.09
3.05
f1 (ppm)

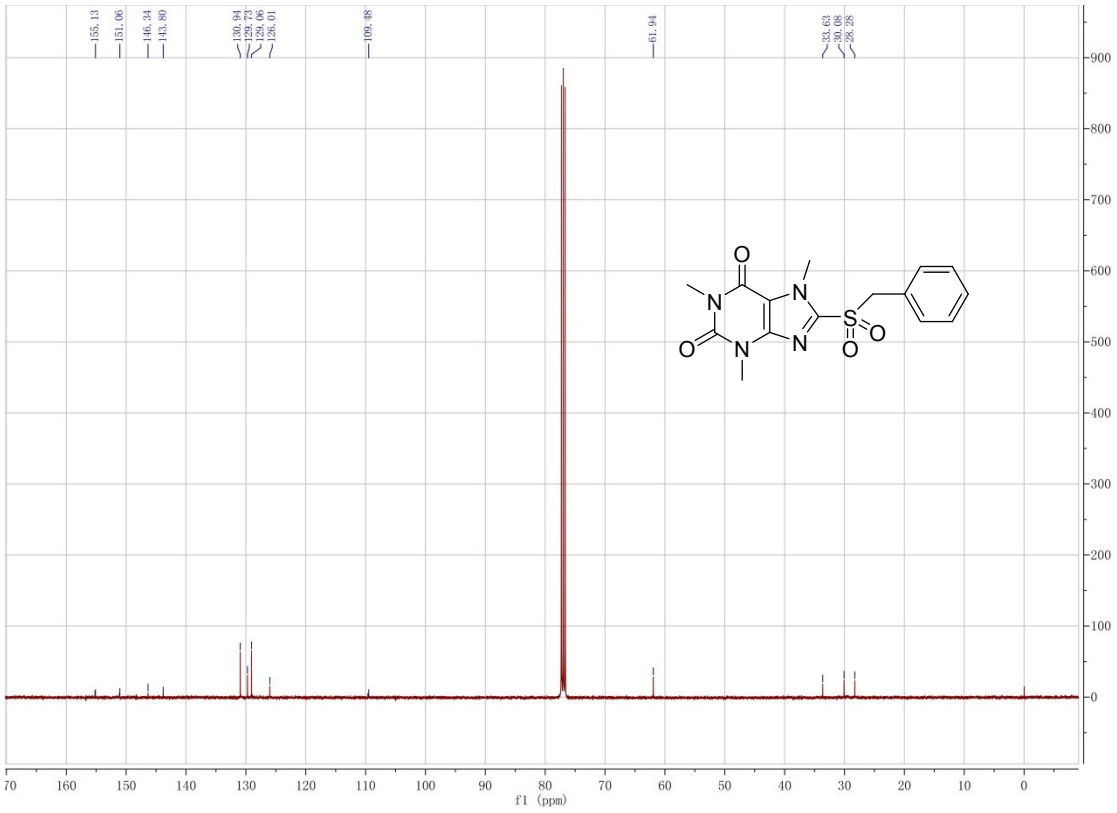

155.34
151.22
146.22
145.36
109.66
77.29
76.97
76.65
56.52
34.18
29.93
28.24
15.86
12.89
f1 (ppm)

Compound 25

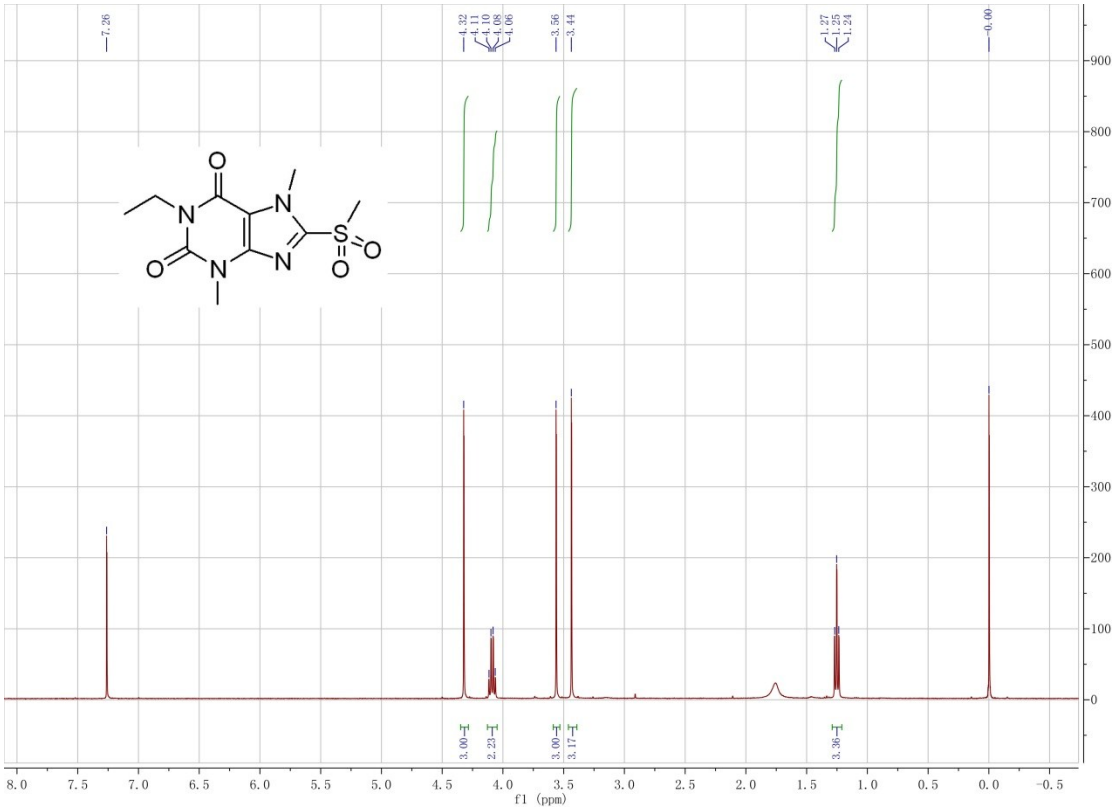

7.26
4.34
3.81
3.79
3.78
3.76
3.75
3.74
3.73
3.71
3.58
3.58
3.42
1.44
1.43
0.01
3.00
1.17
3.04
3.11
3.22
3.09
f1 (ppm)

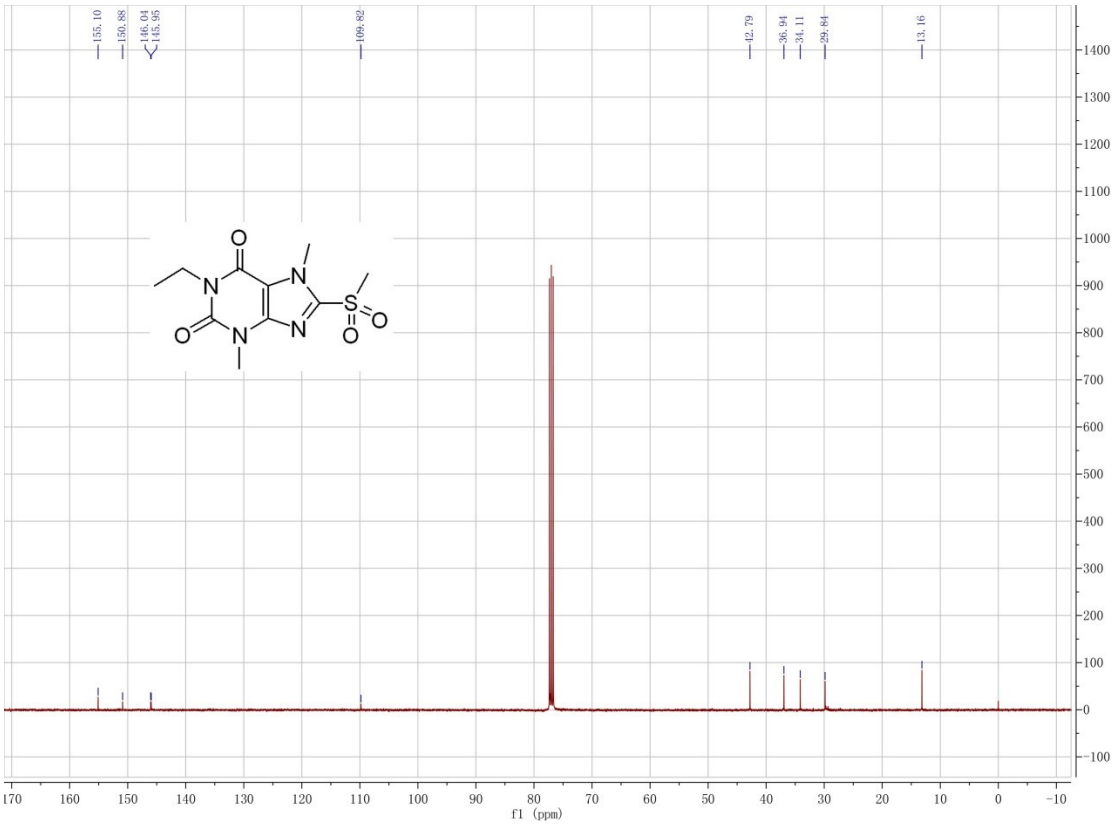
Compound 26


7.40
7.39
7.36
7.34
7.34
7.32
7.26
7.26
7.17
7.15
7.15
4.66
3.71
3.64
3.49
3.40
0.00
0.00
1.02
1.12
1.21
1.08
0.88
2.00
3.01
3.09
2.96
f1 (ppm)

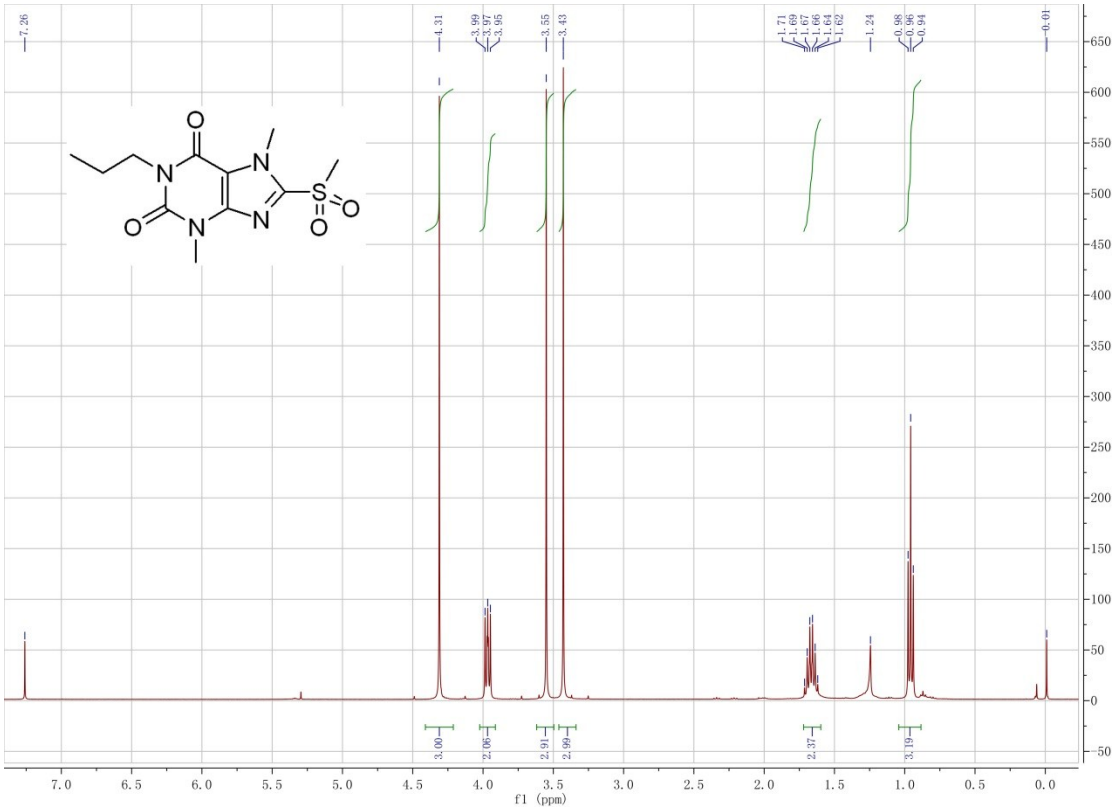

155.13
151.06
146.34
143.80
130.94
129.73
129.06
126.01
109.48
61.94
33.63
30.08
28.28
f1 (ppm)

Compound 27

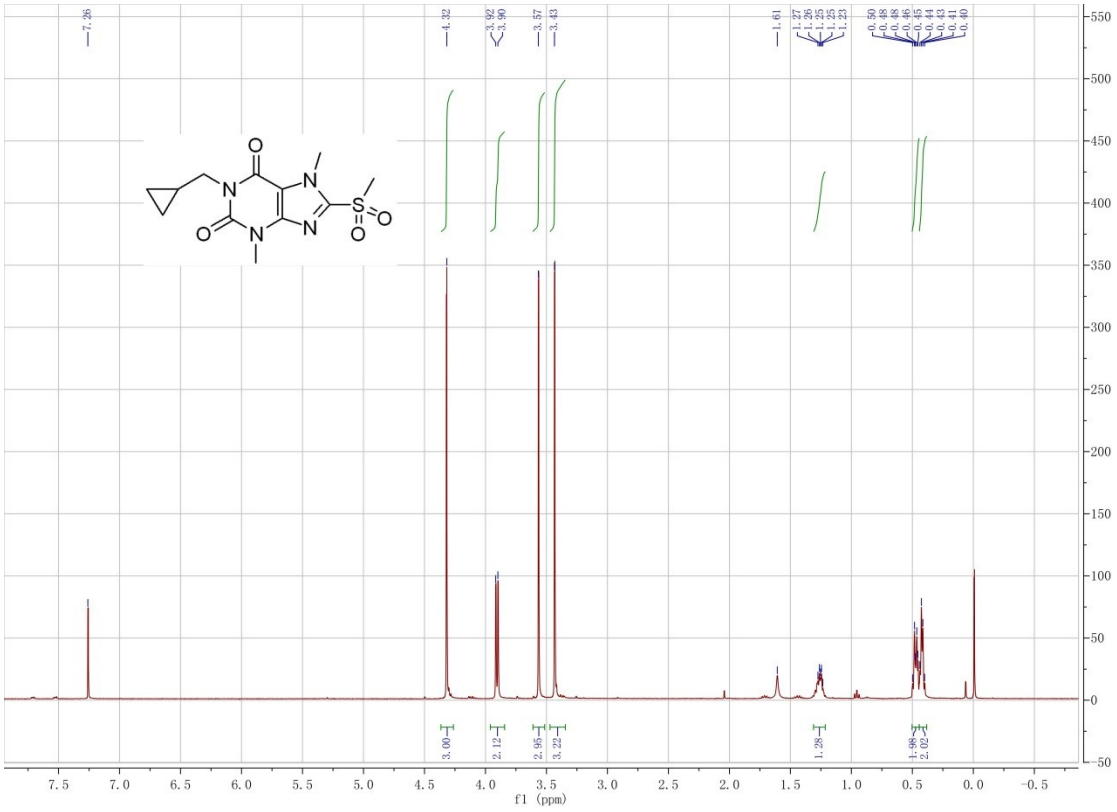

7.26
4.32
4.11
4.10
4.08
4.06
3.56
3.44
1.27
1.25
1.24
0.00
3.00
2.23
3.00
3.17
3.36
f1 (ppm)

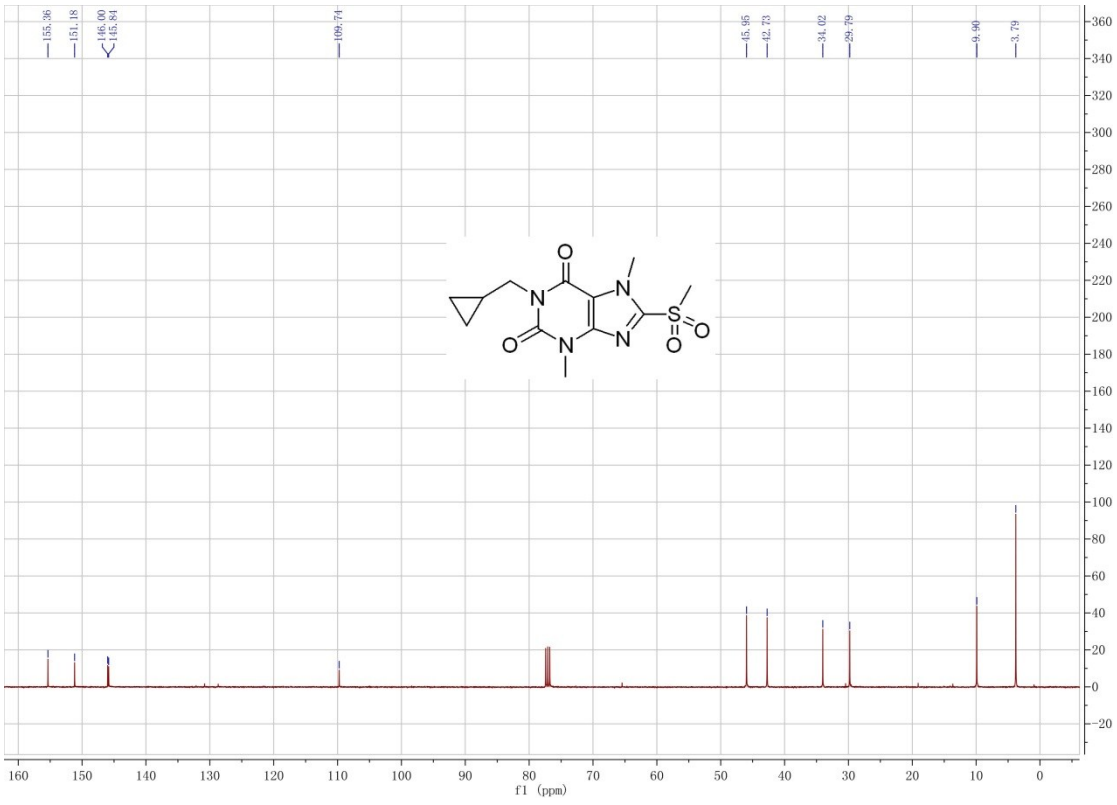


Compound 28

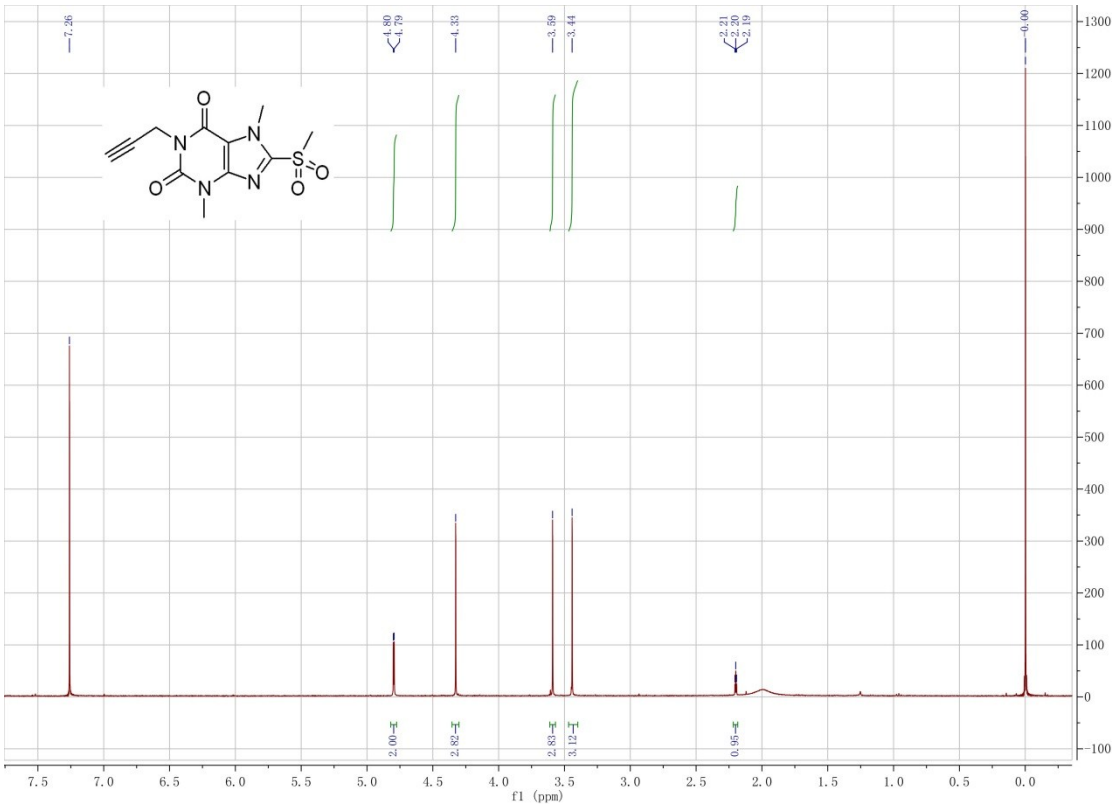


Compound 29

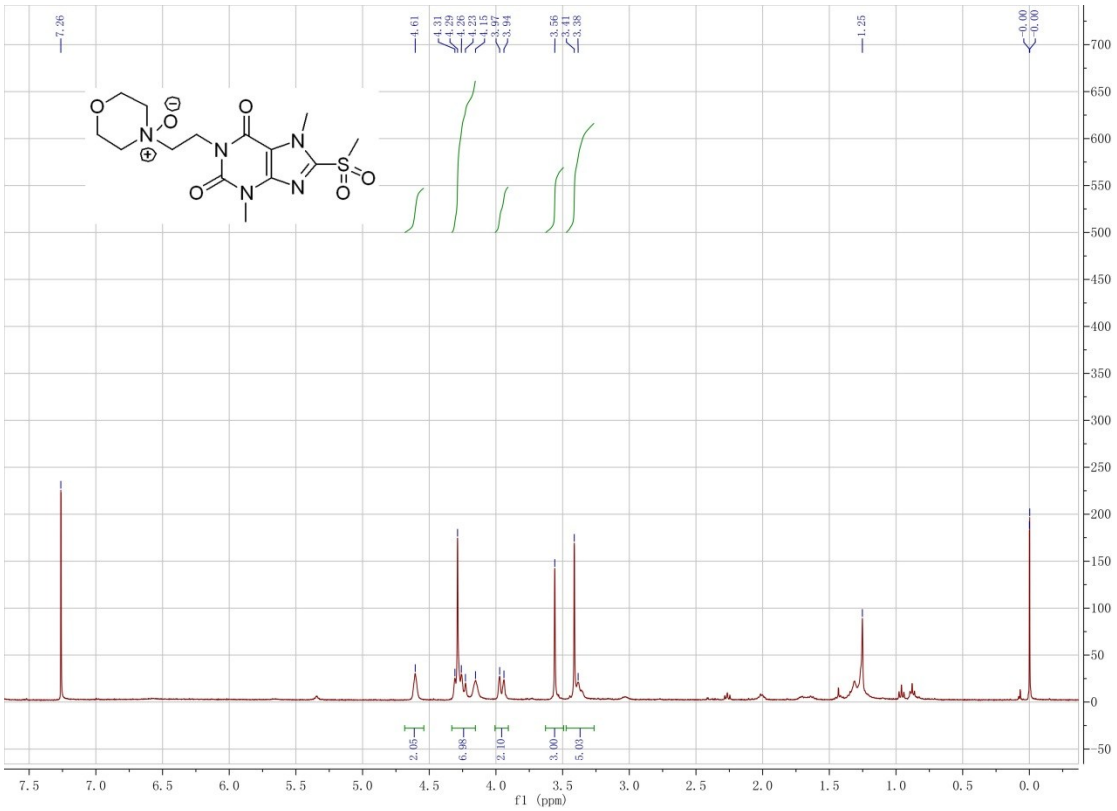

7.26
4.32
3.92
3.90
3.57
3.43
1.61
1.27
1.26
1.25
1.25
1.23
0.50
0.48
0.48
0.46
0.45
0.44
0.43
0.41
0.40
3.00
2.12
2.95
3.22
1.28
1.98
2.02
f1 (ppm)


155.36
151.18
146.00
145.84
109.74
45.95
42.73
34.02
29.79
9.90
3.79
f1 (ppm)

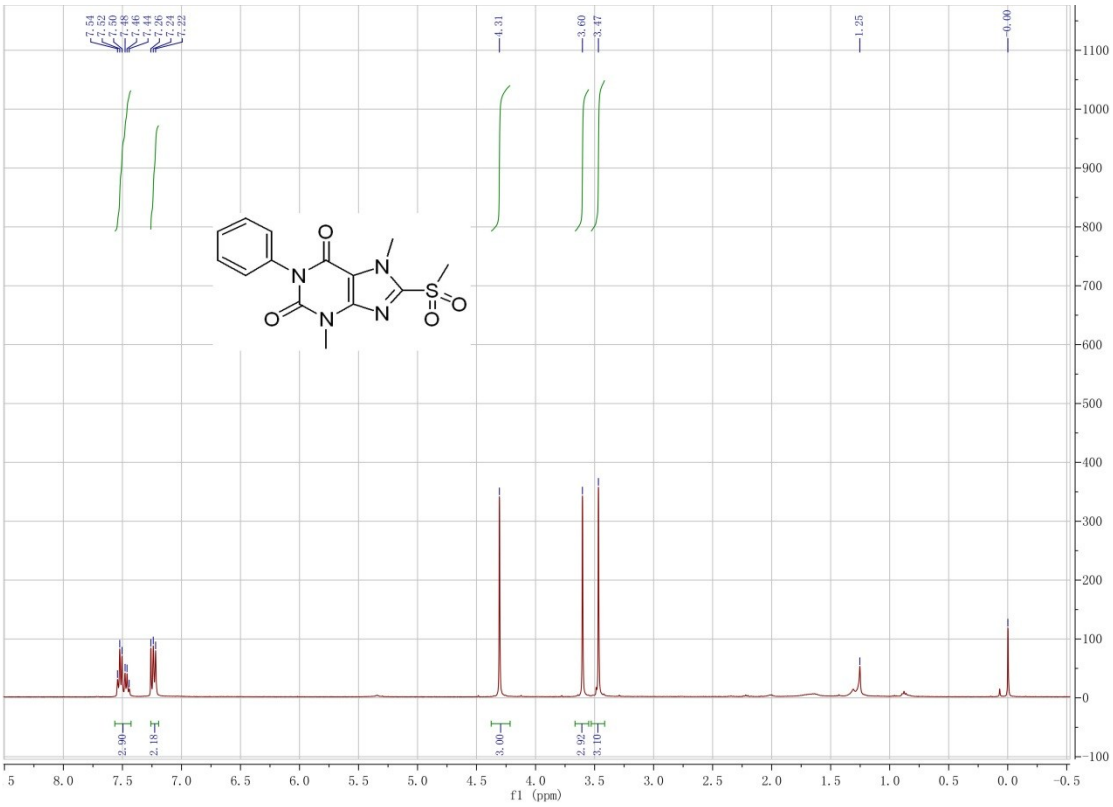
Compound 30



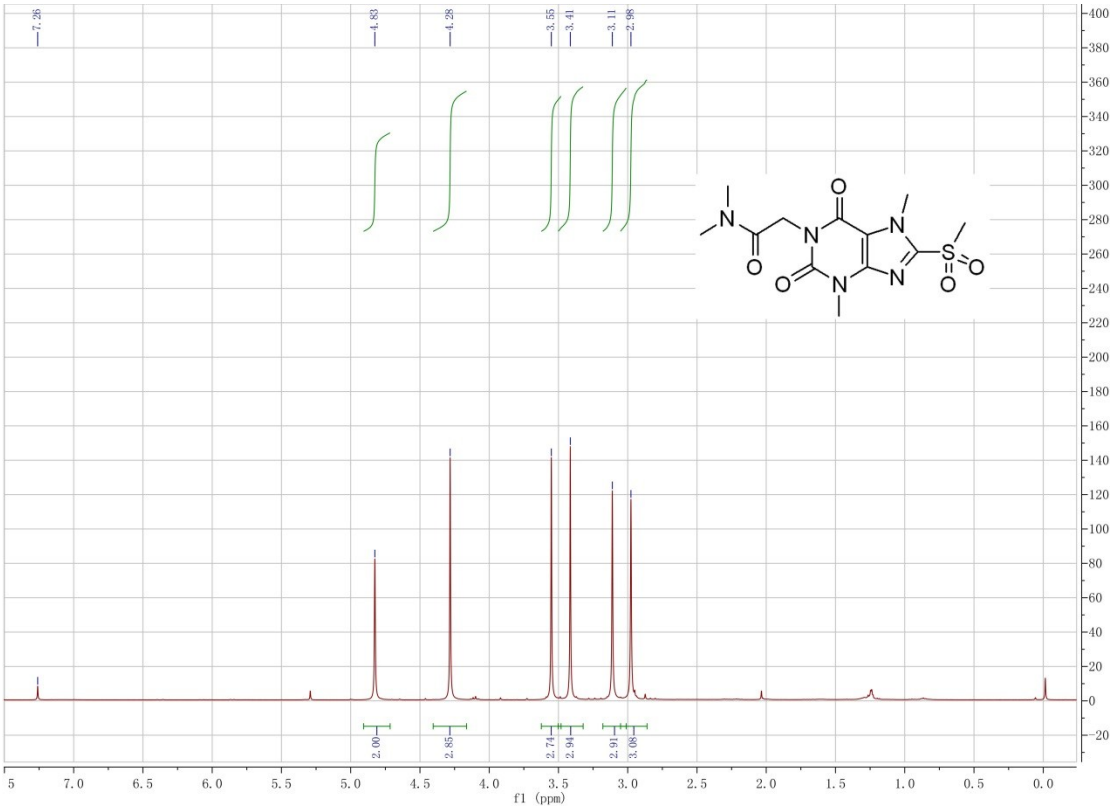
Compound 31

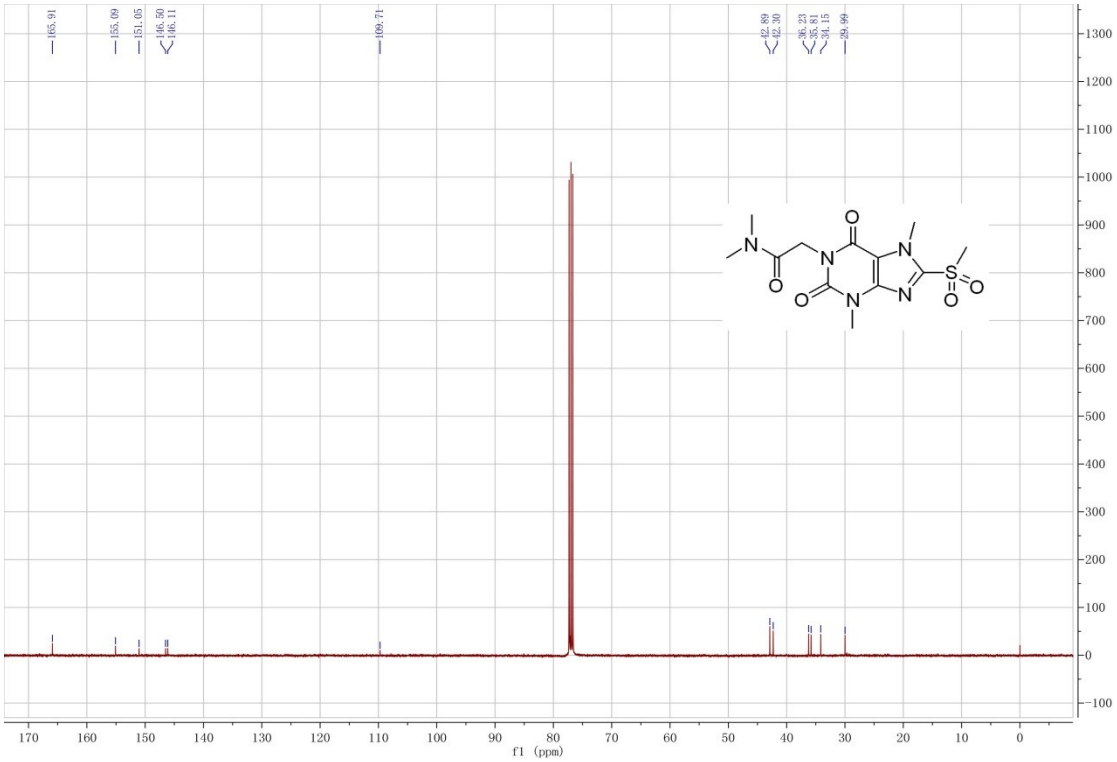


Compound 32

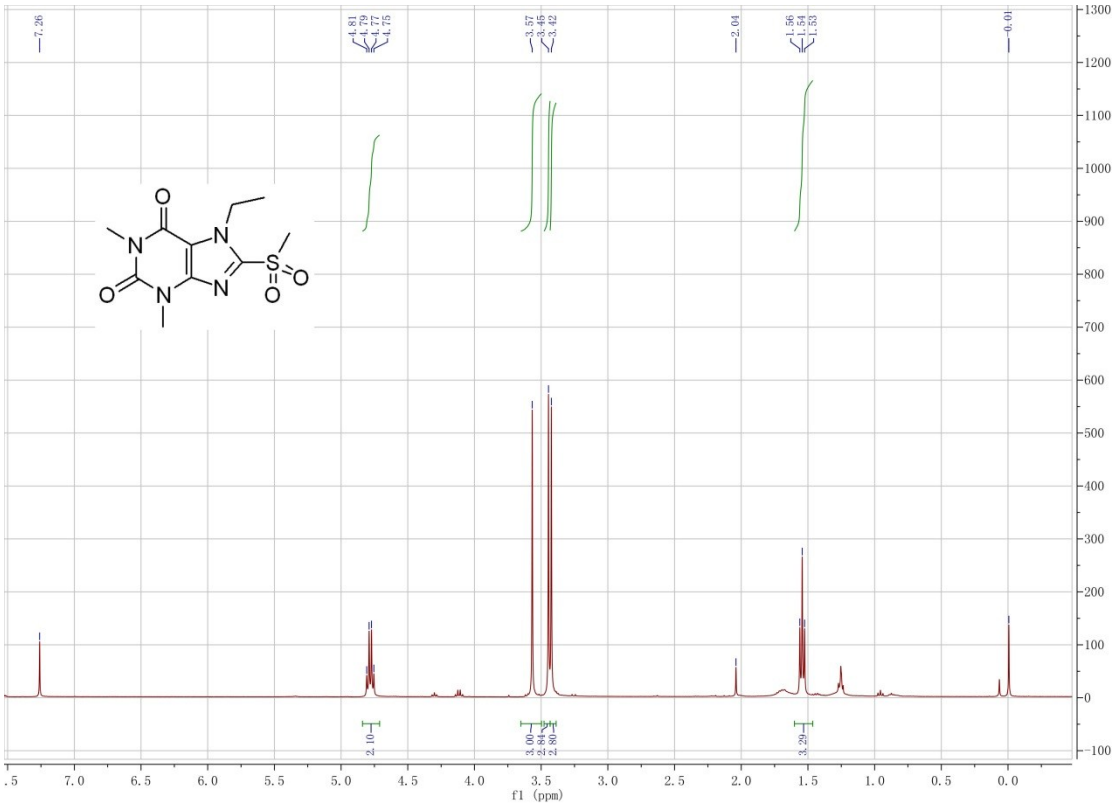

7.54
7.52
7.50
7.48
7.46
7.44
7.26
7.24
7.22
4.31
3.60
3.47
1.25
0.00
2.90
2.18
3.00
2.92
3.10
f1 (ppm)

Compound 33

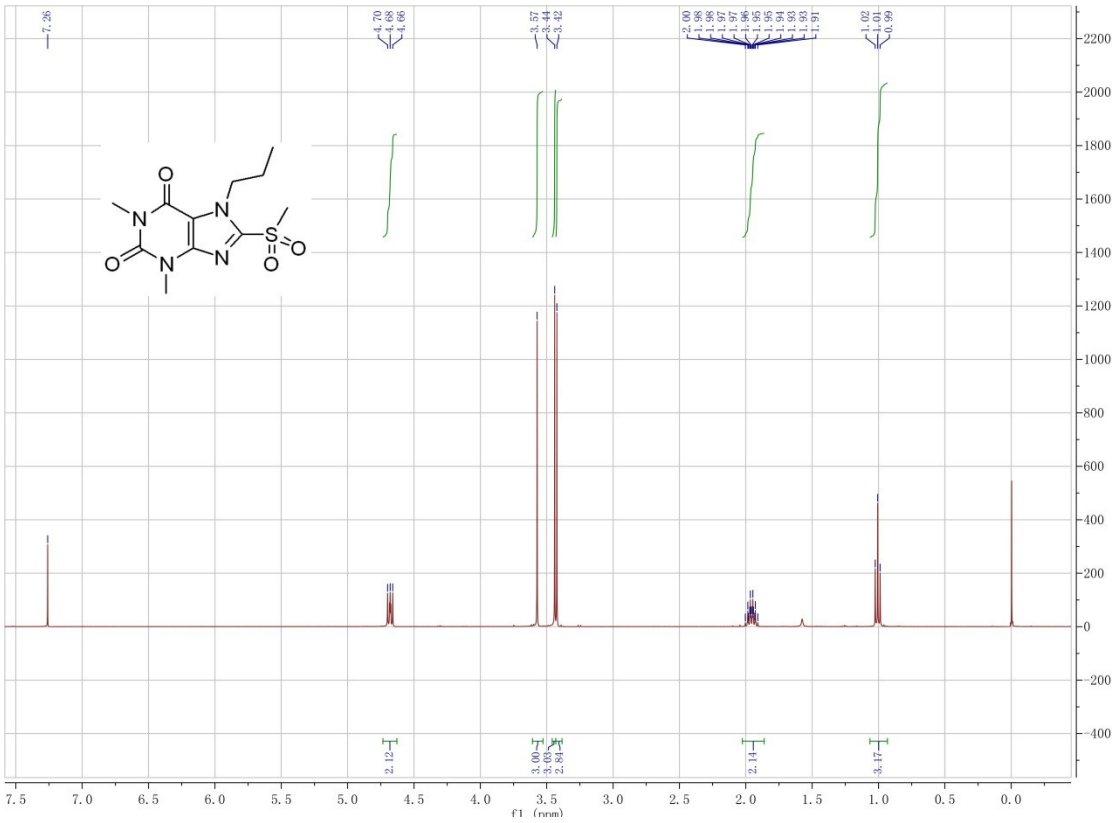

7.26
4.83
4.28
3.55
3.41
3.11
2.98
2.00
2.85
2.74
2.94
2.91
3.08
f1 (ppm)

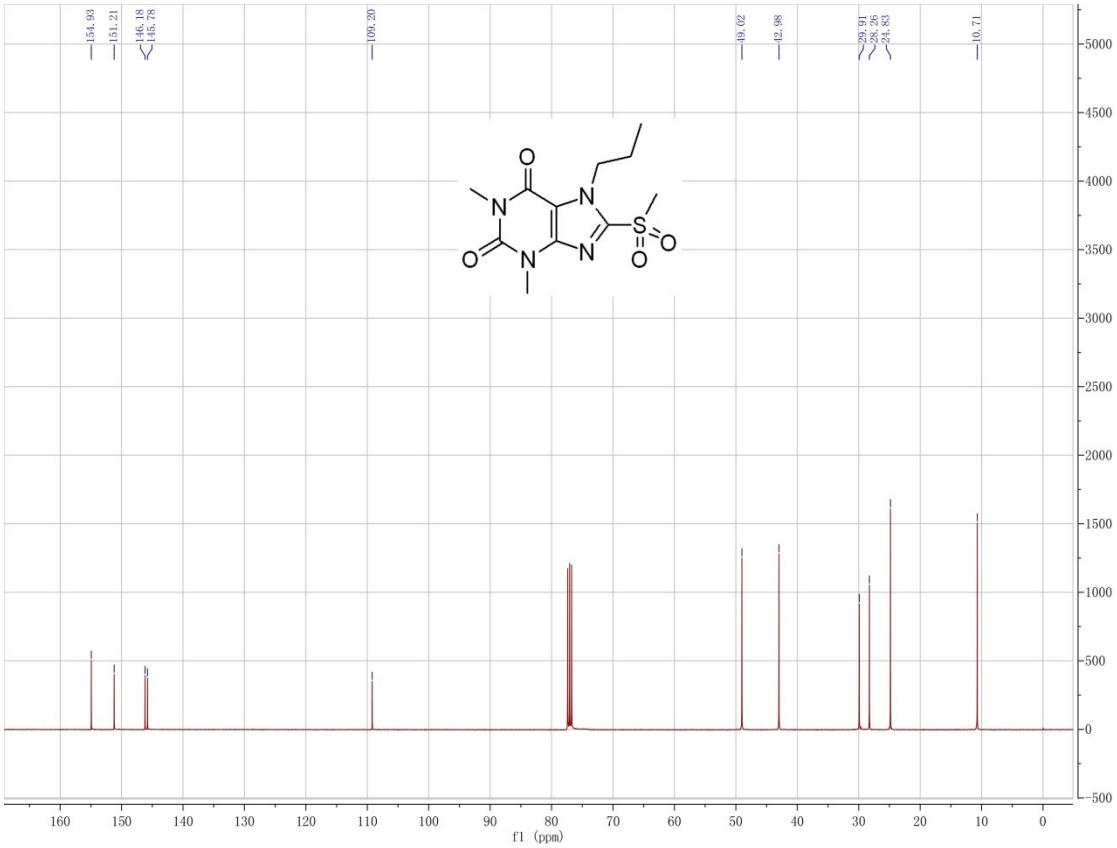


Compound 34

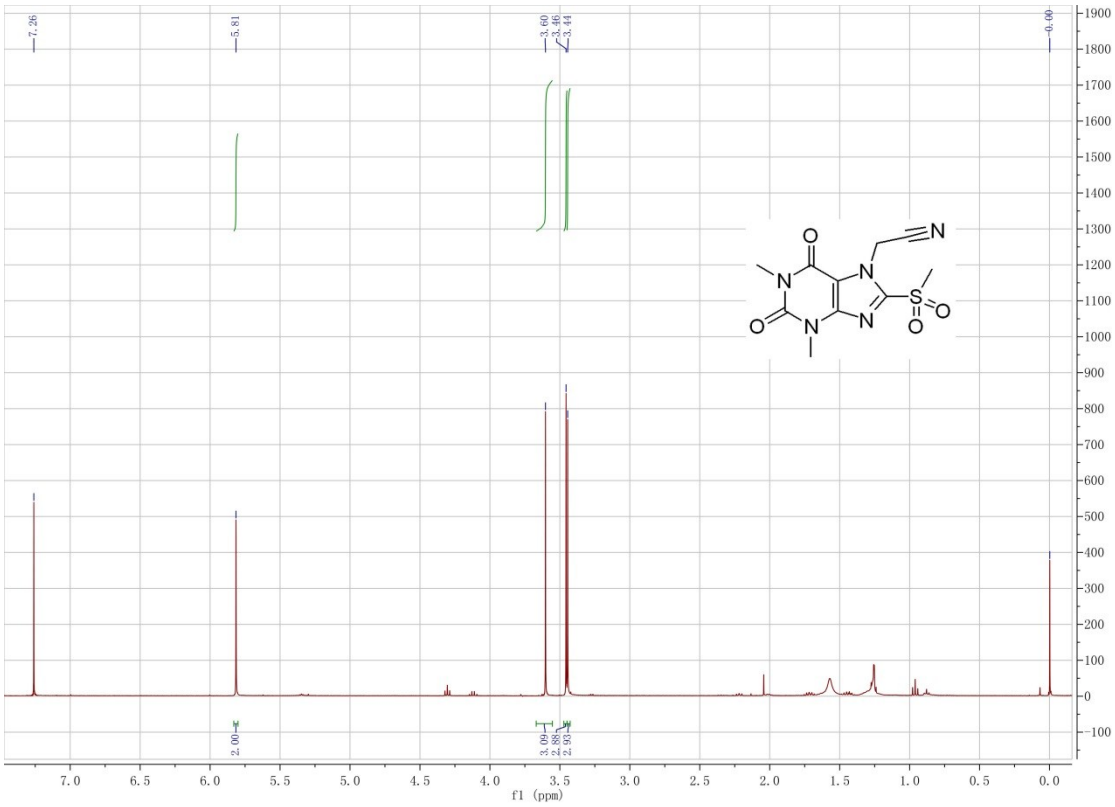


Compound 35

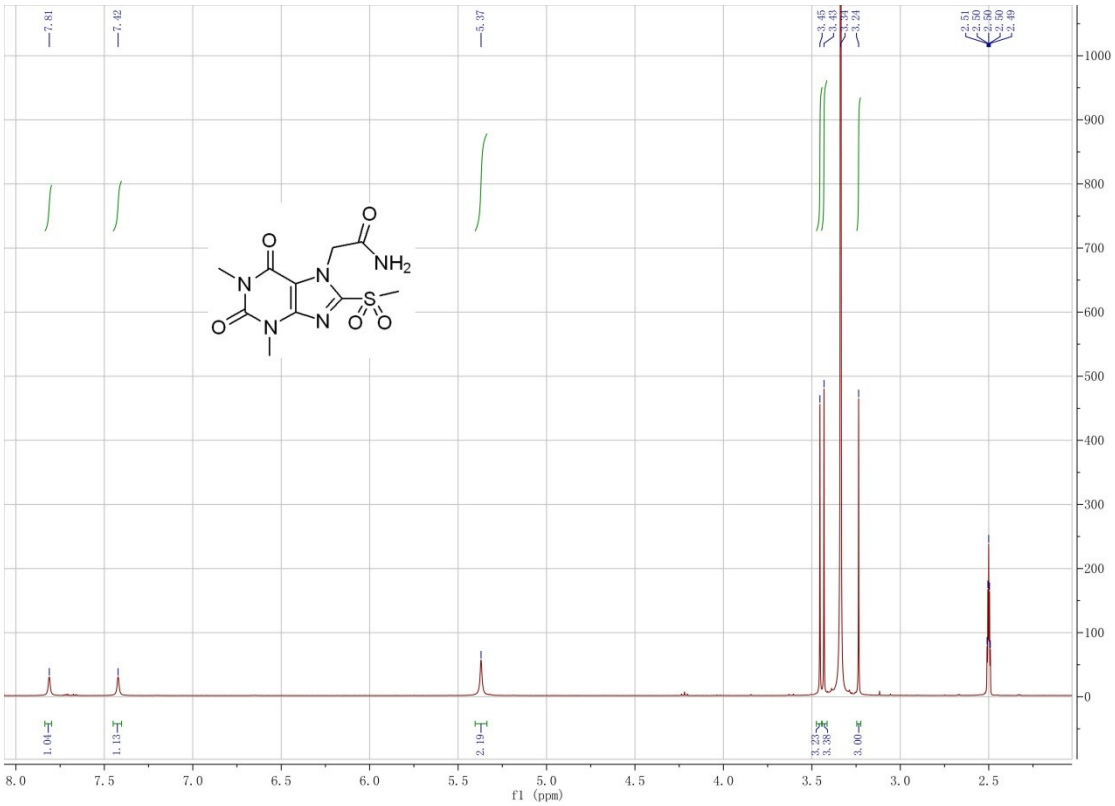

7.26
4.70
4.68
4.66
3.57
3.44
3.42
2.00
1.98
1.98
1.97
1.97
1.96
1.95
1.95
1.94
1.93
1.93
1.91
1.02
1.01
0.99
2.12
3.00
2.84
2.14
3.47
f1 (ppm)


154.93
151.21
146.18
145.78
109.20
49.02
42.98
29.91
28.26
24.83
10.71
f1 (ppm)

Compound 36


7.26
5.81
3.60
3.46
3.44
0.00
2.00
3.09
2.88
2.93
f1 (ppm)

Compound 37


7.81
7.42
5.37
3.45
3.43
3.31
3.24
2.51
2.50
2.50
2.49
1.04
1.13
2.19
3.23
3.38
3.00
f1 (ppm)