Supporting Information

Crystallographic and SAR Analyses Reveal High Requirement to Selectively and Potently Inhibit SIRT2 Deacetylase and Decanoylase

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Content

Supplementary Experimental Section

Chemistry.

Unless otherwise noted, all of the commercially available starting materials, reagents, and solvents and reagents were used without further purification. For all target compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC). HPLC analysis was performed on a Waters 2695 HPLC system equipped with a Kromasil C18 column (4.6 mm × 250 mm, 5 um). Analytical thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254, with detection by UV light (λ = 254 nm). ^{1}H and ^{13}C spectra were recorded on a Bruker AV-400 (Bruker Company, Germany) instrument at room temperature (rt). Chemical shifts (δ) are quoted in ppm (parts per million) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants (*J* values) were given in hertz (Hz). High-resolution mass spectra (HRMS) were determined using a SCIEX X500 QTOF mass spectrometer. Melting points were measured on an electrothermal melting point apparatus without correction.

General procedure for the preparation of 1a–1b and 12a-12l.

A mixture of 4-iodoaniline (**2a**, 438 mg, 2.0 mmol), ethane-1,2-dithiol (188 mg, 2.0 mmol), copper sulfate pentahydrate (25 mg, 0.1 mmol), and potassium hydroxide (560 mg, 10.0 mmol) in DMSO/H_2O (4 mL / 0.4 mL) was stirred for 8 h at 110℃ under the argon atmosphere. After the reaction cooled to room temperature, iodobenzene (**3**, 530 mg, 2.6 mmol) in DMF (2 mL) was added to the above mixture, and then reacted for another 18h at 120℃. Upon completion of the reaction as determined by TLC, The mixture was partitioned between water (70 mL) and ethyl acetate (3× 40 mL). The organic layer was washed by saturated sodium chloride aqueous (50 mL) and dried over magnesium sulfate anhydrous, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (*V*(PA):*V*(EA)=6:1) to give the brown intermediate **4a** (300 mg) in 74% yield.

To a solution of 4-(phenylthio)aniline (**4a**, 100 mg, 0.5 mmol) in dichloromethane (4 mL), 2-bromoacetyl bromide (130 μL, 1.5 mmol) and triethylamine (346 μL, 2.5 mmol) were added and the miture was stirred at 0℃ for 40min. When TLC indicated that the reaction was finished, the reaction solution was concentrated and the crude product was purified by column chromatography (*V*(PA):*V*(EA)=8:1) to give the yellow

intermediate **5a** (145 mg), in 90% yield.

4,6-Dimethylpyrimidine-2-thiol (47 mg, 0.34 mmol) and potassium tert-butoxide (63 mg, 0.56 mmol) were dissolved in DMF (3 mL), and the solution was stirred at room temperature for 0.5h. Next, 2-bromo-N-(4-(phenylthio)phenyl)acetamide (**5a**, 90 mg, 0.28 mmol) was added to the above reaction and stirred overnight. Upon completion of the reaction as determined by TLC, the reaction mixture was poured into 50 mL water and extracted with ethyl acetate (3× 20 mL). Then, the combined organic layers were washed with brine, dried, and concentrated. The residue was purified by column chromatography (*V*(PA):*V*(EA)=3:1) to give the desired target compound **1a** (100 mg) in 93% yield. 96.8% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d$_6$*): δ 10.40 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 2H), 7.26-7.20 (m, 3H), 6.98 (s, 1H), 4.06 (s, 2H), 2.33 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d$_6$*): δ 169.7, 167.5, 167.3, 139.6, 137.2, 133.9, 129.9, 129.3, 127.4, 127.0, 120.6, 116.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{20}N_3OS_2$ $[M+H]^+$ 382.1042, found 382.1045.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(3-(phenylthio)phenyl)acetamide ***(1b)*** 97.9% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d$_6$*): δ 10.32 (s, 1H), 7.61 (s, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.47 - 7.45 (m, 1H), 7.43-7.29 (m, 6H), 6.96 (s, 1H), 4.02 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d$_6$*): δ 169.7, 167.4, 167.3, 140.5, 135.9, 134.9, 131.4, 130.3, 130.1, 128.0, 125.7, 121.1, 118.5, 116.5, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{20}N_3OS_2$ $[M+H]^+$ 382.1042, found 382.1040.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(pyridin-3-ylthio)phenyl)acetamide ***(12a)*** 97.3% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d$_6$*): δ 10.44 (s, 1H), 8.44 (s, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.37 - 7.33 (m, 1H), 6.96 (s, 1H), 4.06 (s, 2H), 2.33 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d$_6$*): δ 169.7, 167.4, 167.3, 149.6, 148.0, 140.0, 137.0, 134.5, 134.1, 126.1, 124.8, 120.7, 116.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{19}H_{19}N_4OS_2$ $[M+H]^+$ 383.0995, found 383.0988.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(thiophen-3-ylthio)phenyl)acetamide ***(12b)*** 97.5% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d$_6$*): δ 10.32 (s, 1H), 7.70 - 7.64 (m, 1H), 7.61 - 7.54 (m, 3H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.03 (dd, *J* = 4.8, 1.2 Hz, 1H), 6.95 (s, 1H), 4.03 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d$_6$*): δ 169.7, 167.4, 167.1, 138.6, 130.8, 130.6, 130.0, 128.5, 127.9, 120.5, 116.5, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{18}H_{18}N_3OS_3$ $[M+H]^+$ 388.0607, found 388.0605.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(naphthalen-1-ylthio)phenyl)acetamide ***(12c)*** 97.3% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d$_6$*): δ 10.37 (s, 1H), 8.31 - 8.21 (m, 1H), 8.05 - 7.87 (m, 2H), 7.66 - 7.55 (m, 4H), 7.52 - 7.45 (m, 2H), 7.29 (d, *J*

= 8.8 Hz, 2H), 6.94 (s, 1H), 4.05 (s, 2H), 2.32 (s, 6H) ppm. ^{3}C NMR (100 MHz, *DMSO-d_6*): δ 169.7, 167.4, 167.1, 138.9, 134.2, 132.6, 132.3, 132.0, 130.5, 129.2, 128.8, 128.4, 127.5, 127.0, 126.5, 124.8, 120.6, 116.5, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{24}H_{22}N_3OS_2$ $[M+H]^+$ 432.1199, found 432.1198.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(naphthalen-2-ylthio)phenyl)acetamide (**12d**)* 97.8% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d_6*): δ 10.43 (s, 1H), 7.93 - 7.85 (m, 2H), 7.85 - 7.75 (m, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.55 - 7.47 (m, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.32 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.96 (s, 1H), 4.07 (s, 2H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d_6*): δ 169.7, 167.4, 167.2, 139.6, 134.5, 133.8, 133.6, 132.0, 129.4, 128.1, 127.7, 127.6, 127.5, 127.3, 126.6, 120.6, 116.5, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{24}H_{22}N_3OS_2$ $[M+H]^+$ 432.1199, found 432.1196.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(m-tolylthio)phenyl)acetamide (**12e**)* 97.3% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d_6*): δ 10.41 (s, 1H), 7.64 (d, *J*= 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.26 - 7.17 (m, 1H), 7.09 - 6.93 (m, 4H), 4.06 (s, 2H), 2.34 (s, 6H), 2.25 (s, 3H) ppm. ^{13}C NMR (100 MHz, *DMSO-d_6*): δ 169.8, 167.4, 167.3, 139.5, 139.2, 136.8, 133.7, 129.9, 129.7, 127.9, 127.7, 126.6, 120.6, 116.6, 36.0, 23.8, 21.3 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3OS_2$ $[M+H]^+$ 396.1199, found 396.1190.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-((3-(trifluoromethyl)phenyl)thio)phenyl)acetamide (**12f**)* 98.6% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d_6*): δ 10.51 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.64 - 7.34 (m, 6H), 6.97 (s, 1H), 4.08 (s, 2H), 2.33 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d_6*): δ 169.7, 167.4, 167.4, 140.5, 140.3, 135.3, 131.7, 130.8, 124.9, 123.7, 123.7, 123.0, 123.0, 120.7, 116.5, 36.0, 23.7ppm. HRMS: m/z calcd for $C_{21}H_{19}F_3N_3OS_2$ $[M+H]^+$ 450.0916, found 450.0906.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-((3-methoxyphenyl)thio)phenyl)acetamide (**12g**)* 96.6% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d_6*): δ 10.43 (s, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.24 (t, *J* = 8.0 Hz, 1H), 6.97 (s, 1H), 6.85 - 6.67 (m, 3H), 4.07 (s, 2H), 3.71 (s, 3H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d_6*): δ 169.7, 167.4, 167.3, 160.2, 139.8, 138.7, 134.3, 130.7, 126.9, 121.1, 120.6, 116.6, 114.3, 112.4, 55.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S_2$ $[M+H]^+$ 412.1148, found 412.1136.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-(p-tolylthio)phenyl)acetamide (**12h**)* 98.0% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d_6*): δ 10.36 (s, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.16 (s, 4H), 6.95 (s, 1H), 4.05 (s, 2H), 2.33 (s, 6H), 2.27 (s, 3H) ppm. ^{13}C NMR (100 MHz, *DMSO-d_6*): δ 169.7, 167.4, 167.2, 139.1, 137.1, 132.8, 132.6, 130.6, 130.5, 128.9, 120.5, 116.5, 36.0, 23.8, 21.0 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3OS_2$ $[M+H]^+$ 396.1199, found 396.1201.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-((4-(trifluoromethyl)phenyl)thio)phenyl)acetamide (**12i**)* 97.5% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.50 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 4.08 (s, 2H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 169.7, 167.5, 167.4, 144.6, 140.8, 135.9, 131.7, 127.3, 126.5, 126.4, 124.1, 120.9, 116.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{19}F_3N_3OS_2$ $[M+H]^+$ 450.0916, found 450.0912.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-((4-methoxyphenyl)thio)phenyl)acetamide (**12j**)* 97.6% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.32 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 3H), 4.04 (s, 2H), 3.77 (s, 3H), 2.33 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 169.7, 167.4, 167.0, 159.6, 138.4, 134.2, 130.9, 130.7, 125.3, 120.4, 116.5, 115.6, 55.7, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S_2$ $[M+H]^+$ 412.1148, found 412.1149.

*2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(4-((4-(trifluoromethoxy)phenyl)thio)phenyl)acetamide (**12k**)* 97.5% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.46 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.35 - 7.22 (m, 4H), 6.97 (s, 1H), 4.07 (s, 2H), 2.33 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 169.7, 167.4, 167.4, 147.1, 140.2, 137.4, 134.7, 130.1, 126.1, 122.5, 120.7, 116.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{19}F_3N_3O_2S_2$ $[M+H]^+$ 466.0865, found 466.0860.

*N-(4-((4-acetylphenyl)thio)phenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide (**12l**)* 97.3% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.50 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 4.08 (s, 2H), 2.52 (s, 3H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 197.3, 169.7, 167.5, 145.4, 140.7, 135.8, 134.5, 129.5, 126.7, 126.6, 124.3, 120.8, 116.6, 36.0, 27.0, 23.8 ppm. HRMS: m/z calcd for $C_{22}H_{22}N_3O_2S_2$ $[M+H]^+$ 424.1148, found 424.1148.

General procedure for the preparation of 8a–8b.

To a solution of 4-(phenylthio)aniline (**4a**, 100 mg, 0.5 mmol) in dichloromethane (3 mL) at 0℃ was added 3-chlorobenzenecarboperoxoic acid (*m*-CPBA, 86 mg, 0.5 mmol), then the mixture was allowed to warm to room temperature and stirred for 1h. The resulting solution was partitioned between sodium bicarbonate solution (30 mL) and dichloromethane (3×20 mL). Then, the combined organic layers were dried by magnesium sulfate anhydrous, and concentrated. The residue was purified by column chromatography (*V*(PA):*V*(EA)=3:1) to product the intermediate **6a** (61 mg, 56%).

Using the intermediate **6a**, the title compound **8a** was synthesized via condensation reaction (88% yield), and nucleophilic substitution reaction (92% yield) in turn, as similar as that for compound **1a**. 98.1% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.52 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.66 (t, *J* = 4.8 Hz, 4H), 7.55 - 7.45 (m, 3H), 6.92 (s, 1H), 4.04 (s, 2H), 2.29 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 169.6, 167.6, 167.4, 146.5, 142.1, 140.0, 131.4, 129.9, 126.0, 124.5, 120.1, 116.5, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{20}N_3O_2S_2$ $[M+H]^+$ 398.0991, found 398.0988.

Similarly, to a solution of 4-(phenylthio)aniline (**4a**, 100 mg, 0.5 mmol) in dichloromethane (3 mL) at 0℃ was added *m*-CPBA (259 mg, 1.5 mmol), then the mixture was stirred for 1h at room temperature. The finished reaction was extracted, dried, and concentrated. The residue was purified by column chromatography (*V*(PA):*V*(EA)=1:1) to product the intermediate **6b** (99 mg, 85%). Using the intermediate **6b**, the title compound **8b** was synthesized via condensation reaction (92% yield), and nucleophilic substitution reaction (91% yield) in turn. 98.0% HPLC purity. ^{1}H NMR (400 MHz, *DMSO-d*$_6$): δ 10.71 (s, 1H), 7.97 - 7.88 (m, 4H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.71 - 7.57 (m, 3H), 6.94 (s, 1H), 4.07 (s, 2H), 2.30 (s, 6H) ppm. ^{13}C NMR (100 MHz, *DMSO-d*$_6$): δ 169.6, 168.0, 167.4, 144.1, 142.1, 135.2, 133.9, 130.2, 129.3, 127.5, 119.7, 116.6, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{20}N_3O_3S_2$ $[M+H]^+$ 414.0941, found 414.0938.

The ^{1}H NMR, ^{13}C NMR, and mass spectra of all the target compounds are shown in Fig. S13-S28.

Inhibition Assays.

The recombinant proteins, including SIRT2, SIRT1, SIRT3, SIRT5, and SIRT6, were expressed and purified as previously described.[1] The activity assays were tested using RLIK(Ac)AMC and ETDK(Ca)AMC; more details about the activity assays will be reported somewhere. The assay buffer is 25 mM Tris–HCl pH 8.0, 150 mM NaCl, and 10% glycerol. Reaction mixtures of the enzymes (0.1 μM) with the substrate (10 μM RLIK(Ac)AMC or 10 μM ETDK(Ca)AMC) and NAD^+ (400 μM) were incubated for 2 h at 25 ℃ and 140 rpm. Then, a stop solution (50 μL) containing ~2 $U \cdot \mu L^{-1}$ trypsin (purchased from Sigma-Aldrich) and 4 mM nicotinamide was added to stop the reactions, followed by further incubation for 30 min at 25 ℃ and 140 rpm. Then, the fluorescence was obtained using a Thermo microplate reader.

Crystallographic Assays.

The purified SIRT2 proteins (13 mg·mL^{-1} in the buffer of 20 mM Tris/HCl pH 8.0, 150 mM NaCl) were co-crystallized with **1a** (5 mM) at 4℃ by the hanging-drop vapor diffusion method. SIRT2 was preincubated for 2h on ice with **1a**. Then, 2 μL crystallization drops composed of equal volumes of SIRT2 solution and reservoir solution. The crystals were transferred to a cryo-protectant drop containing the reservoir, 19% glycerol and frozen in liquid nitrogen. X-ray diffraction data were obtained at the Shanghai Synchrotron Radiation Facility and processed using HKL2000. Initial phases were obtained by the molecular replacement method using the PHASER[2] subroutine within PHENIX,[3] and structure refinements were carried out using iterative rounds of model building using COOT[4] and PHENIX.[3]

Supplementary Figures

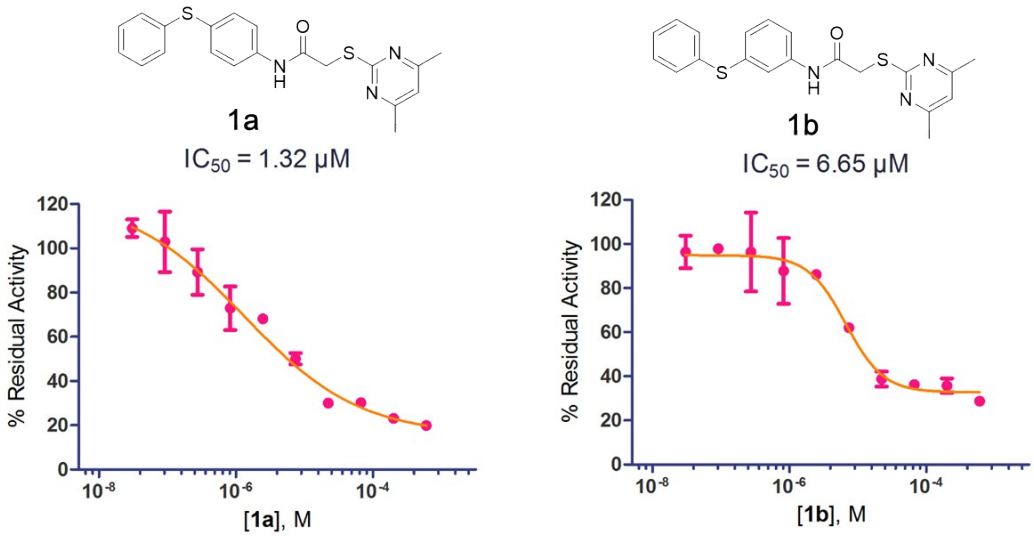


Fig. S1 The IC_{50} curves of **1a** and **1b** obtained by using the acetylated substrate RLIK(Ac)AMC.

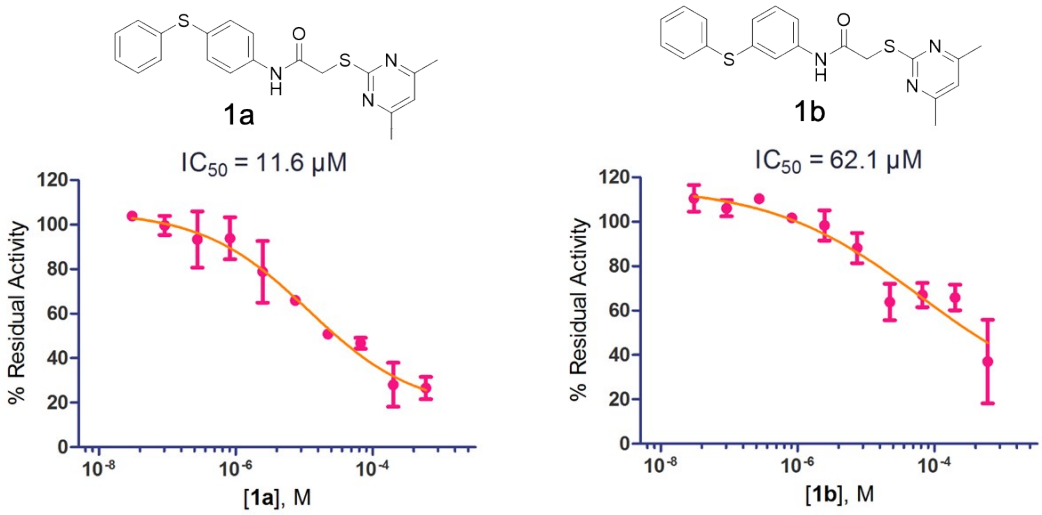


Fig. S2 The IC_{50} curves of **1a** and **1b** obtained by using the canoylated substrate ETDK(Ca)AMC.

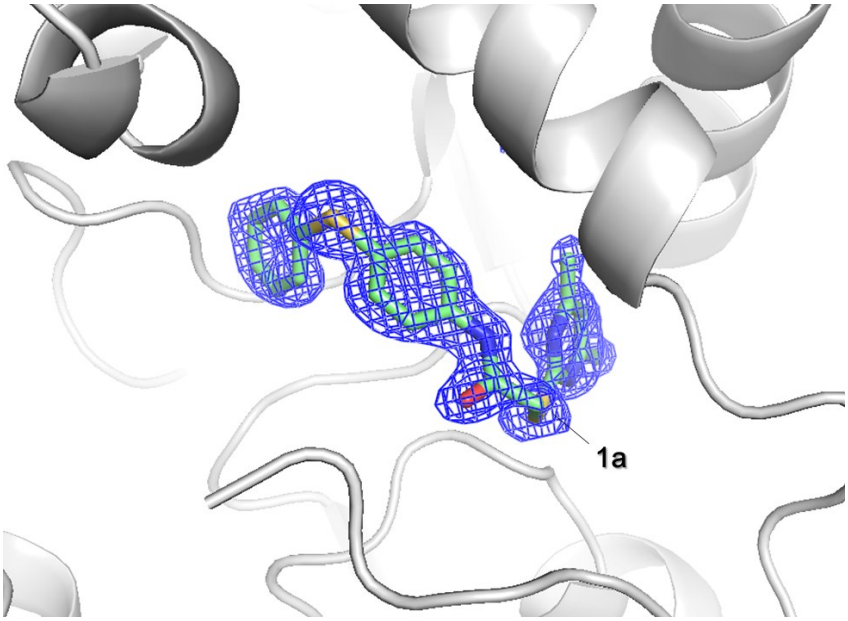


Fig. S3 The mode of **1a** binding as defined by electron density maps. Complex structures of SIRT2:**1a** (PDB ID: 5YQM) with the mF_o-DF_c electron density (OMIT maps) around **1a** (blue mesh, contoured to 3σ) calculated from the final refined model.

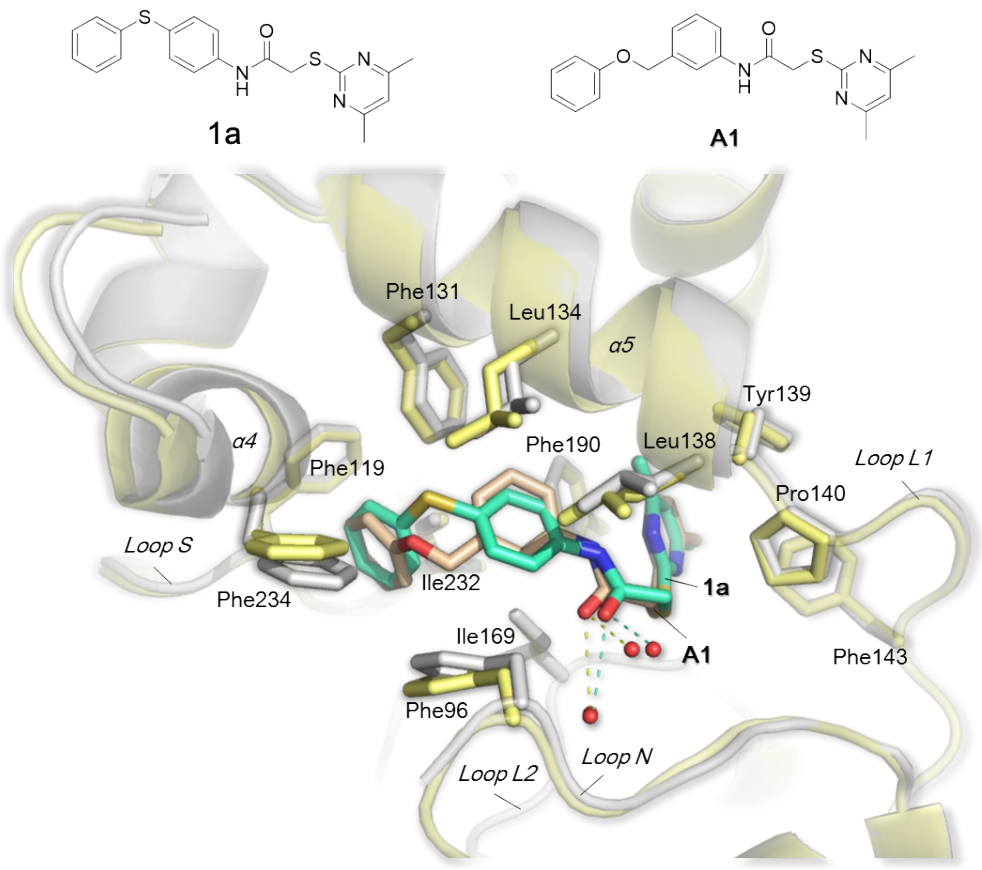


Fig. S4 Comparison of the structures of SIRT2:**1a** (PDB ID 5YQM) and SIRT2:**A1** (PDB ID 5YQL)[1] reveals that these two compounds have a similar binding mode with SIRT2 hydrophobic pocket.

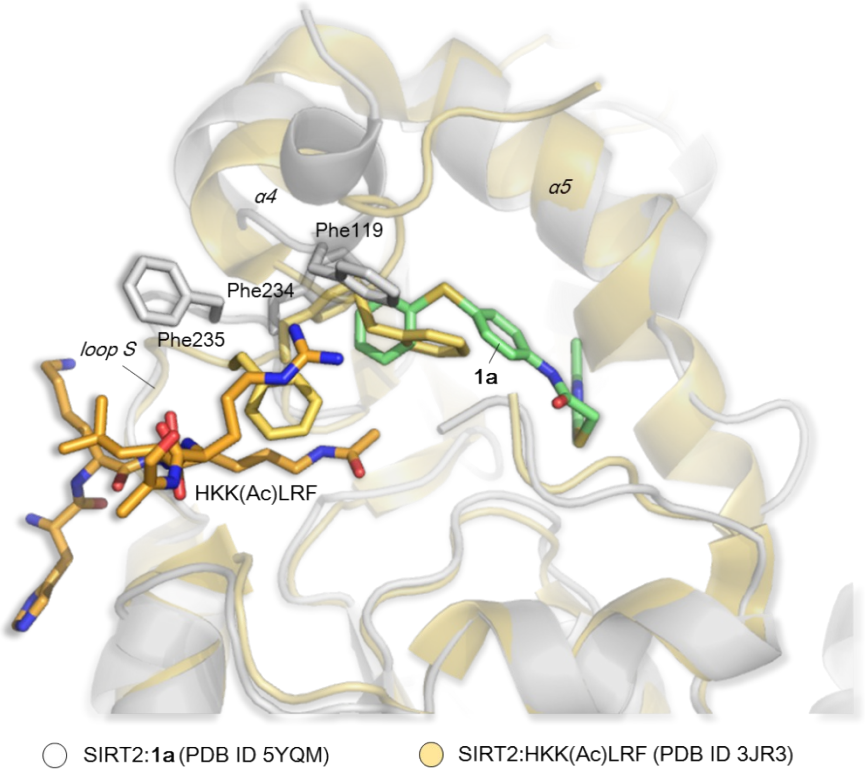


Fig. S5 Superimposition of SIRT2:**1a** (PDB ID 5YQM) with SIRT2:HKK(Ac)LRF (PDB ID 3JR3)[5] reveals that the binding of **1a** induces substantial rearrangements of loop S and α4, e.g. the positional change of the catalytically important residue Phe235.

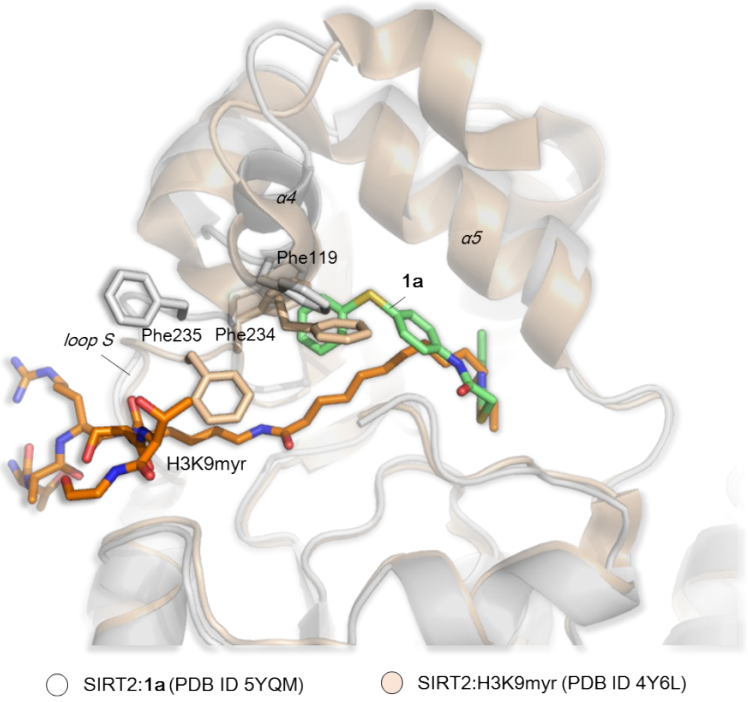


Fig. S6 Superimposition of SIRT2:**1a** (PDB ID 5YQM) with SIRT2:H3K9myr (PDB ID 4Y6L)[6] reveals that **1a** can also disturb the binding of myristoylated substrate via occupying the myristoyl binding pocket.

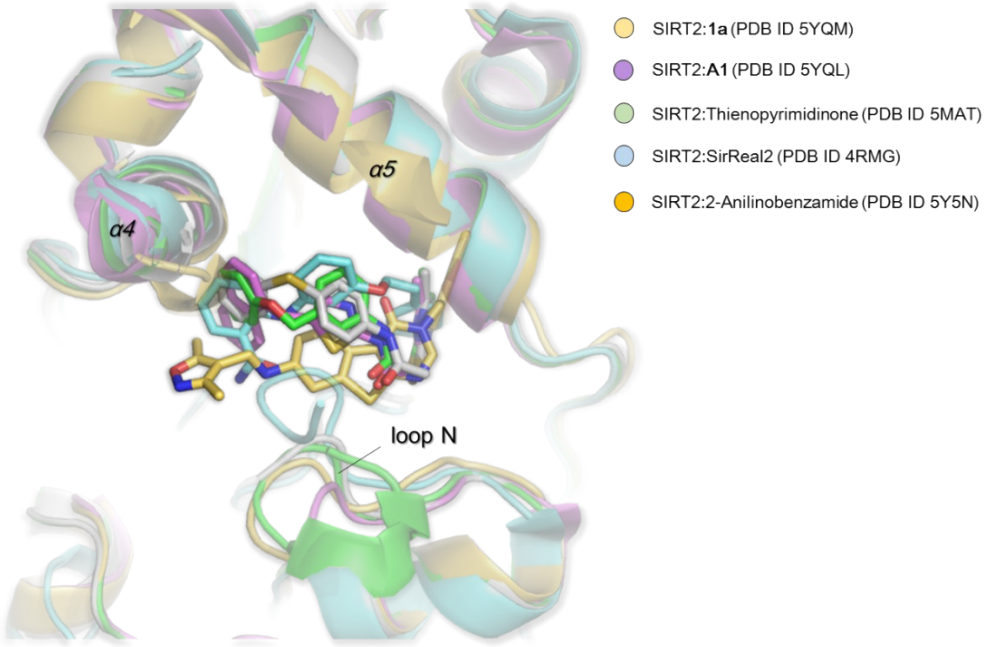


Fig. S7 Comparison of the structures of SIRT2 with structurally different selective inhibitors reveals the common pharmacophore features and evidence for the flexibility

in the conformation of the loop *N*, suggesting that this loop may be important in inhibitor capture.

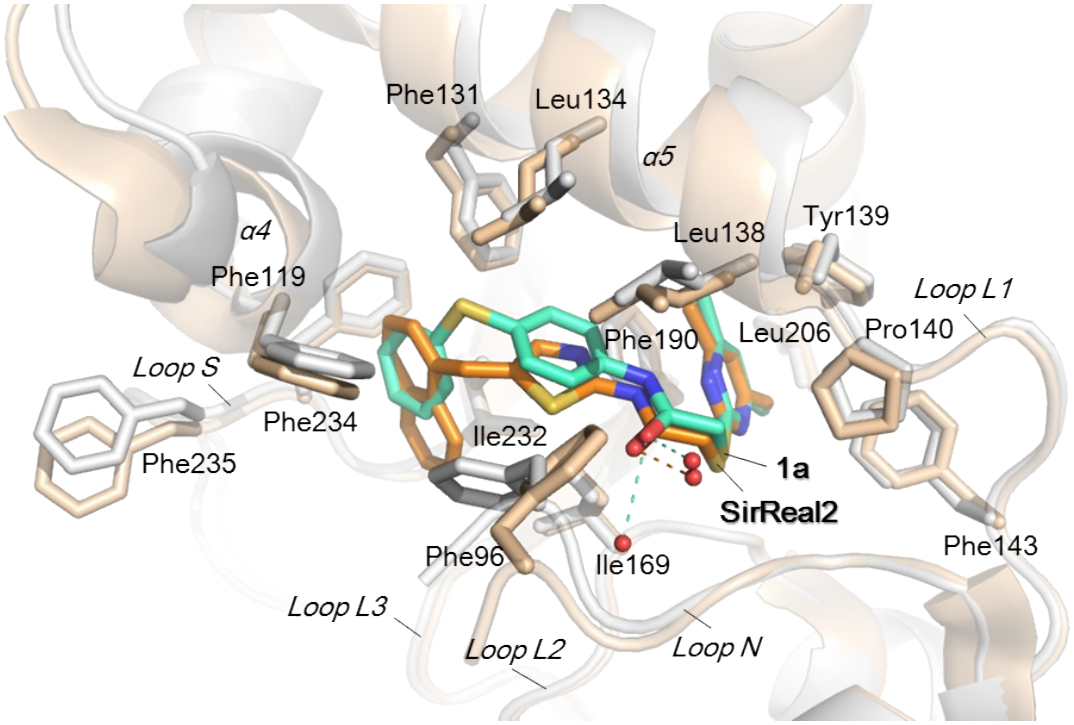


Fig. S8 Comparison of the structures of SIRT2:**1a** (PDB ID 5YQM) and SIRT2:SirReal2 (PDB ID 4RMG)[7] reveals that these two compounds have a similar binding mode with SIRT2 hydrophobic pocket.

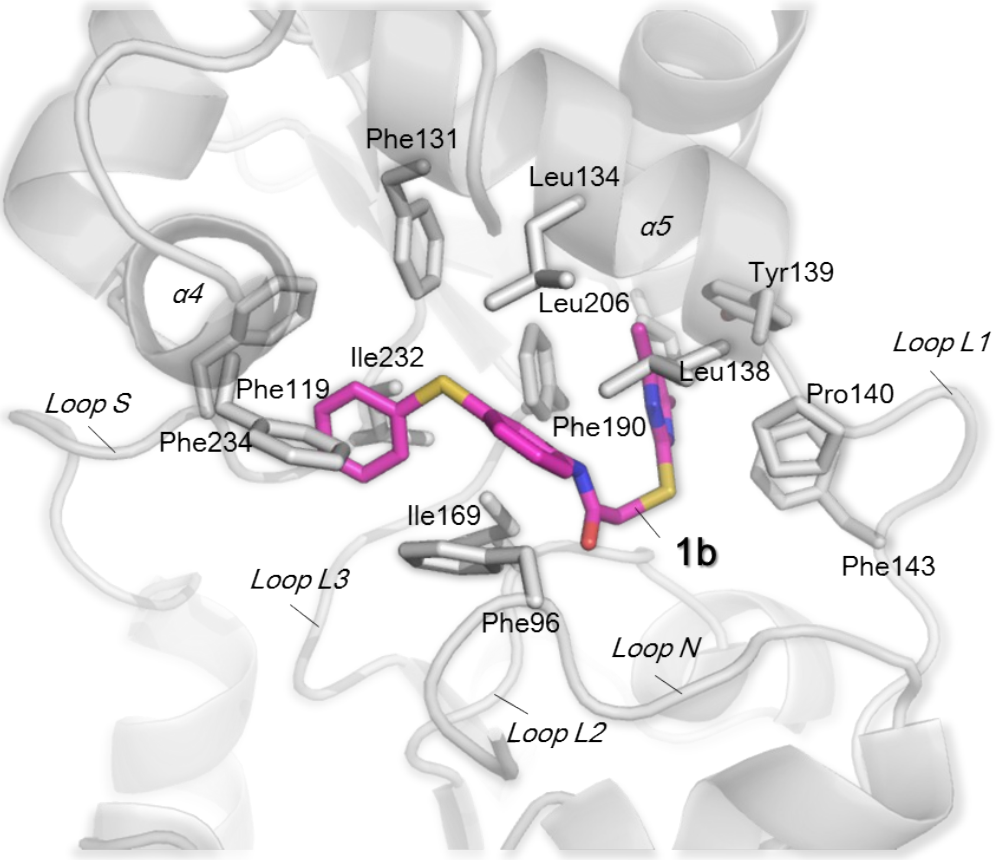


Fig. S9 The predicted binding mode of **1b** with SIRT2 using Autodock Vina.[8]

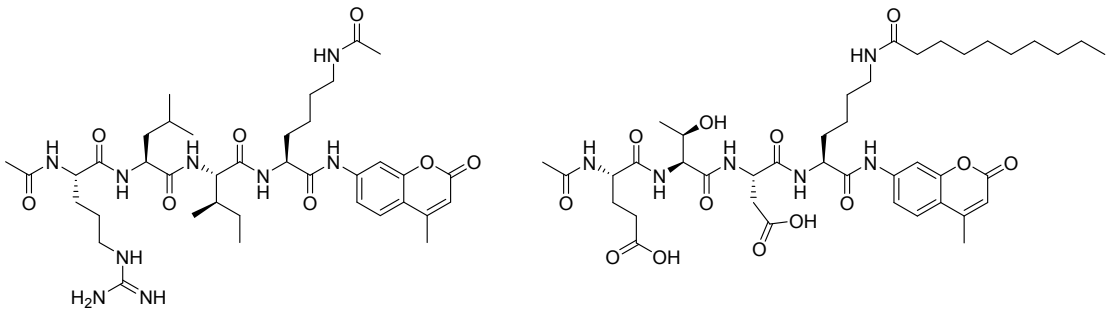


Fig. S10 Chemical structures of the used substrates RLIK(Ac)AMC and ETDK(Ca)AMC.

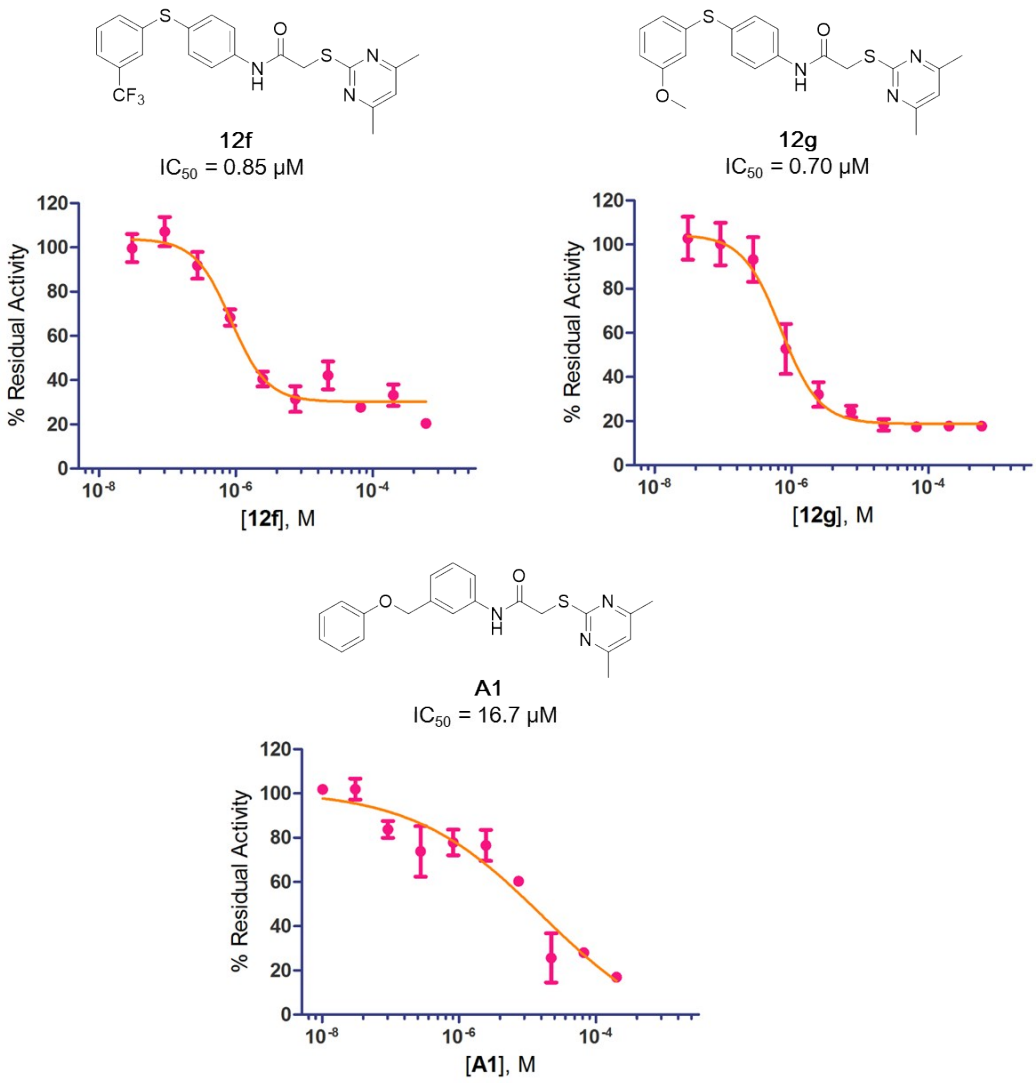


Fig. S11 The IC_{50} curves of **12f**, **12g** and **A1** obtained by using the acetylated substrate RLIK(Ac)AMC.

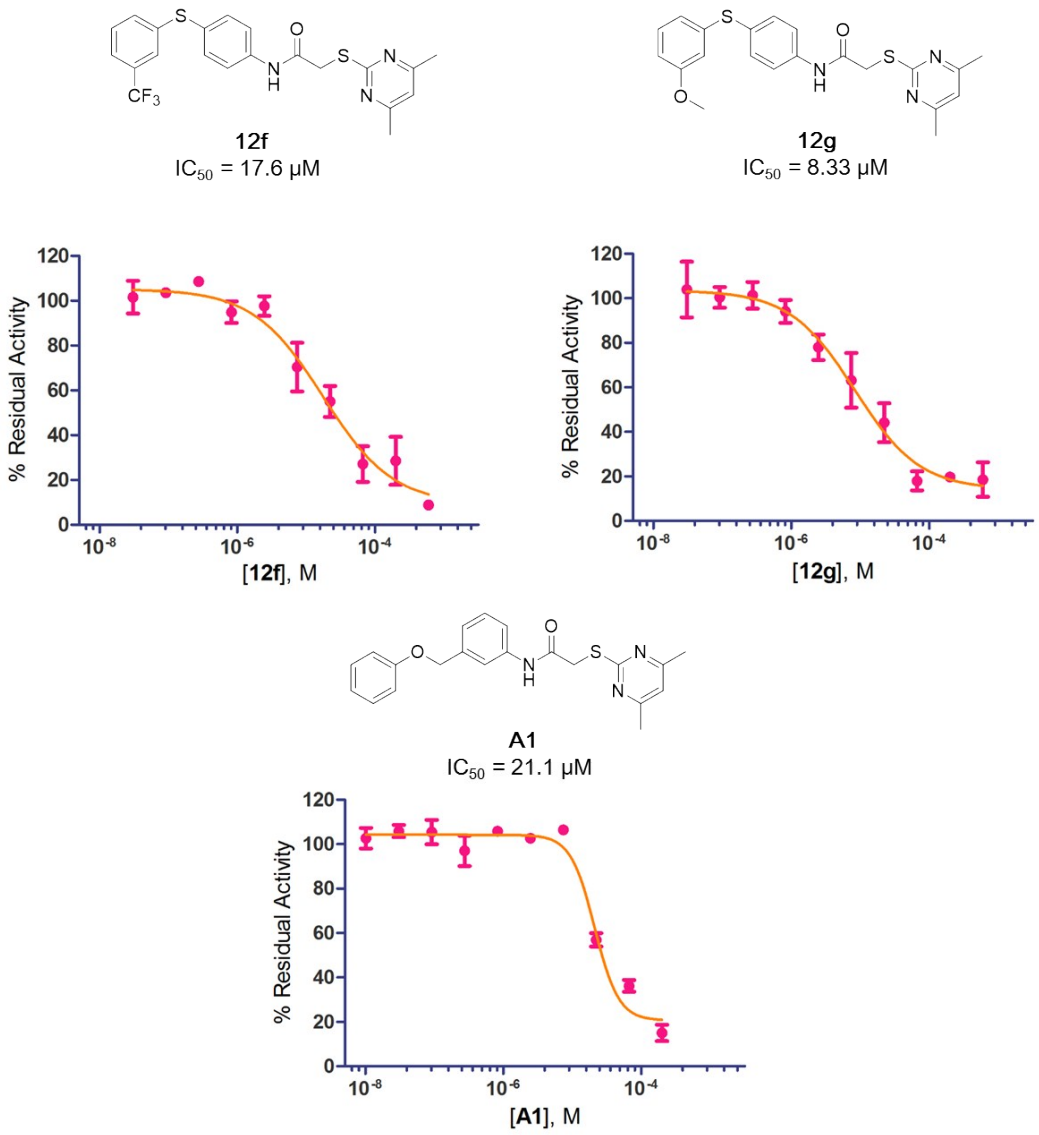
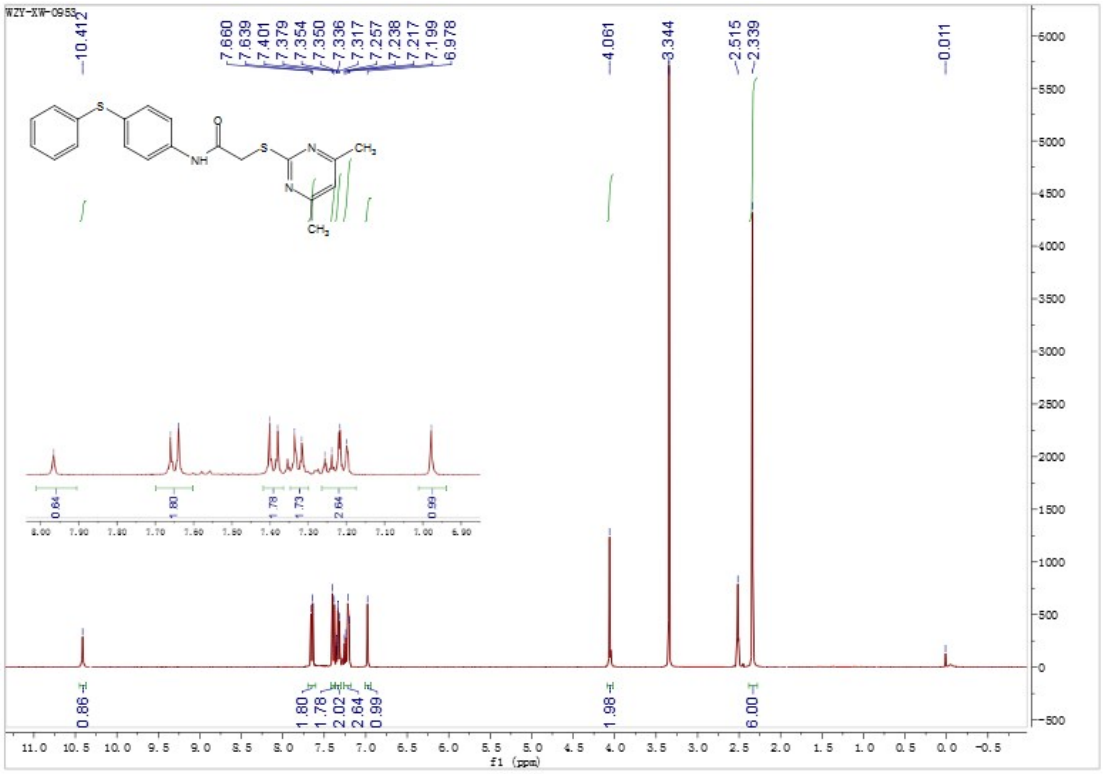
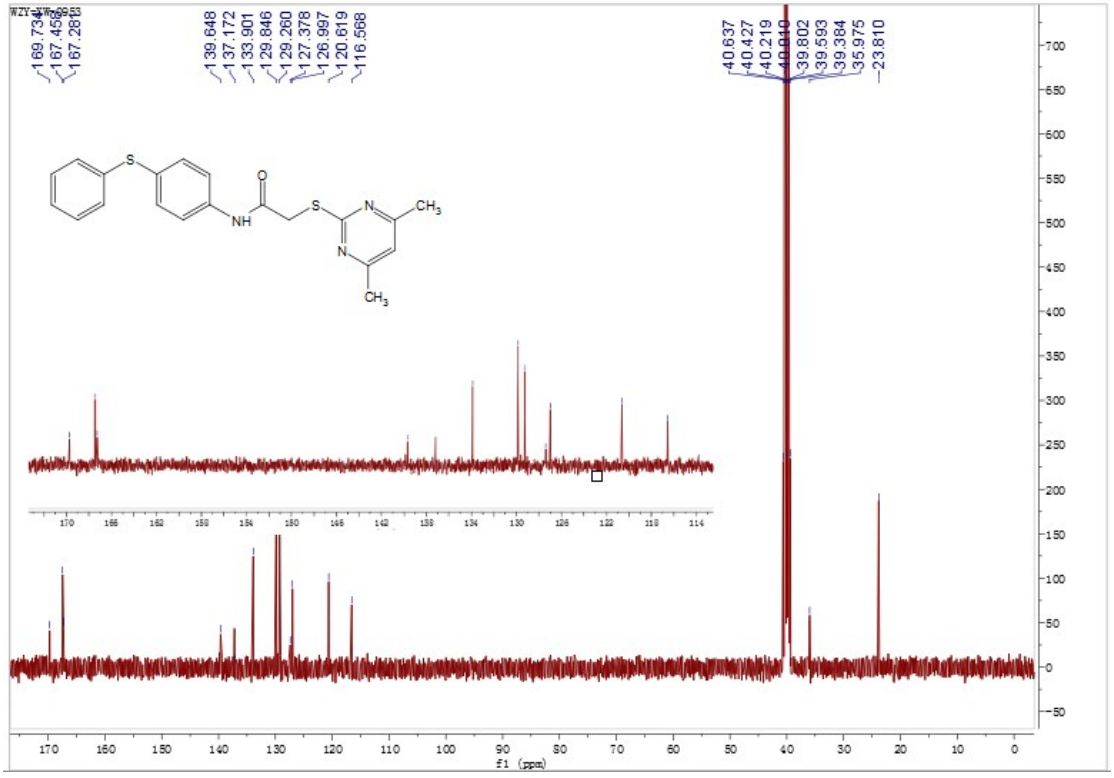


Fig. S12 The IC_{50} curves of **12f**, **12g** and **A1** obtained by using the canoylated substrate ETDK(Ca)AMC.


WZY-XW-0953
10.412
7.660
7.639
7.401
7.379
7.354
7.350
7.336
7.317
7.257
7.238
7.217
7.199
6.978
4.061
3.344
2.515
2.339
0.011
0.86
1.80
1.78
2.02
2.64
0.99
1.98
6.00
f1 (ppm)


169.734
167.458
167.281
139.648
137.172
133.901
129.846
129.260
127.378
126.997
120.619
116.568
40.637
40.427
40.219
39.802
39.593
39.384
35.975
23.810
f1 (ppm)

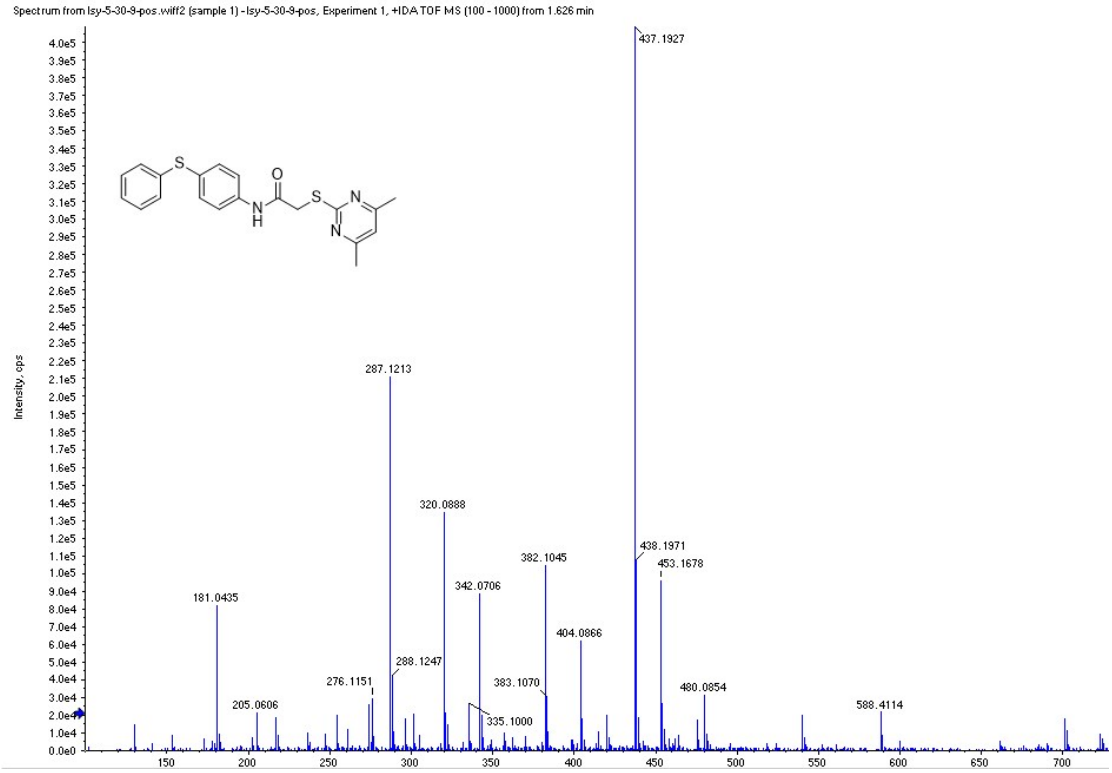
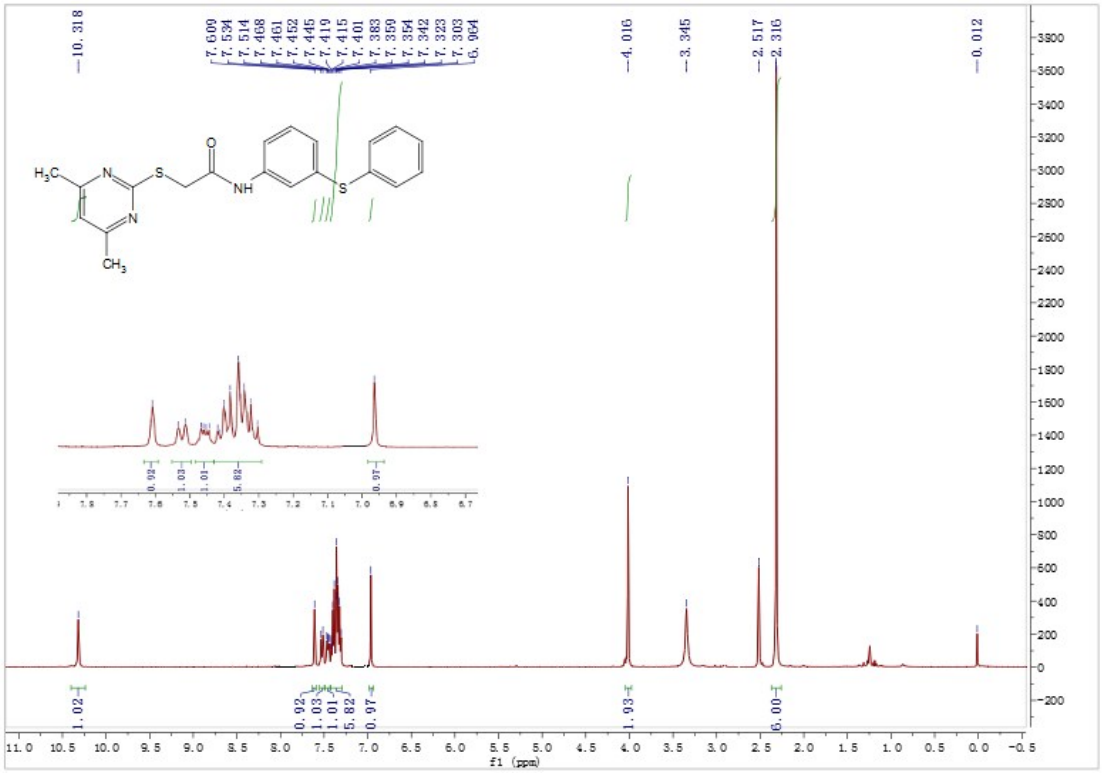


Fig. S13 ^{1}H, ^{13}C NMR, and mass spectra of **1a**.



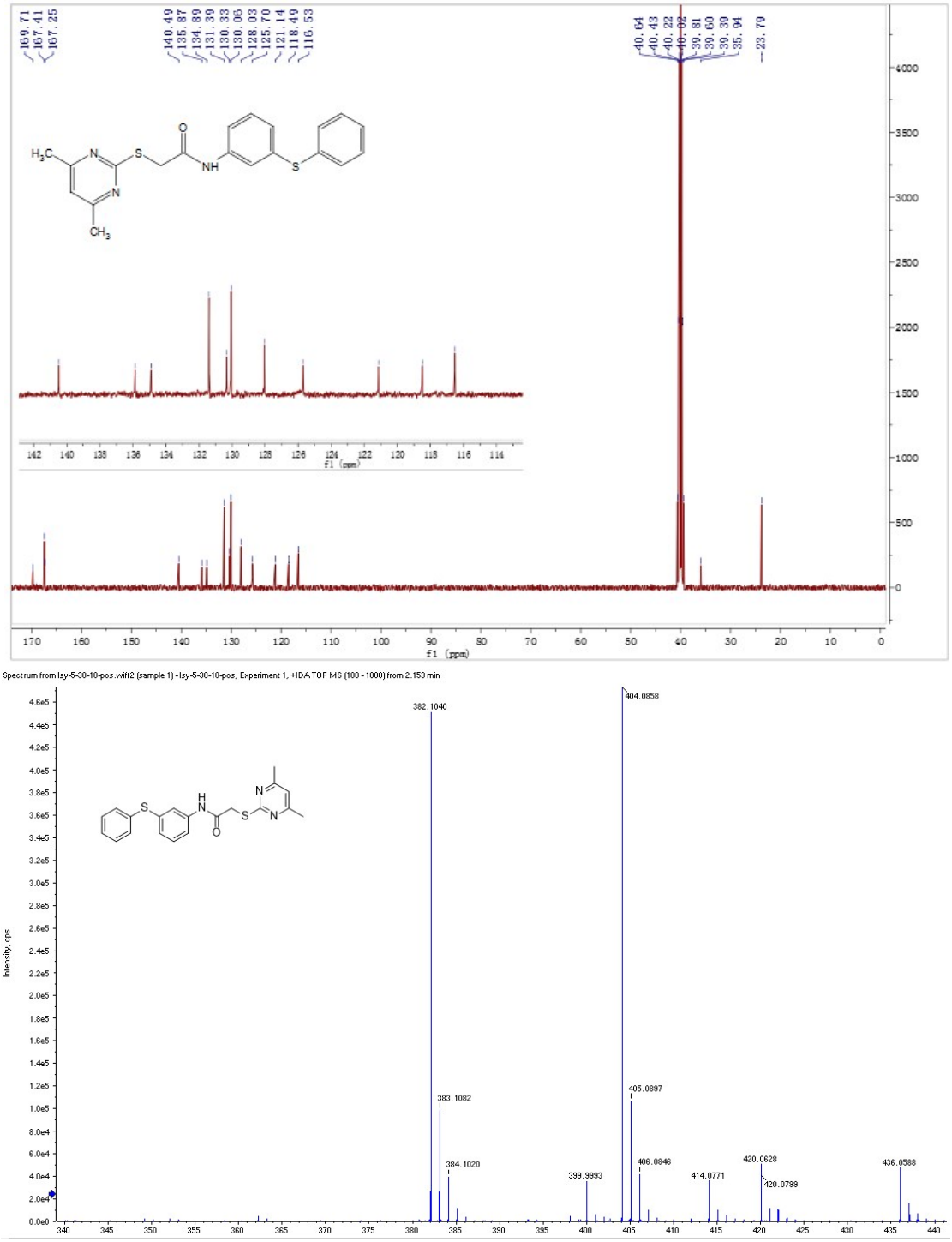
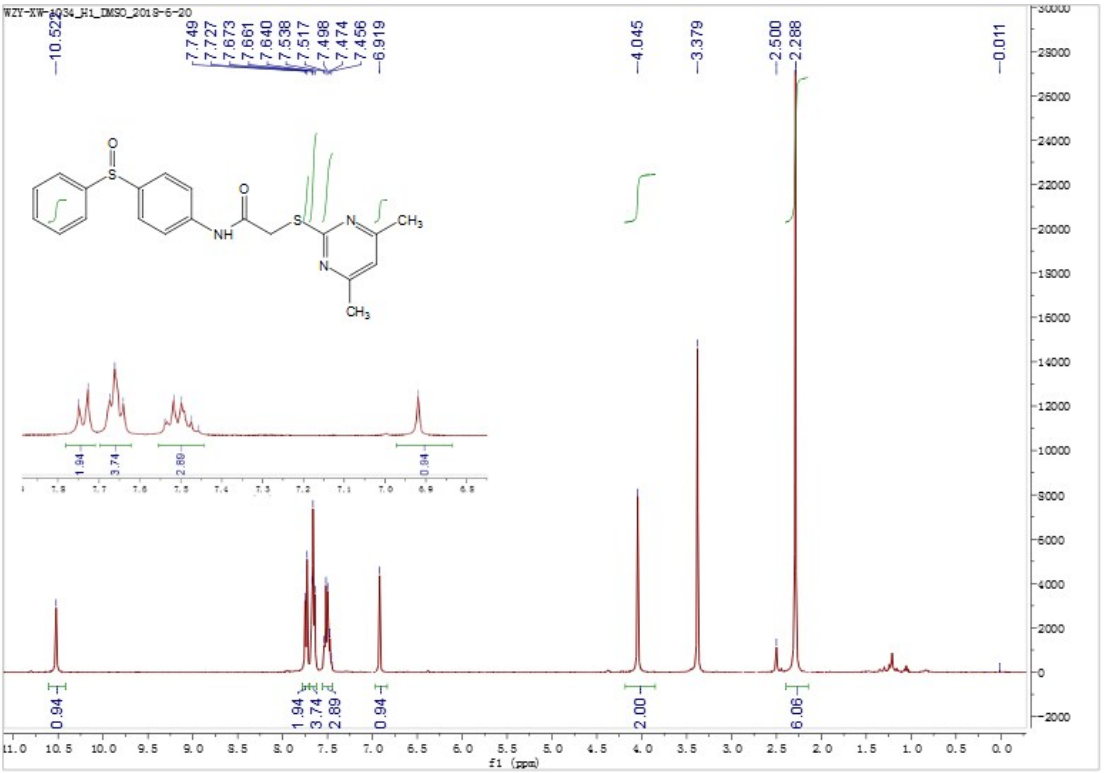
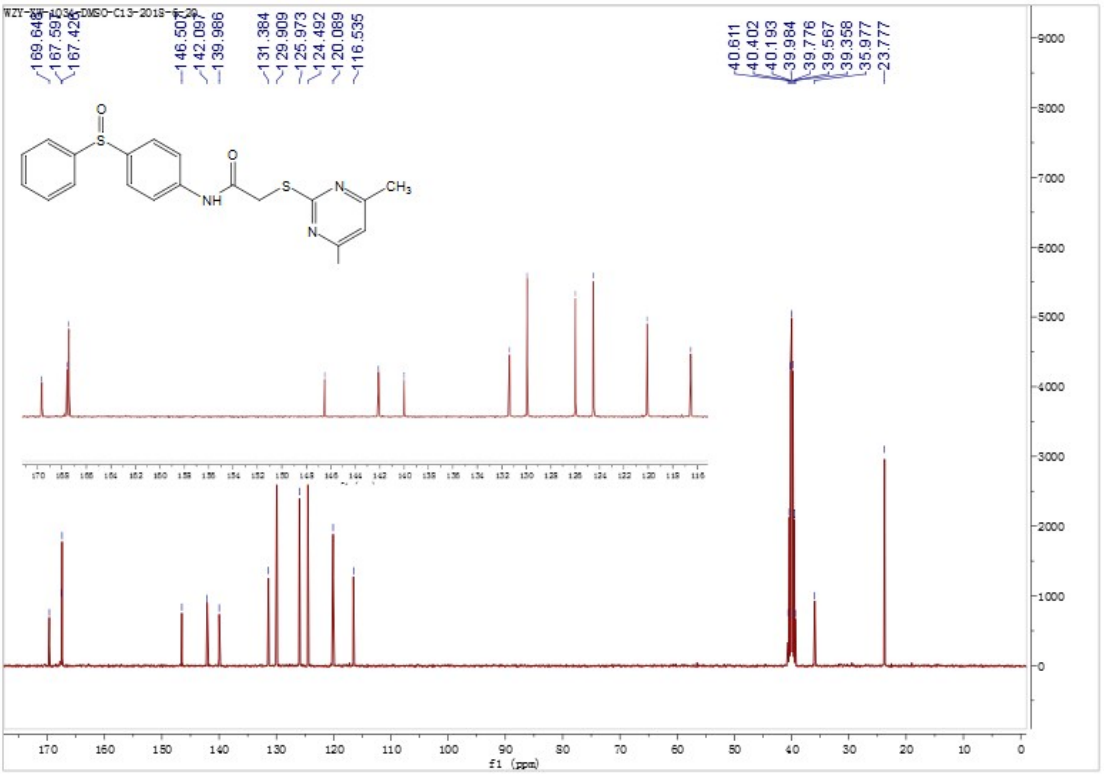


Fig. S14 ^{1}H, ^{13}C NMR, and mass spectra of **1b**.


10.522
7.749
7.727
7.673
7.661
7.640
7.538
7.517
7.498
7.474
7.456
6.919
4.045
3.379
2.500
2.288
0.011
0.94
1.94
3.74
2.89
0.94
2.00
6.06
f1 (ppm)


169.649
167.591
167.426
146.507
142.097
139.986
131.384
129.909
125.973
124.492
120.089
116.535
40.611
40.402
40.193
39.984
39.776
39.567
39.358
35.977
23.777
f1 (ppm)

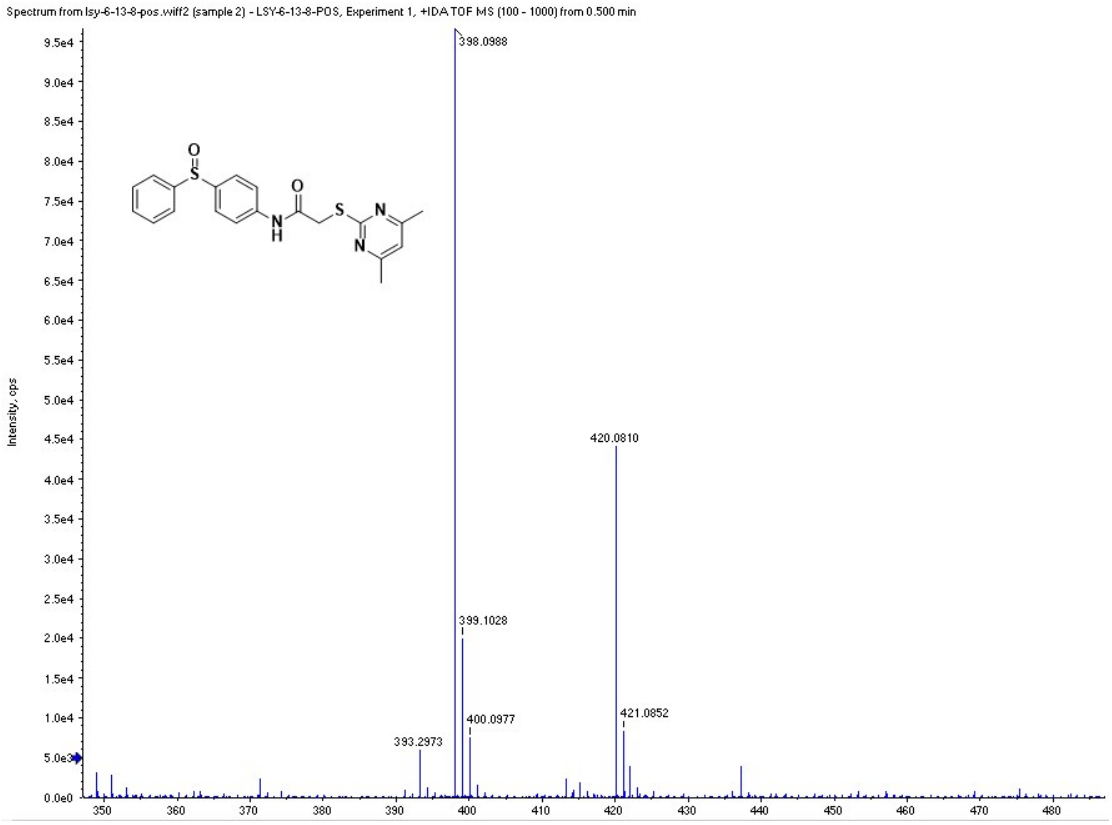
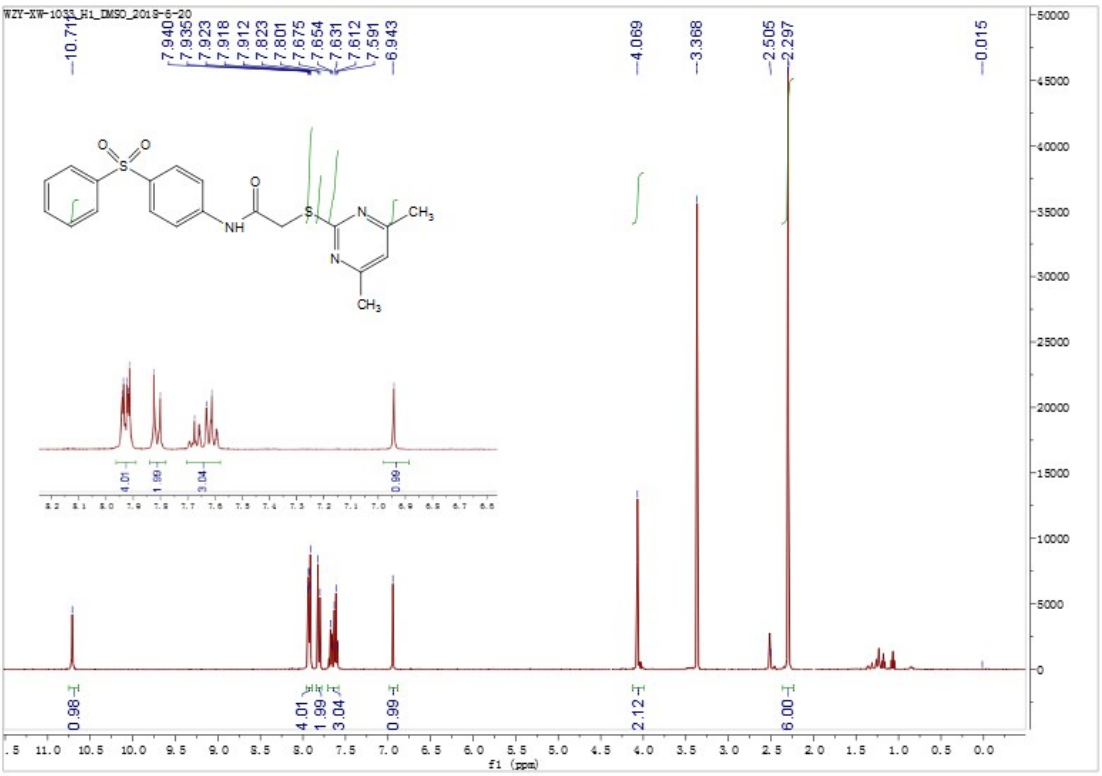
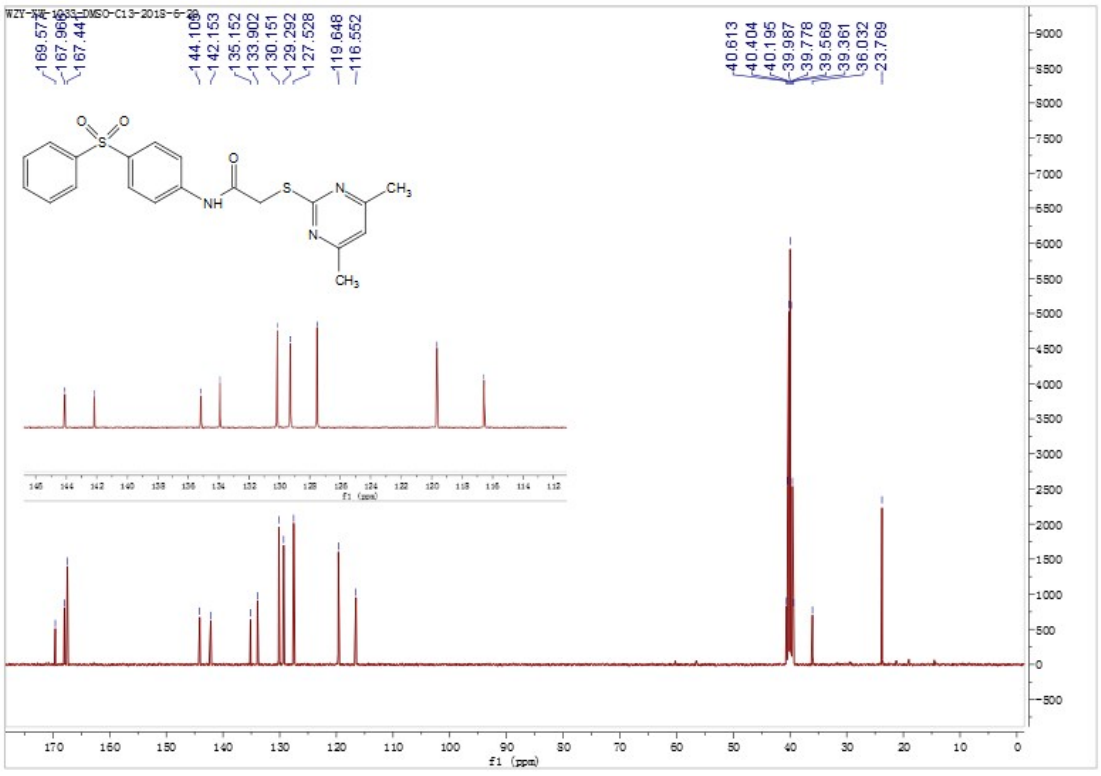


Fig. S15 ^{1}H, ^{13}C NMR, and mass spectra of **8a**.


WZY-XW-1033_H1_DMSO_2018-6-20
10.711
7.940
7.935
7.923
7.918
7.912
7.823
7.801
7.675
7.654
7.631
7.612
7.591
6.943
4.069
3.368
2.505
2.297
0.015
0.98
4.01
1.99
3.04
0.99
2.12
6.00
f1 (ppm)


169.574
167.966
167.447
144.105
142.153
135.152
133.902
130.151
129.292
127.528
119.648
116.552
40.613
40.404
40.195
39.987
39.778
39.569
39.361
36.032
23.769
f1 (ppm)

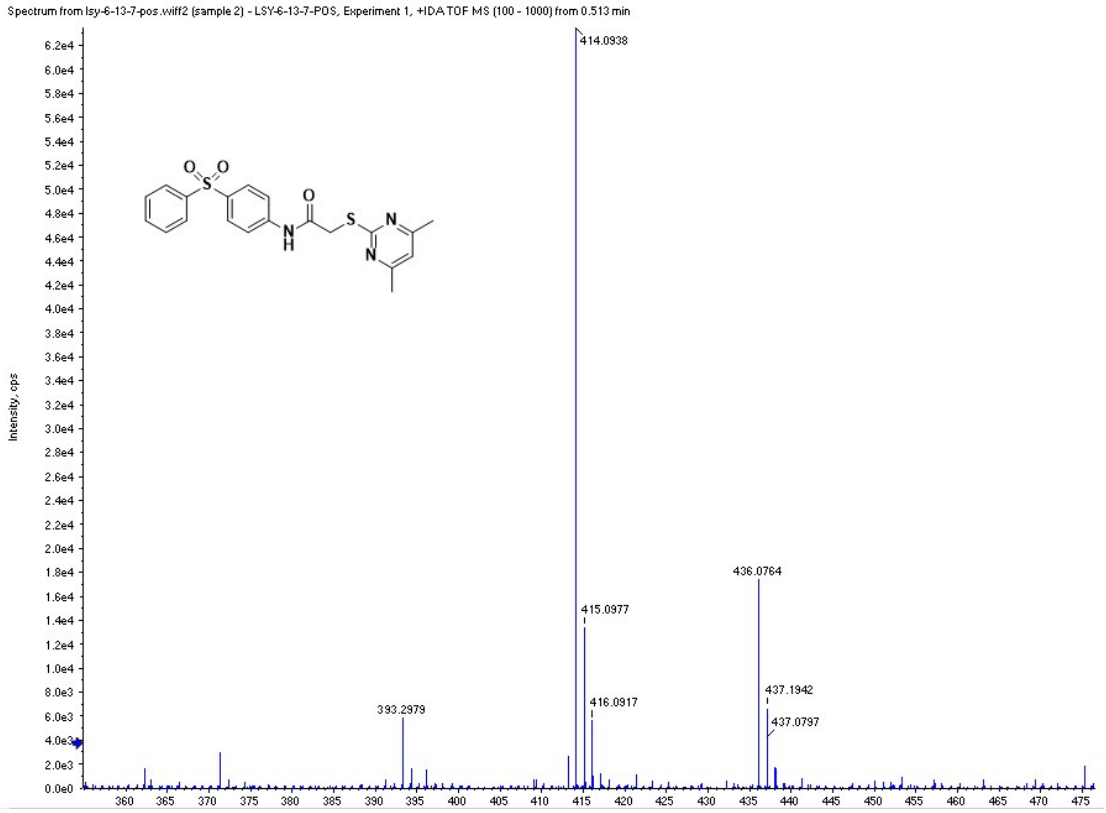
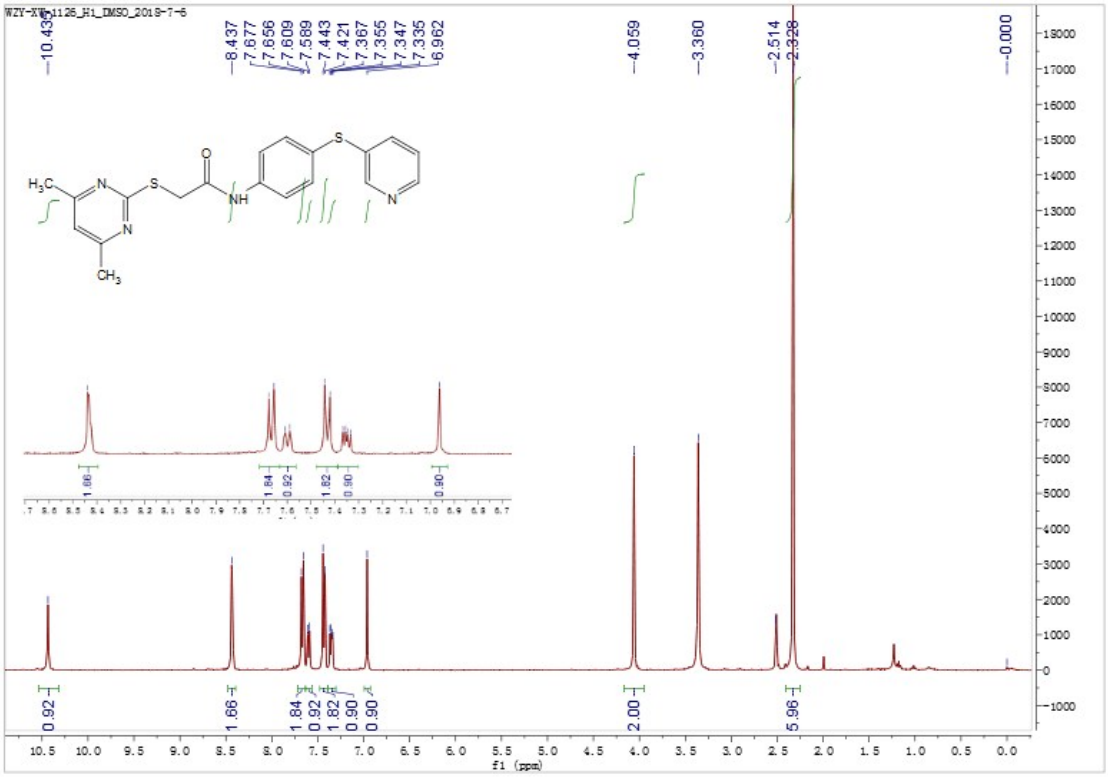
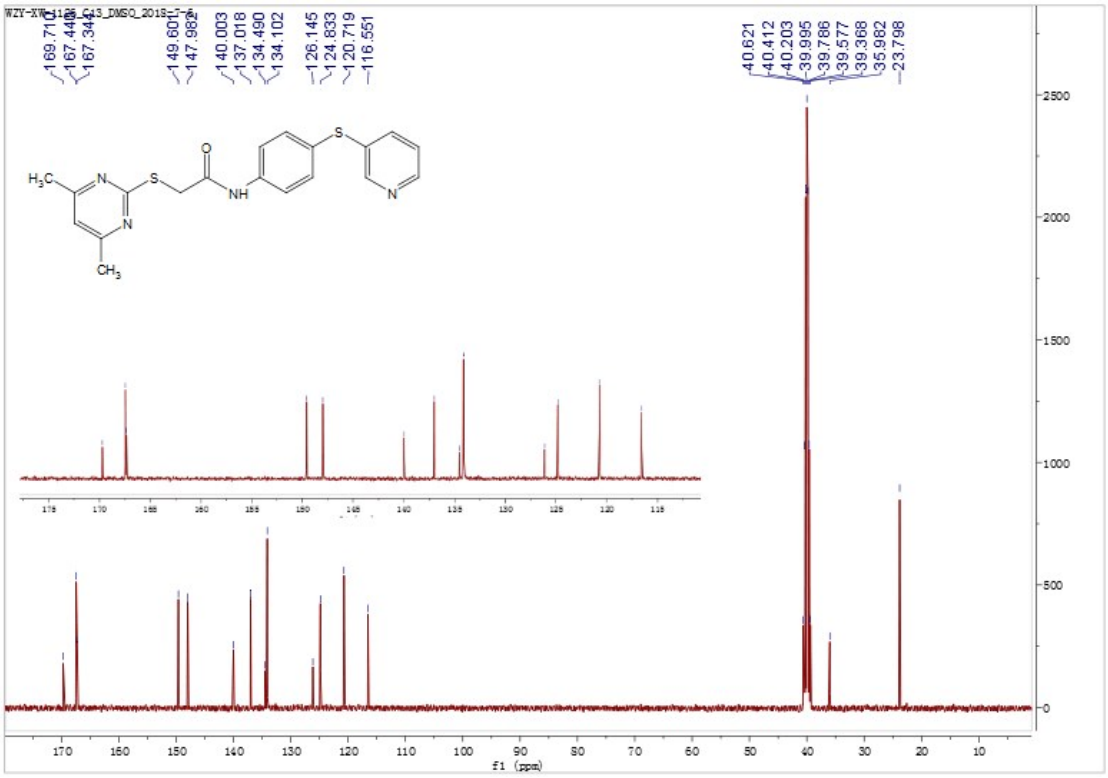


Fig. S16 ^{1}H, ^{13}C NMR, and mass spectra of **8b**.





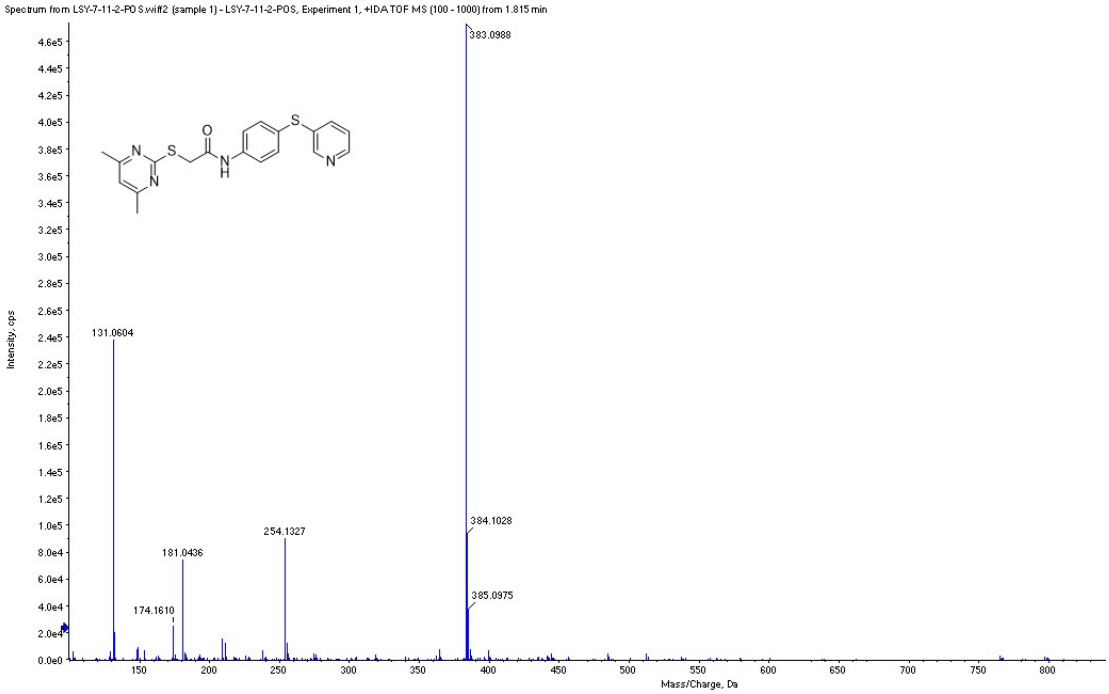
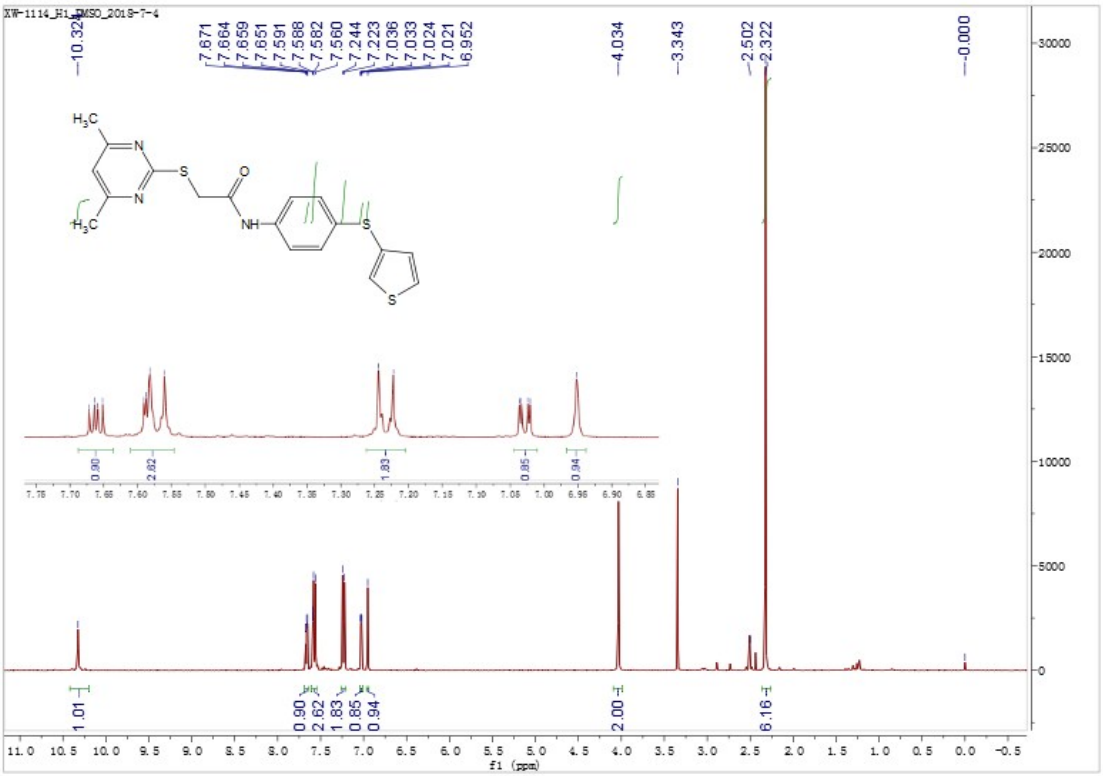
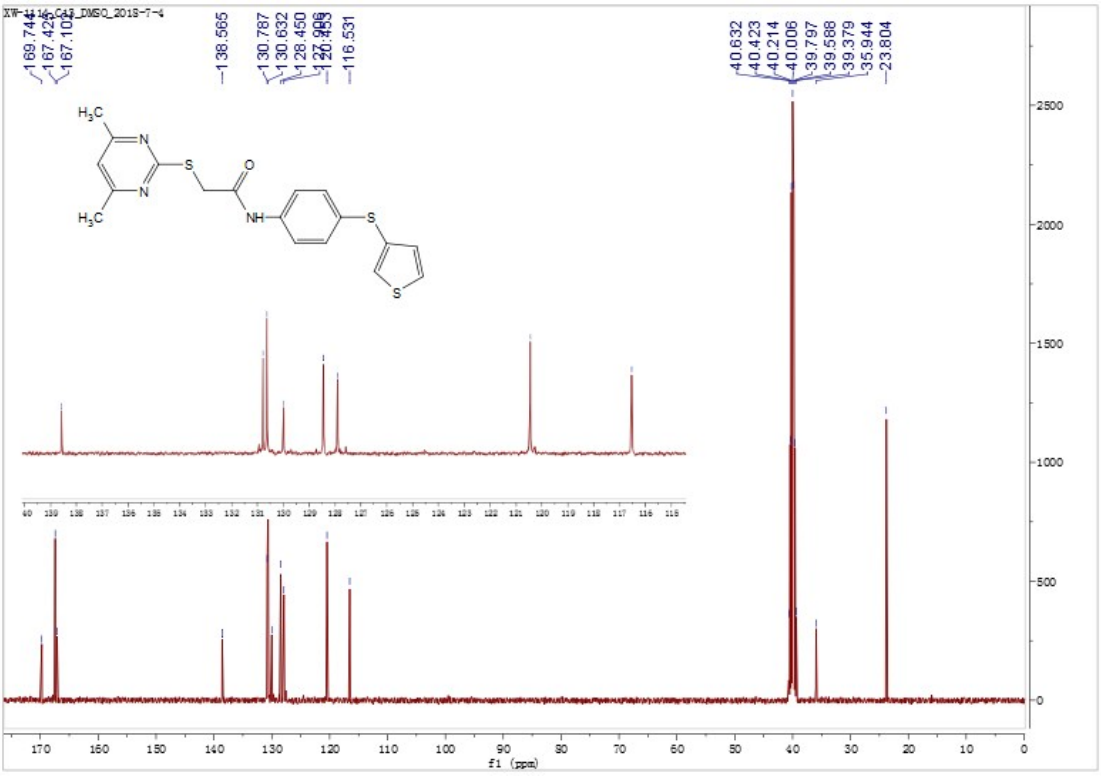


Fig. S17 ^{1}H, ^{13}C NMR, and mass spectra of **12a**.


10.321
7.671
7.664
7.659
7.651
7.591
7.588
7.582
7.560
7.244
7.223
7.036
7.033
7.024
7.021
6.952
4.034
3.343
2.502
2.322
0.000
1.01
0.90
2.62
1.83
0.85
0.94
2.00
6.16
f1 (ppm)


169.744
167.425
167.107
138.565
130.787
130.632
128.450
116.531
40.632
40.423
40.214
40.006
39.797
39.588
39.379
35.944
23.804
f1 (ppm)

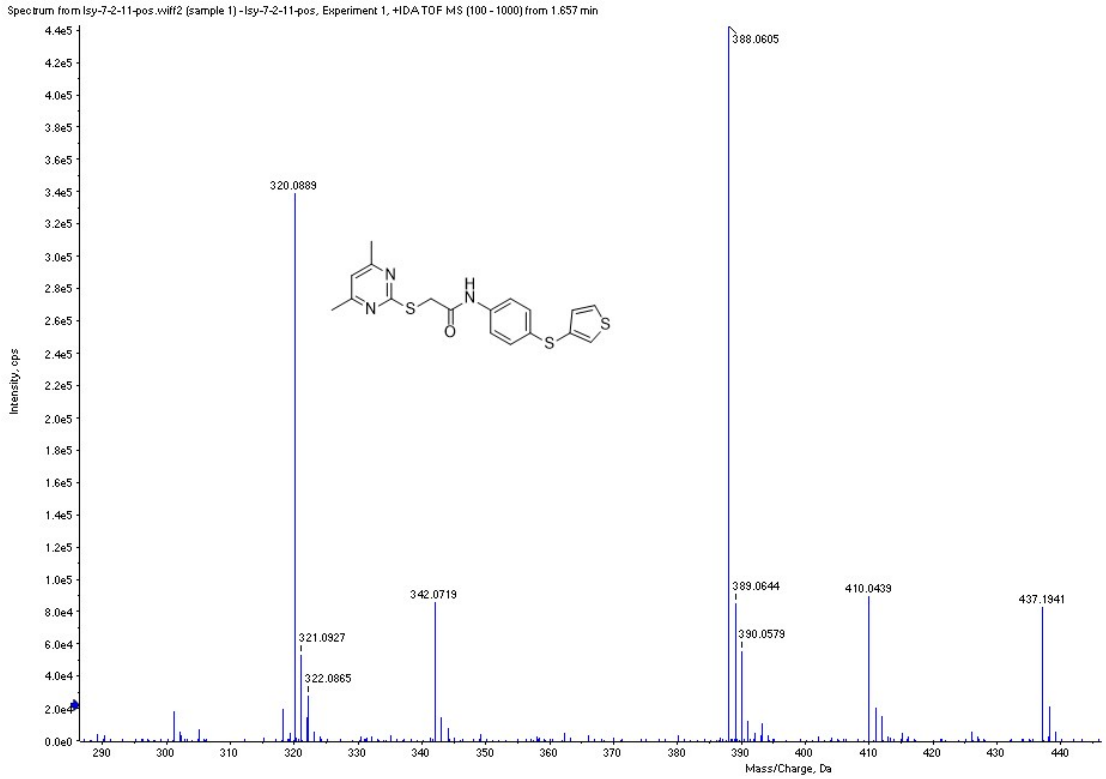
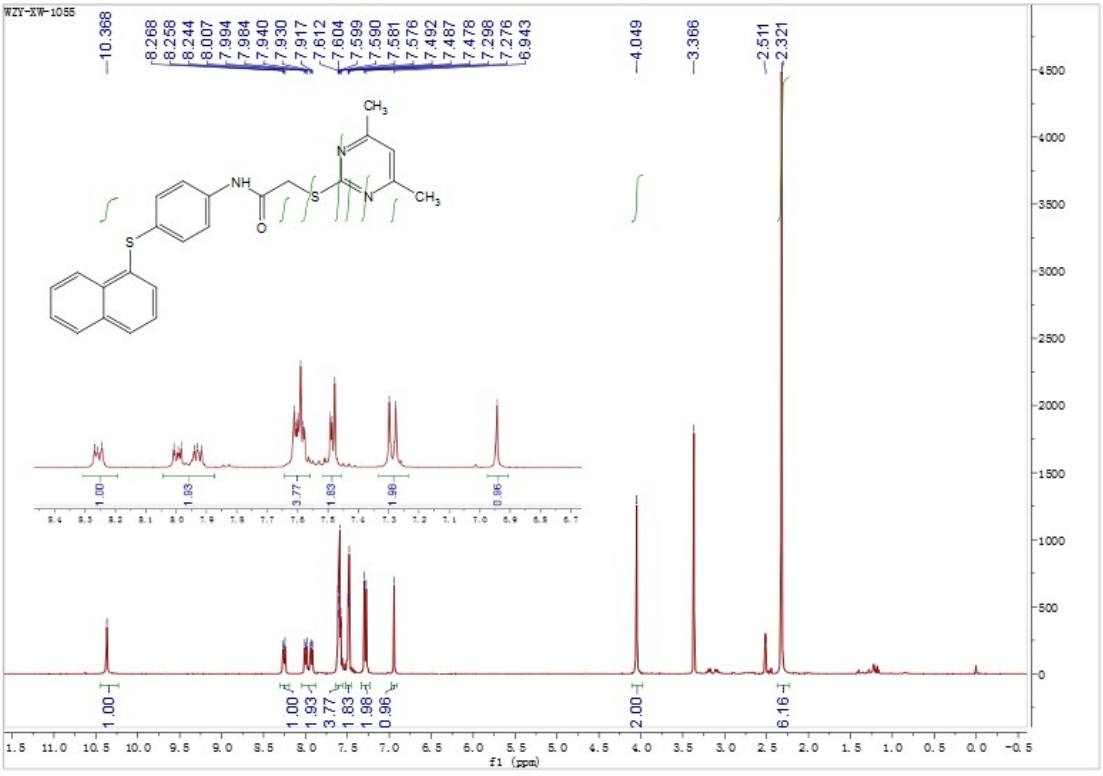


Fig. S18 ^{1}H, ^{13}C NMR, and mass spectra of **12b**.



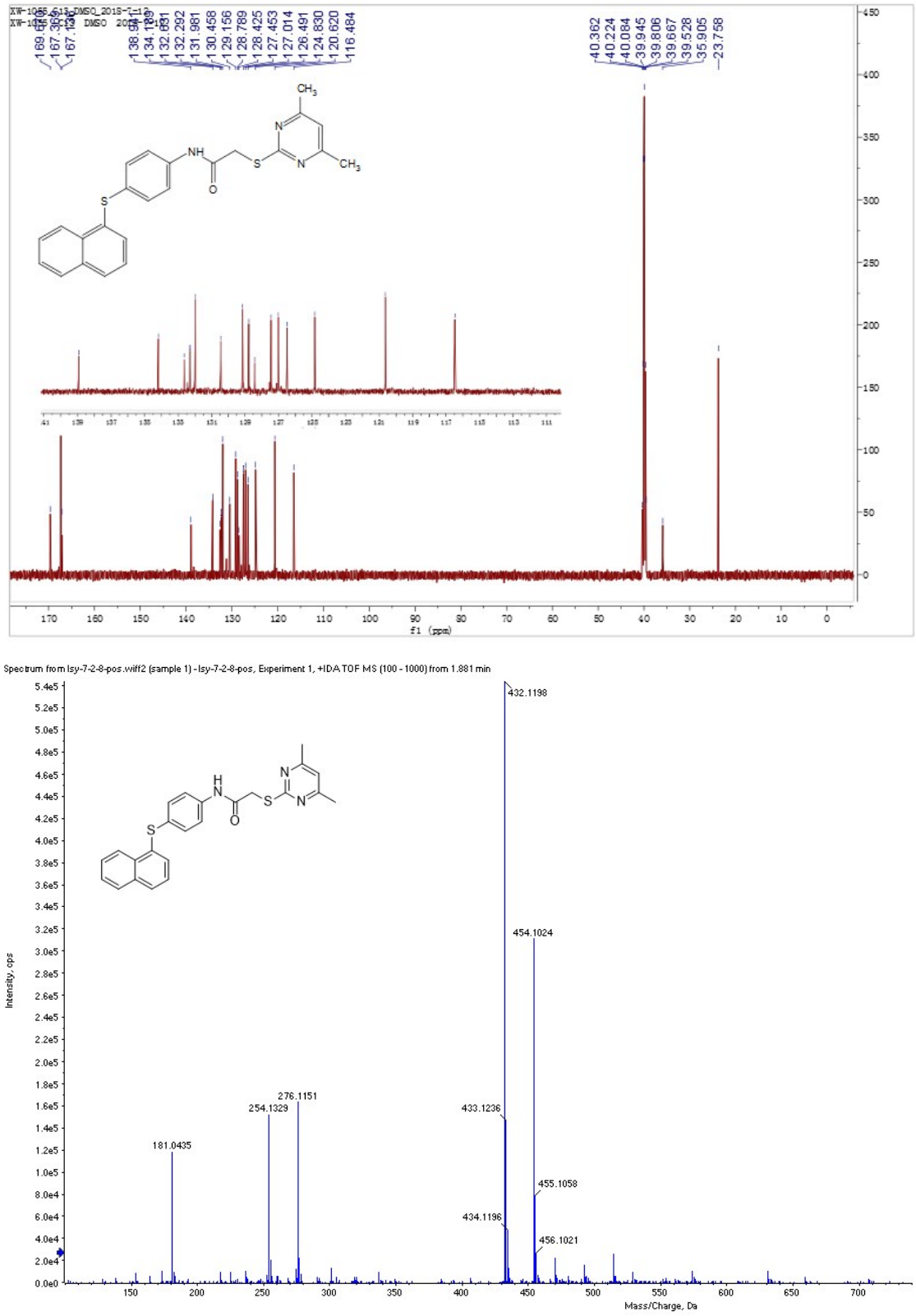
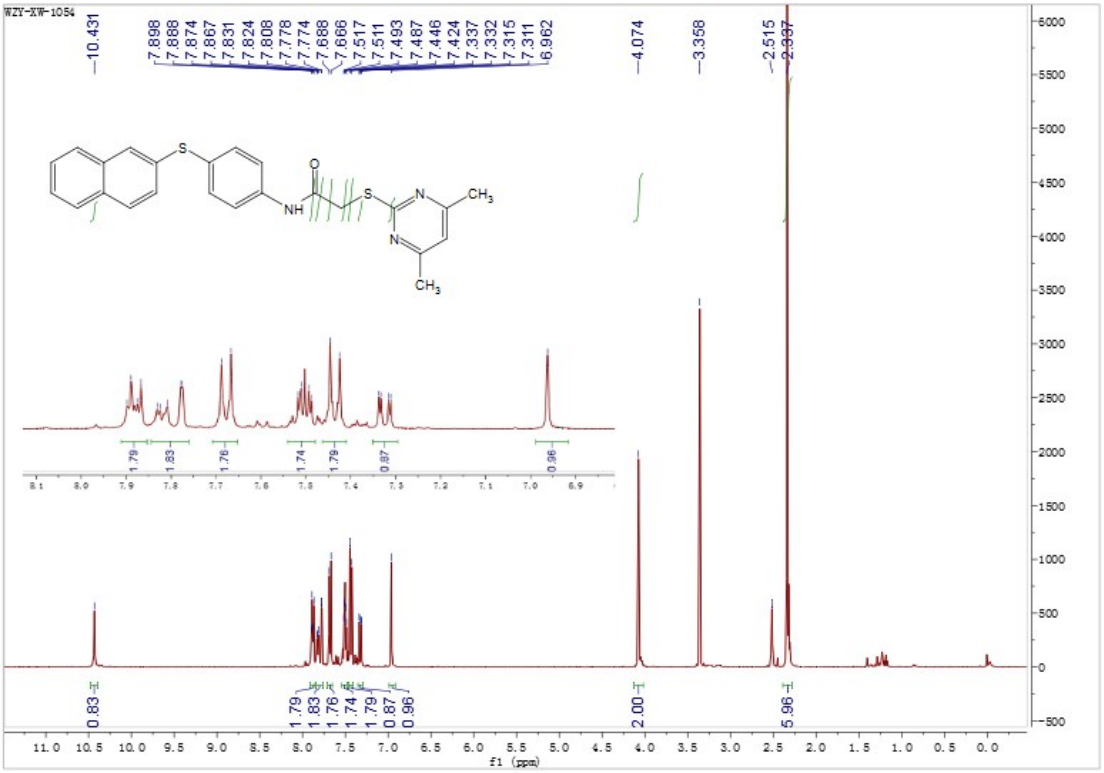
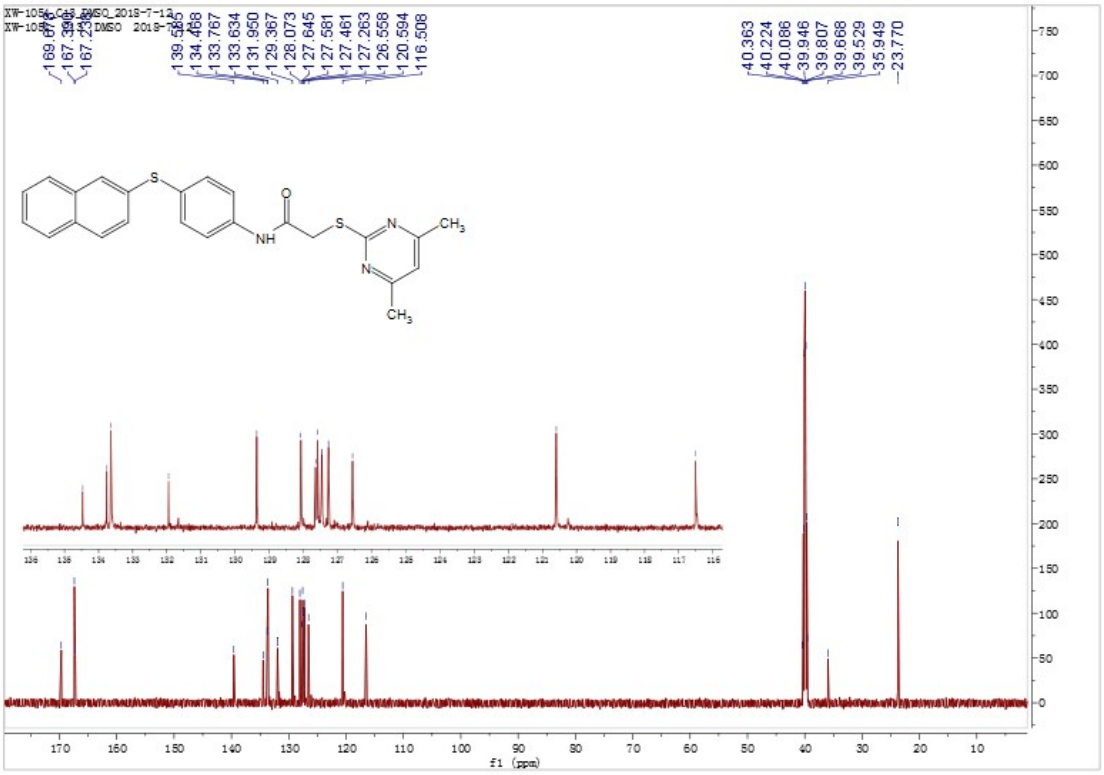


Fig. S19 ^{1}H, ^{13}C NMR, and mass spectra of **12c**.


WZY-XW-1054
10.431
7.898
7.888
7.874
7.867
7.831
7.824
7.808
7.778
7.774
7.688
7.666
7.517
7.511
7.493
7.487
7.446
7.424
7.337
7.332
7.315
7.311
6.962
4.074
3.358
2.515
2.337
0.83
1.79
1.83
1.76
1.74
1.79
0.87
0.96
2.00
5.96
f1 (ppm)


169.677
167.396
167.238
139.535
134.468
133.767
133.634
131.950
129.367
128.073
127.645
127.581
127.461
127.263
126.558
120.594
116.508
40.363
40.224
40.086
39.946
39.807
39.668
39.529
35.949
23.770
f1 (ppm)

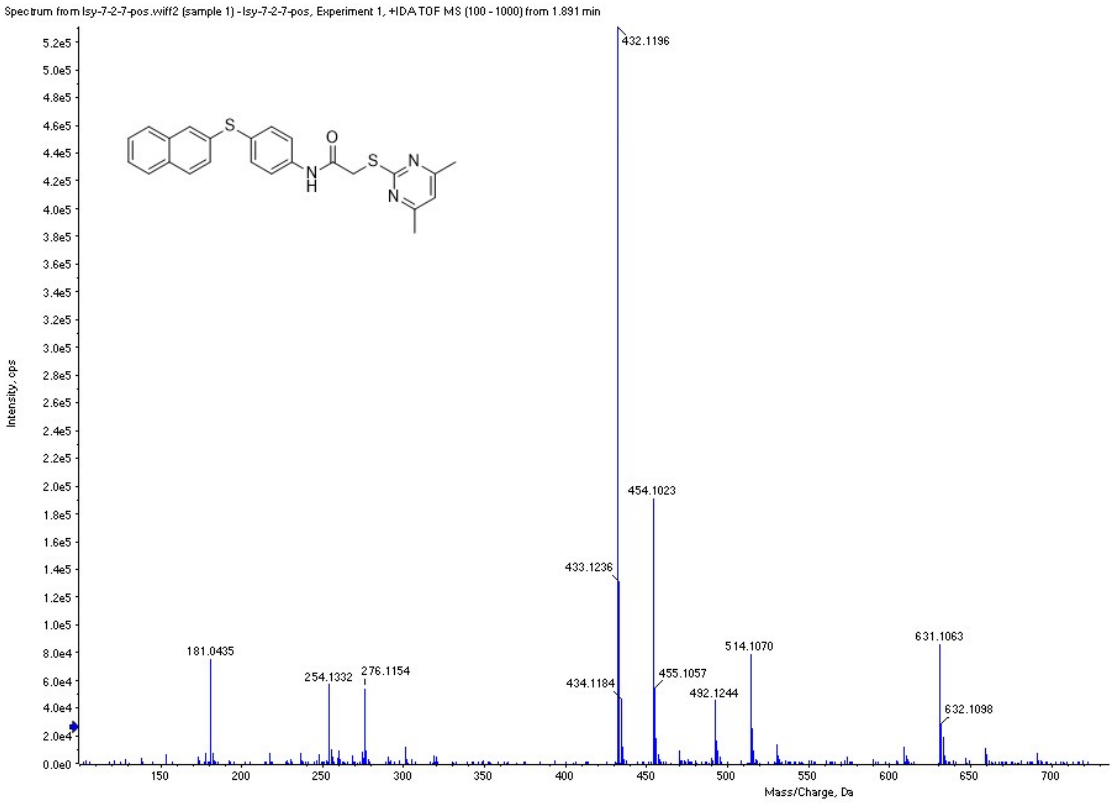
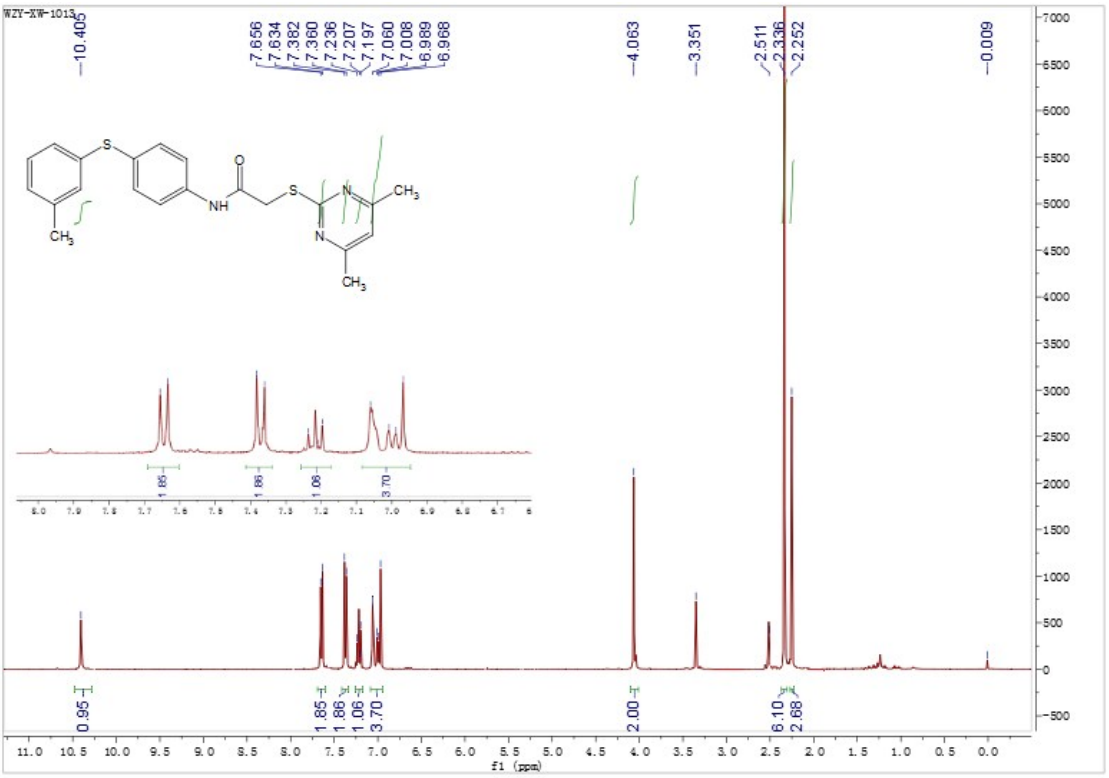
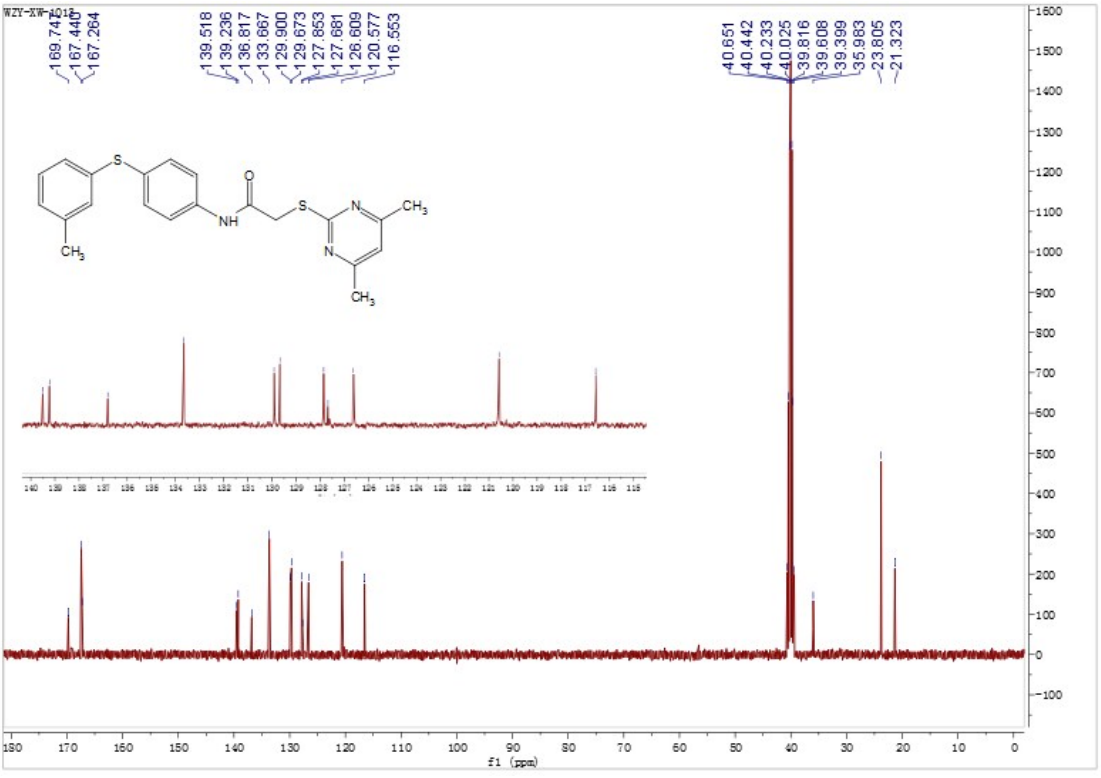


Fig. S20 ^{1}H, ^{13}C NMR, and mass spectra of **12d**.


WZY-XW-1013
10.405
7.656
7.634
7.382
7.360
7.236
7.207
7.197
7.060
7.008
6.989
6.968
4.063
3.351
2.511
2.336
2.252
0.009
S
NH
O
S
N
N
CH3
CH3
CH3
0.95
1.85
1.86
1.06
3.70
2.00
6.10
2.68
f1 (ppm)


169.742
167.440
167.264
139.518
139.236
136.817
133.667
129.900
129.673
127.853
127.681
126.609
120.577
116.553
40.651
40.442
40.233
40.025
39.816
39.608
39.399
35.983
23.805
21.323
S
NH
O
S
N
N
CH3
CH3
CH3
f1 (ppm)

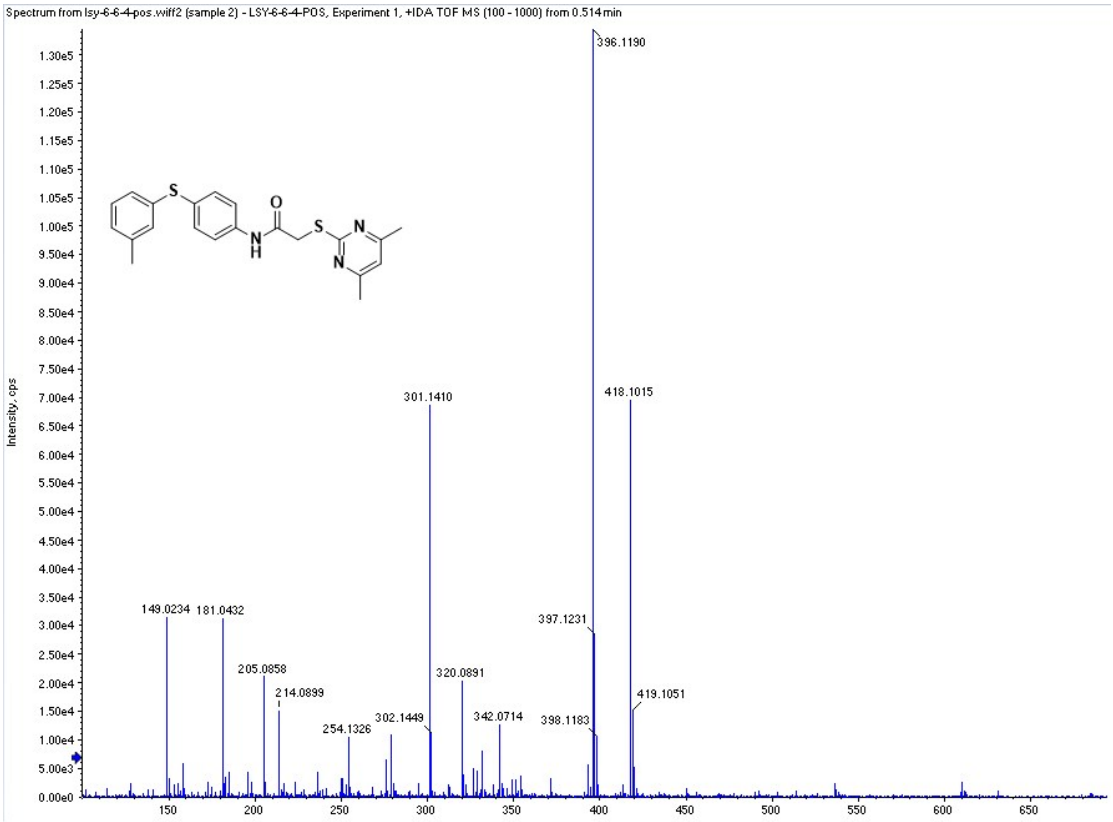
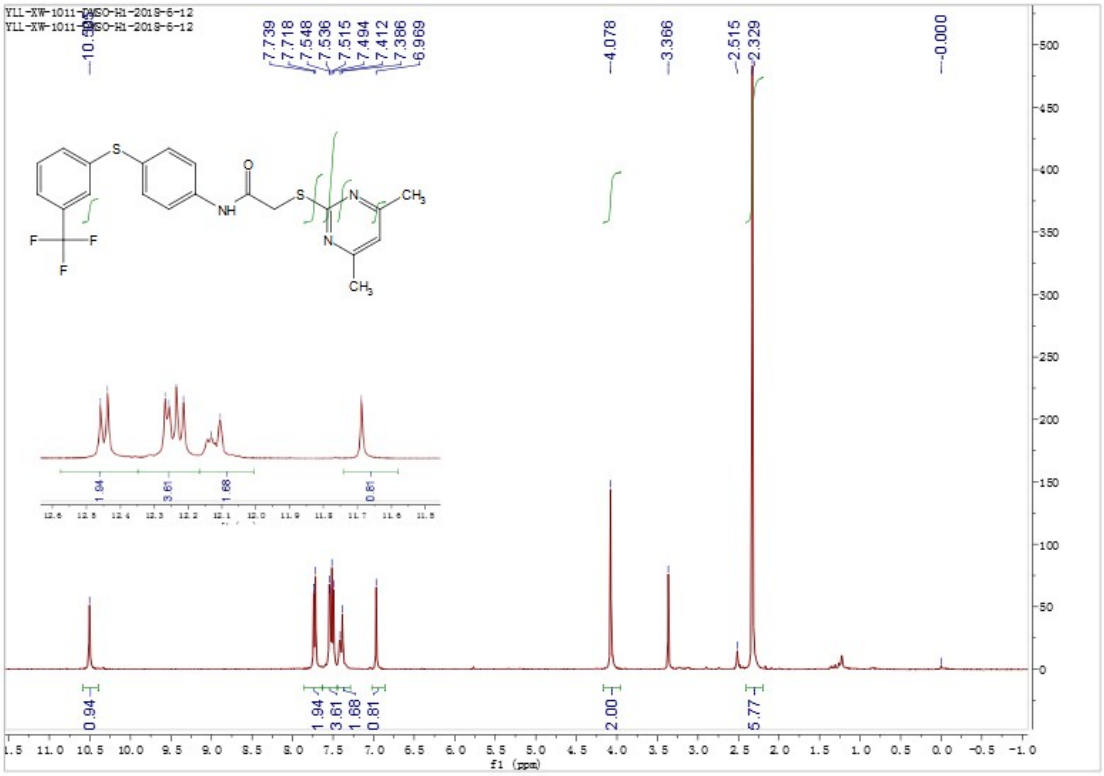


Fig. S21 ^{1}H, ^{13}C NMR, and mass spectra of **12e**.



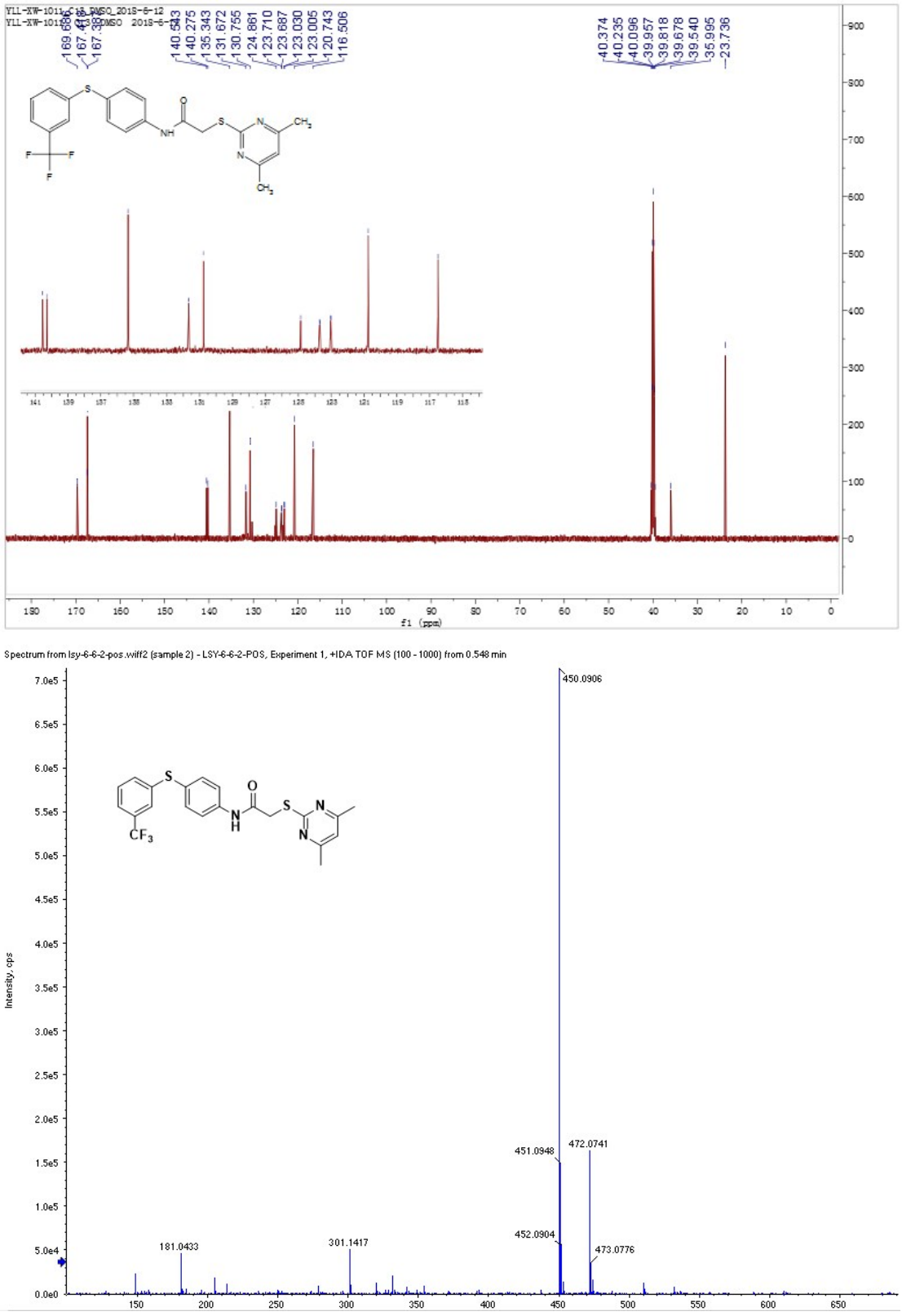
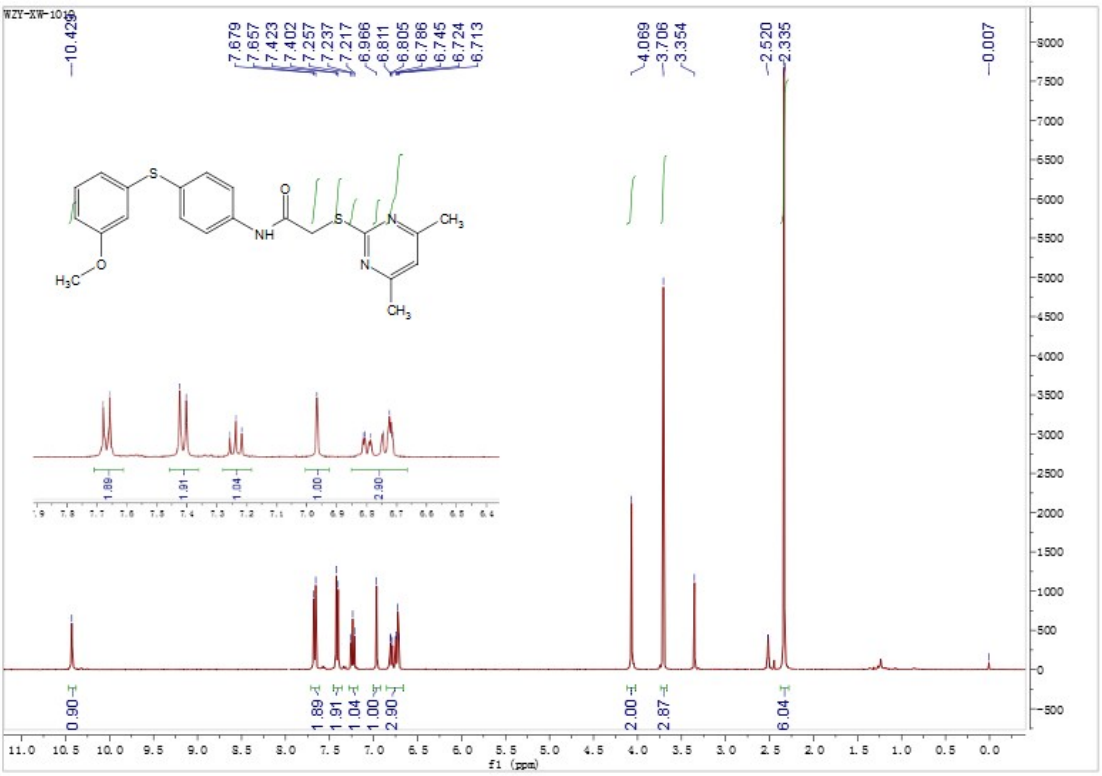
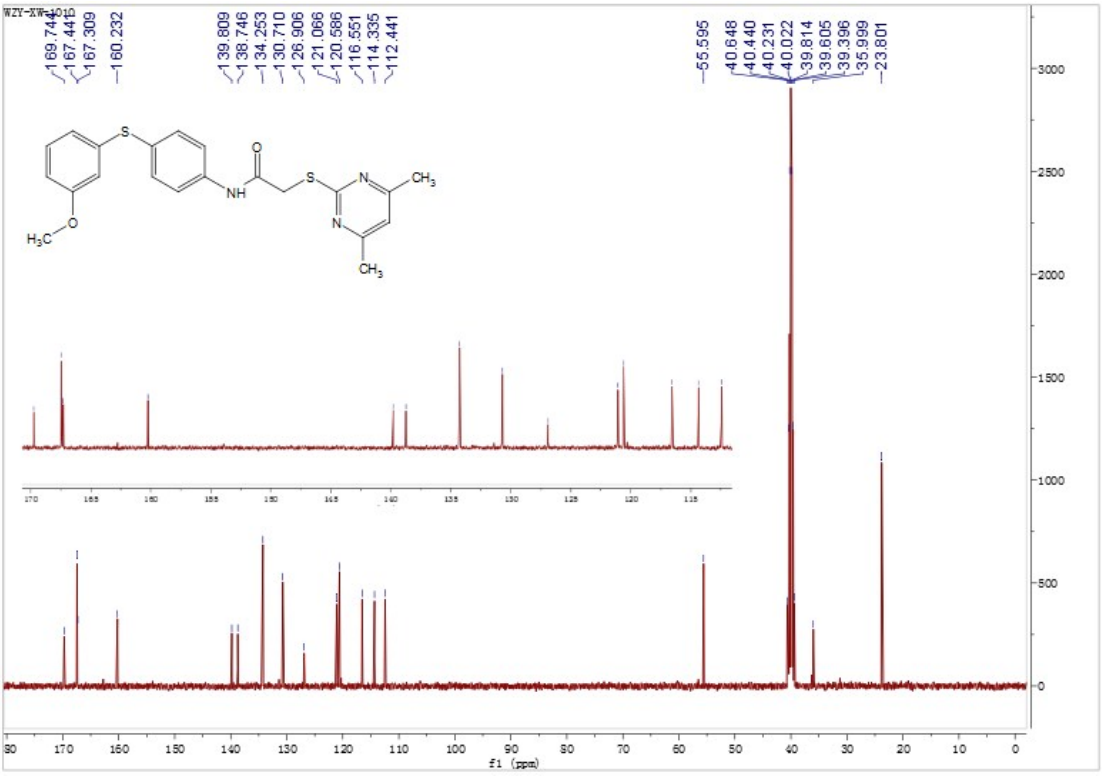


Fig. S22 ^{1}H, ^{13}C NMR, and mass spectra of **12f**.


WZY-XW-1010
10.429
7.679
7.657
7.423
7.402
7.257
7.237
7.217
6.966
6.811
6.805
6.786
6.745
6.724
6.713
4.069
3.706
3.354
2.520
2.335
0.007
S
O
NH
S
N
CH3
N
CH3
H3C
O
1.89
1.91
1.04
1.00
2.90
0.90
1.89
1.91
1.04
1.00
2.90
2.00
2.87
6.04
f1 (ppm)


WZY-XW-1010
169.744
167.441
167.309
160.232
139.809
138.746
134.253
130.710
126.906
121.066
120.586
116.551
114.335
112.441
55.595
40.648
40.440
40.231
40.022
39.814
39.605
39.396
35.999
23.801
S
O
NH
S
N
CH3
N
CH3
H3C
O
f1 (ppm)

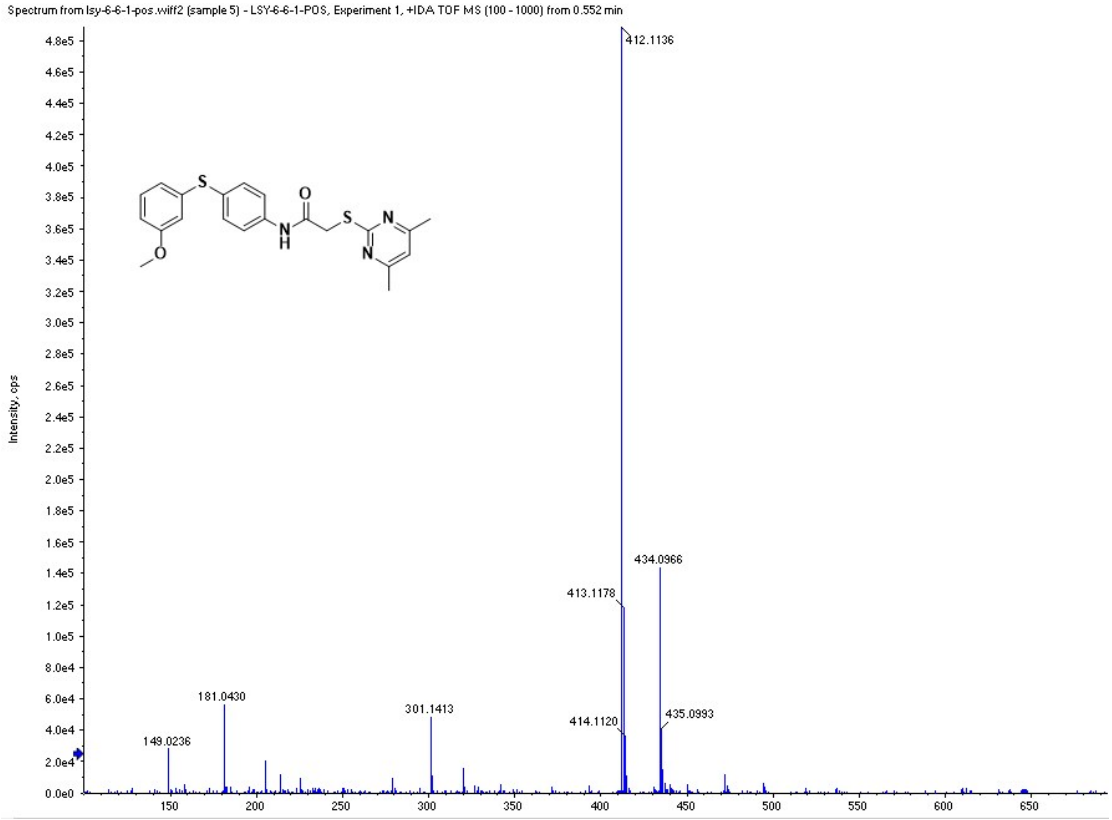
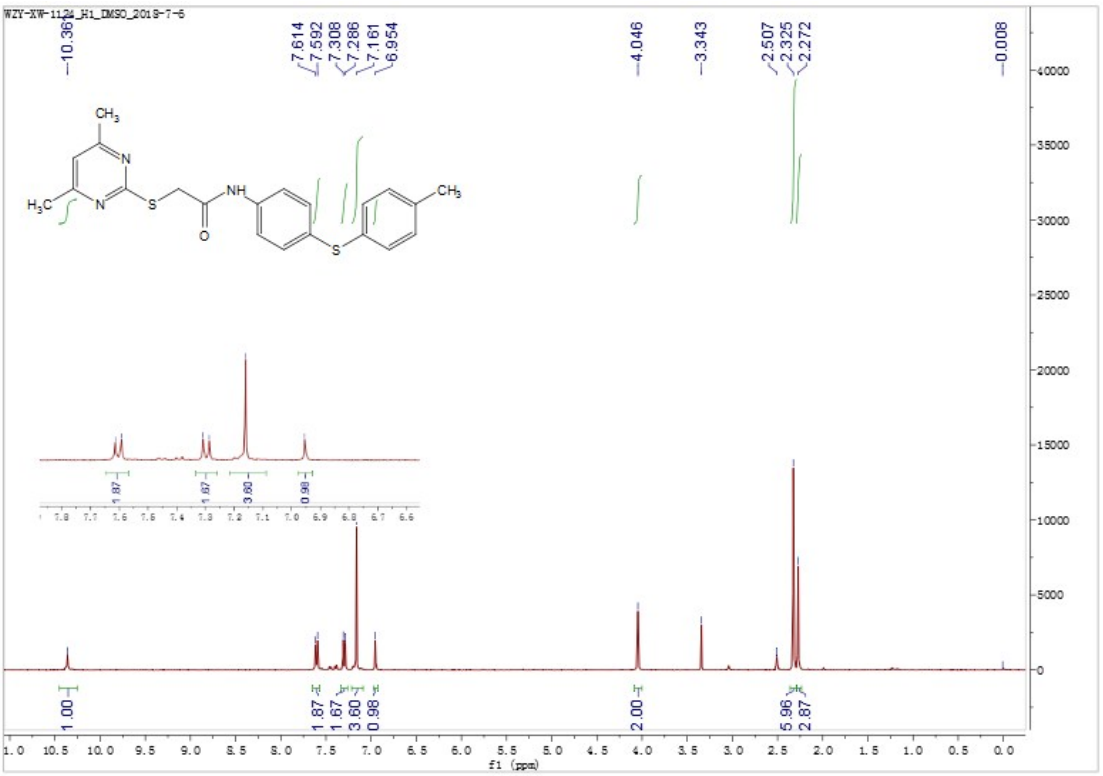
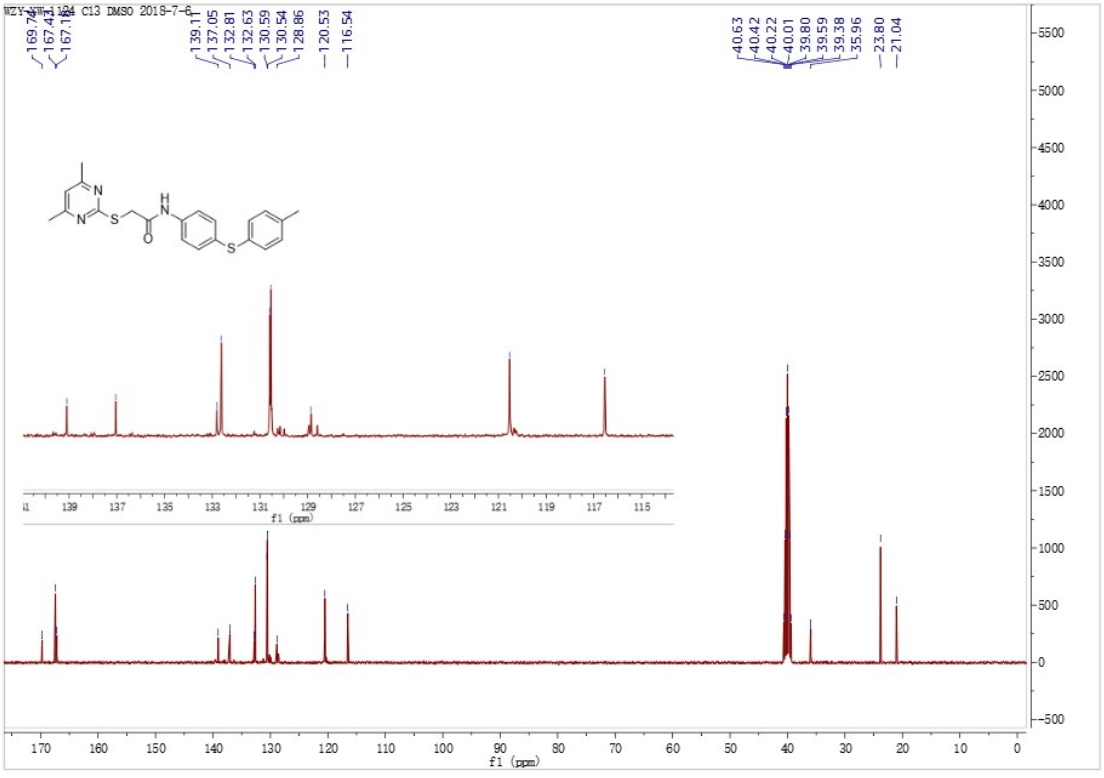


Fig. S23 ^{1}H, ^{13}C NMR, and mass spectra of **12g**.


WZY-XW-1124_H1_DMSO_2018-7-6
10.36
7.614
7.592
7.308
7.286
7.161
6.954
4.046
3.343
2.507
2.325
2.272
0.008
1.87
1.67
3.60
0.98
1.00
2.00
5.96
2.87
f1 (ppm)


169.74
167.41
167.19
139.11
137.05
132.81
132.63
130.59
130.54
128.86
120.53
116.54
40.63
40.42
40.22
40.01
39.80
39.59
39.38
35.96
23.80
21.04
f1 (ppm)

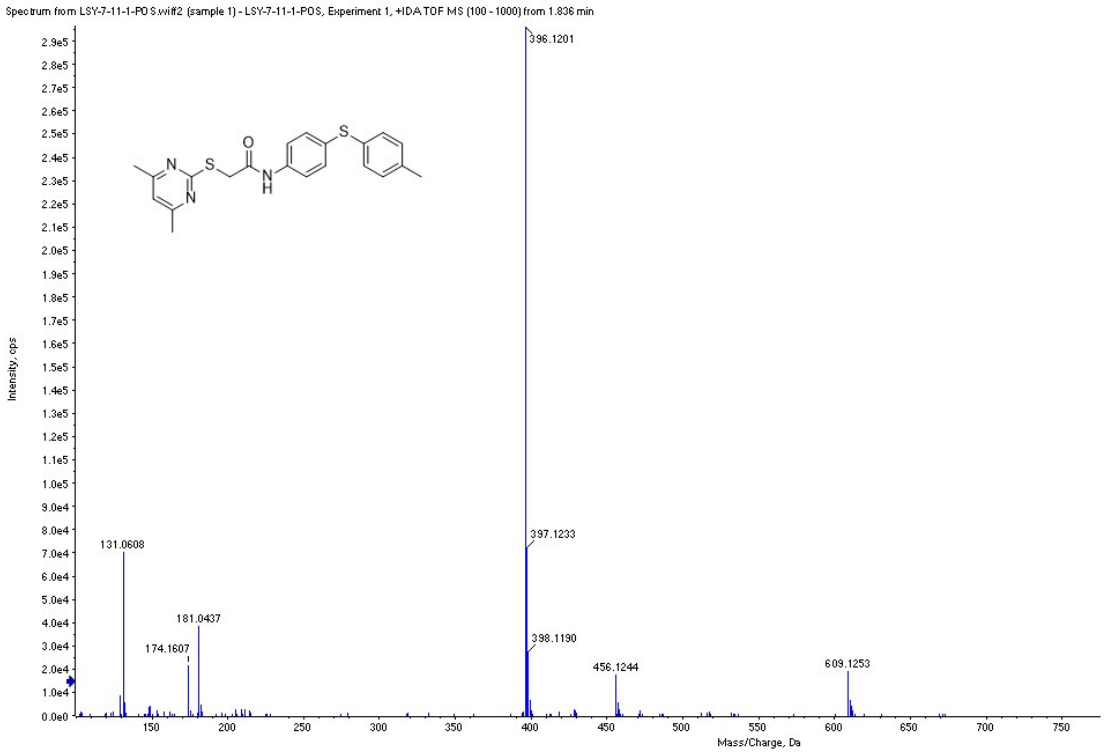
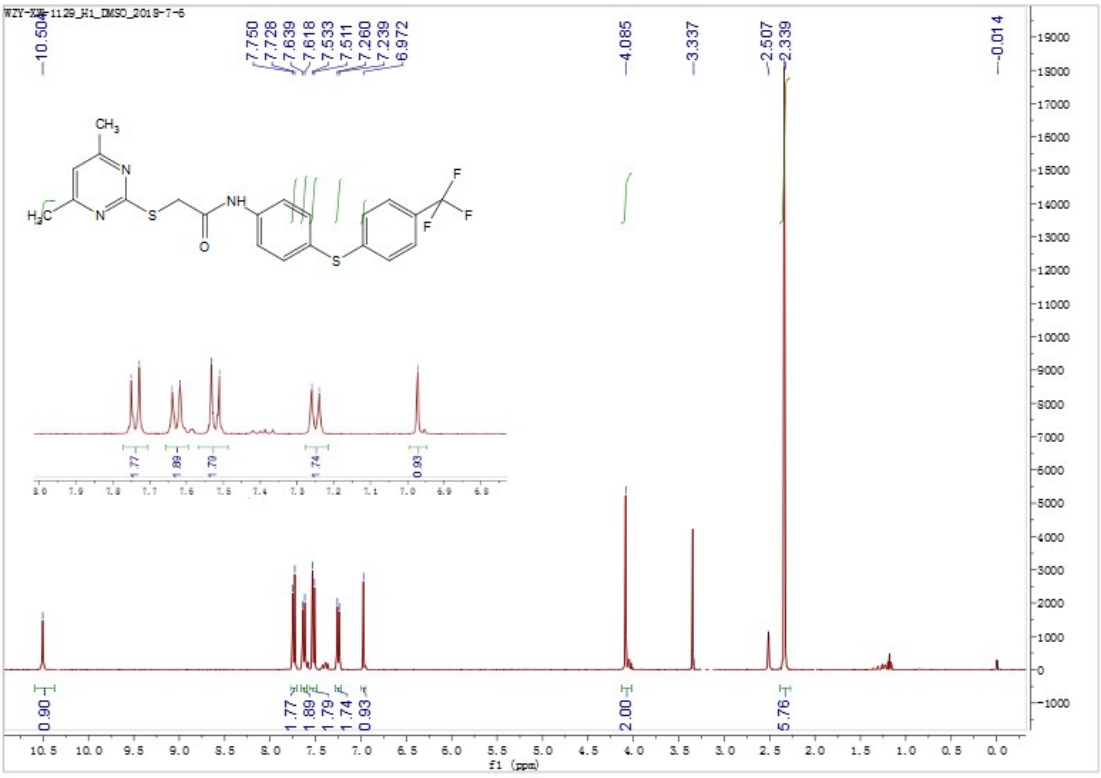


Fig. S24 ^{1}H, ^{13}C NMR, and mass spectra of **12h**.



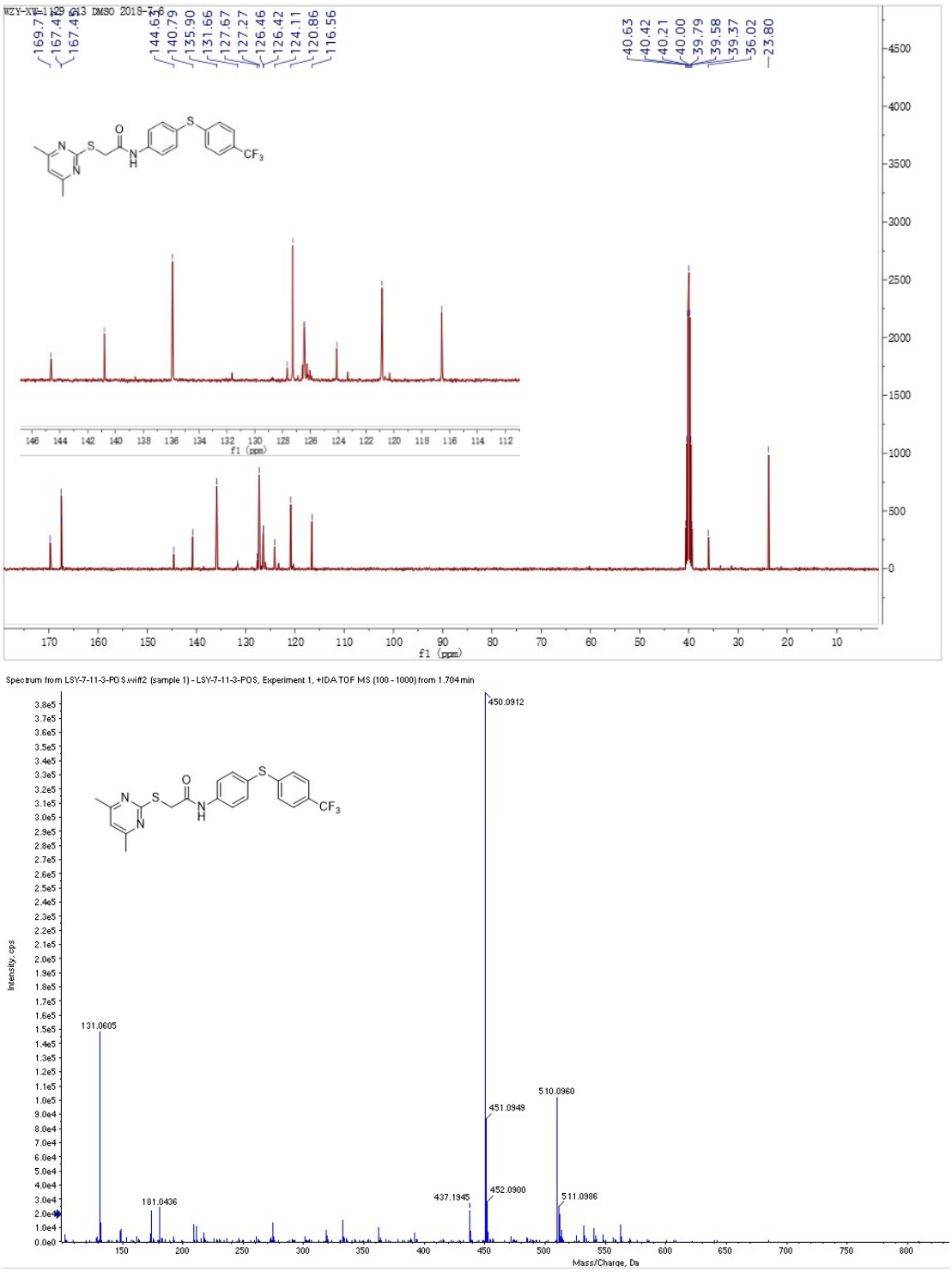
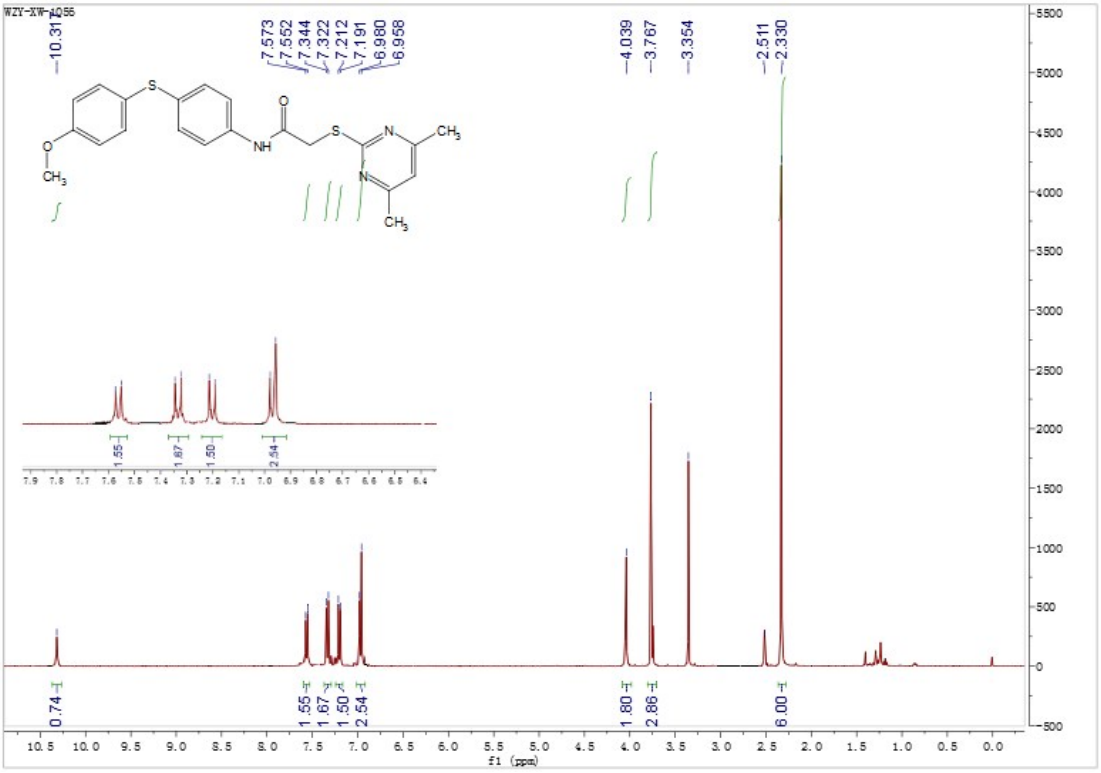
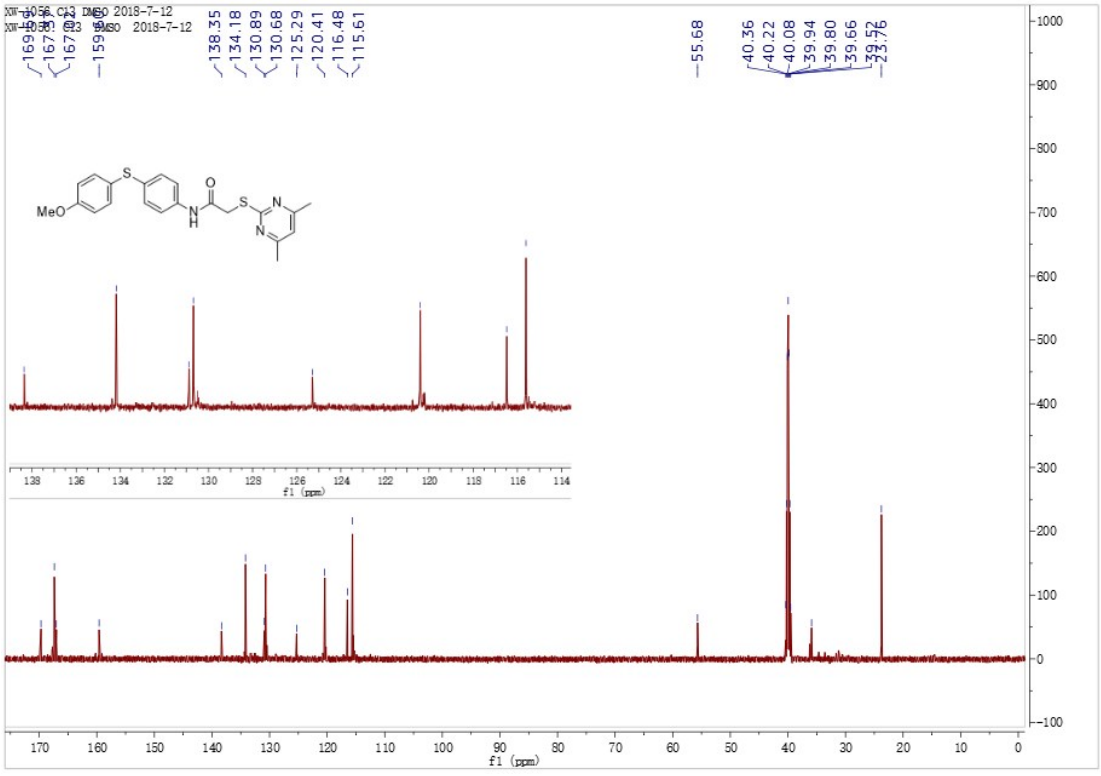


Fig. S25 ^{1}H, ^{13}C NMR, and mass spectra of **12i**.


WZY-XW-A056
10.31
7.573
7.552
7.344
7.322
7.212
7.191
6.980
6.958
4.039
3.767
3.354
2.511
2.330
0.74
1.55
1.67
1.50
2.54
1.80
2.86
6.00
f1 (ppm)


XW-A056-C13 DMSO 2018-7-12
169.38
167.23
159.45
138.35
134.18
130.89
130.68
125.29
120.41
116.48
115.61
55.68
40.36
40.22
40.08
39.94
39.80
39.66
39.52
23.76
MeO
f1 (ppm)

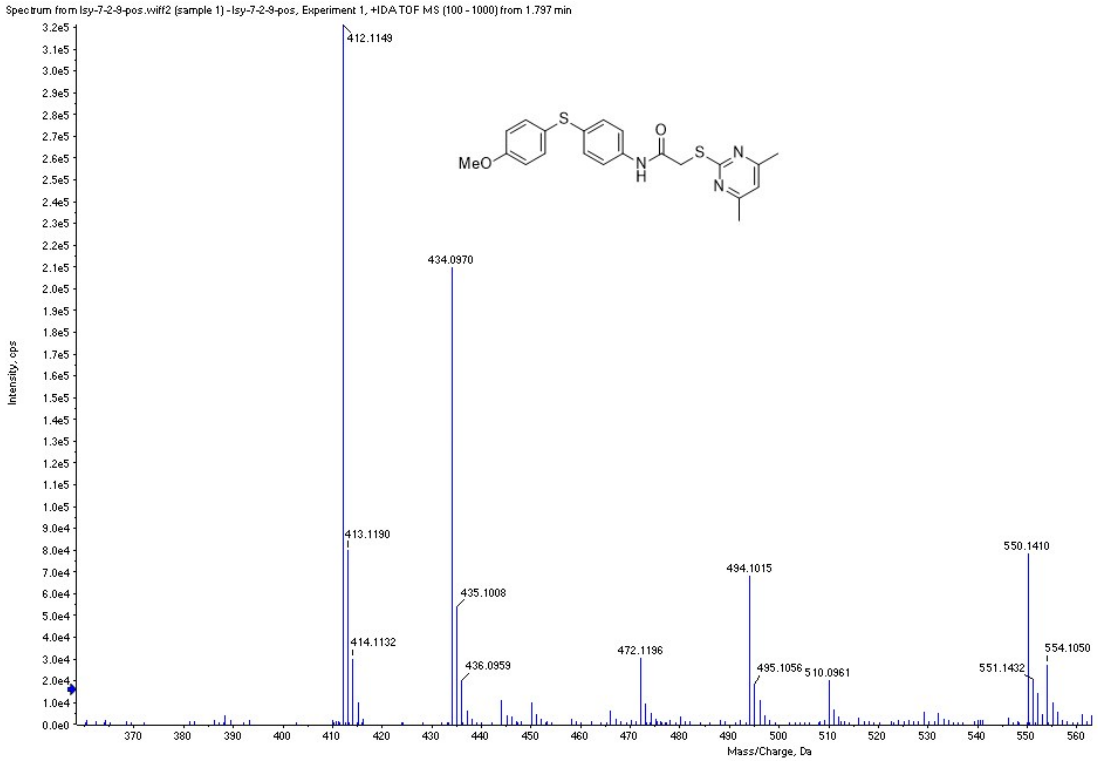
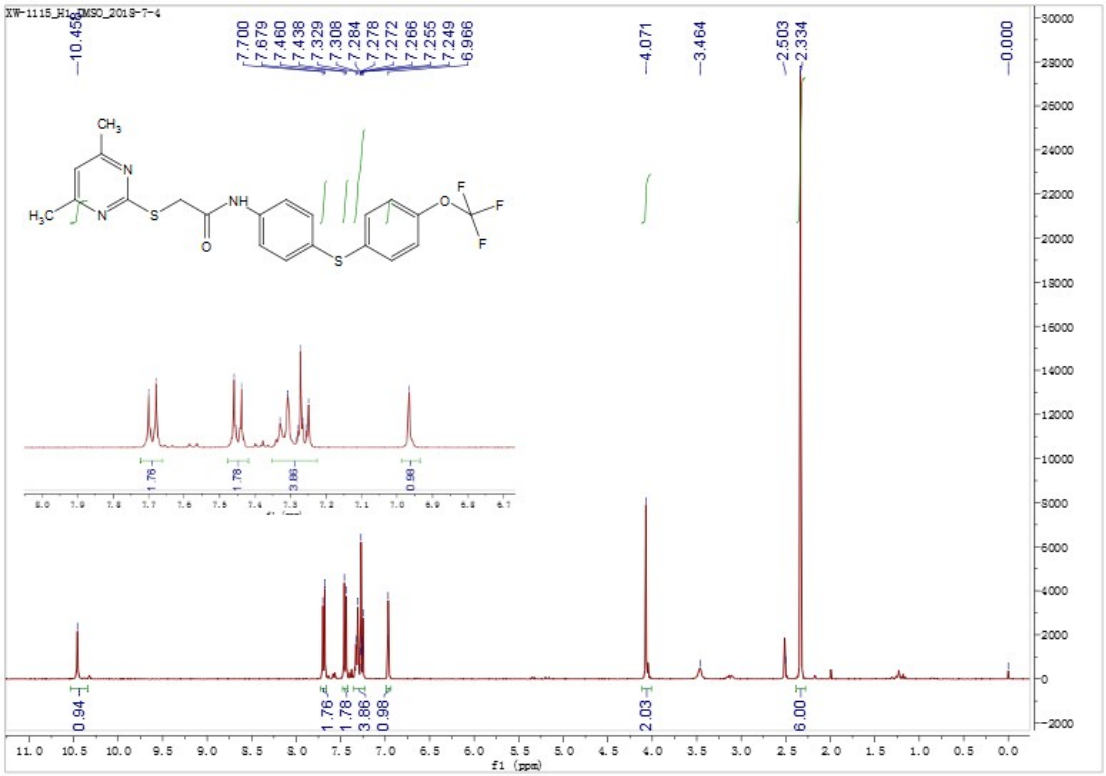
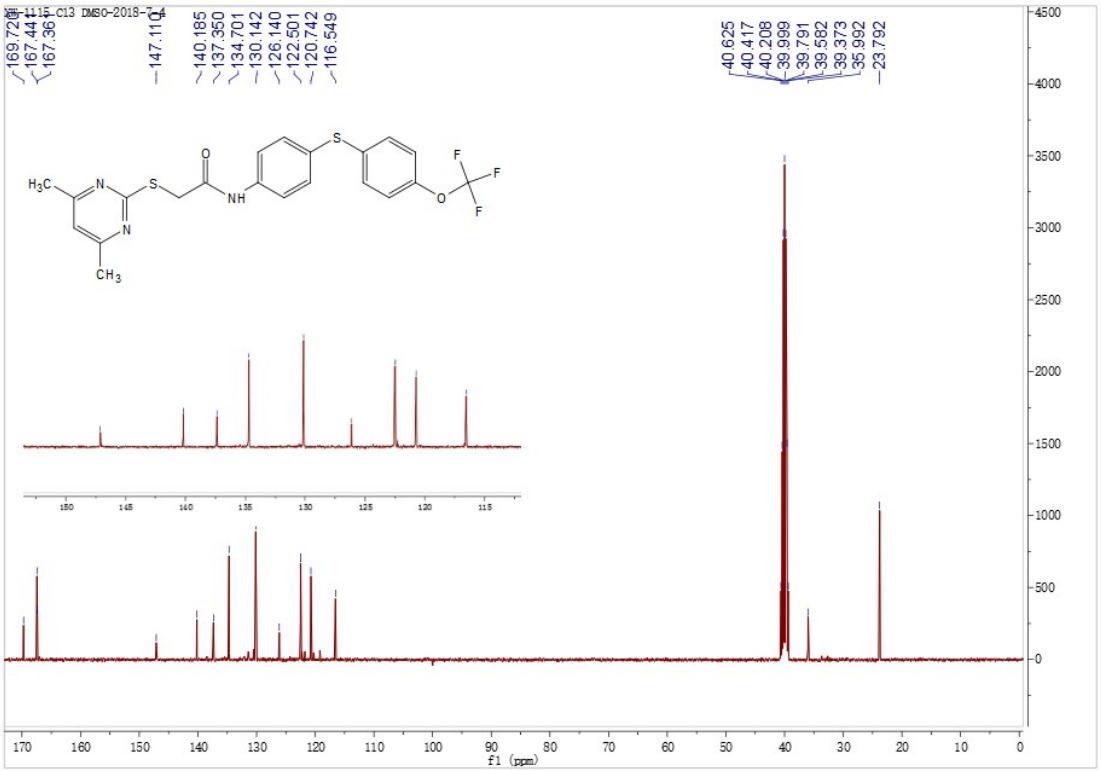


Fig. S26 ^{1}H, ^{13}C NMR, and mass spectra of **12j**.





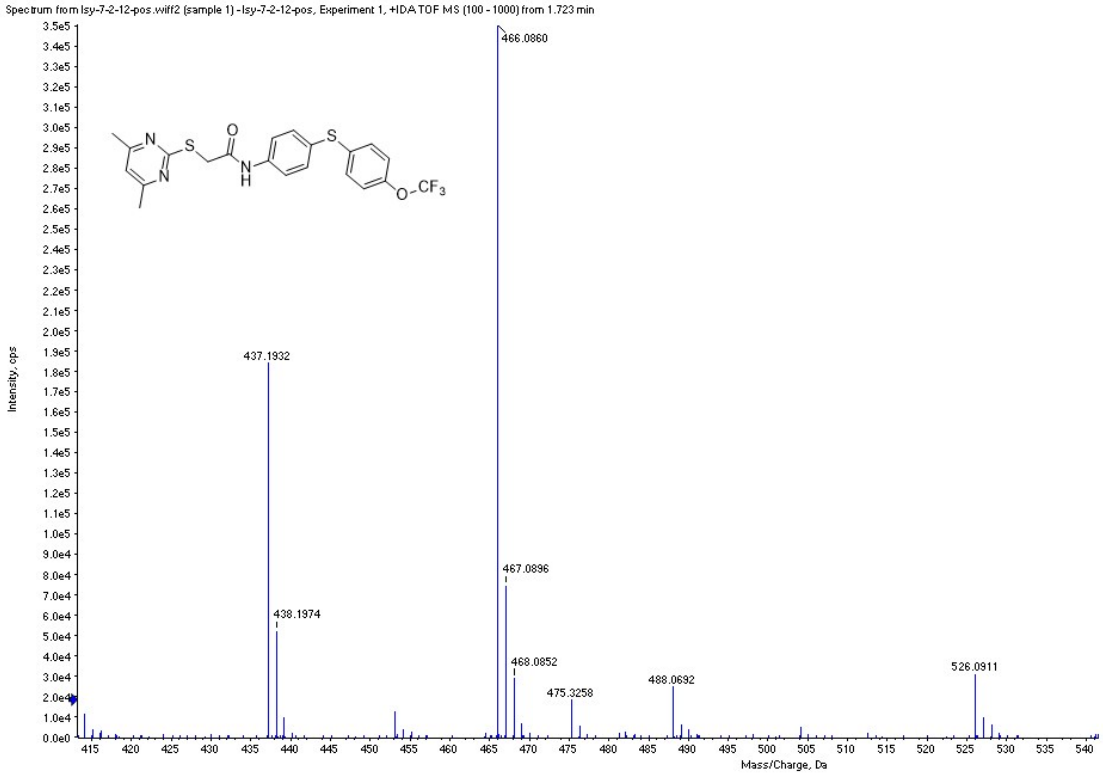
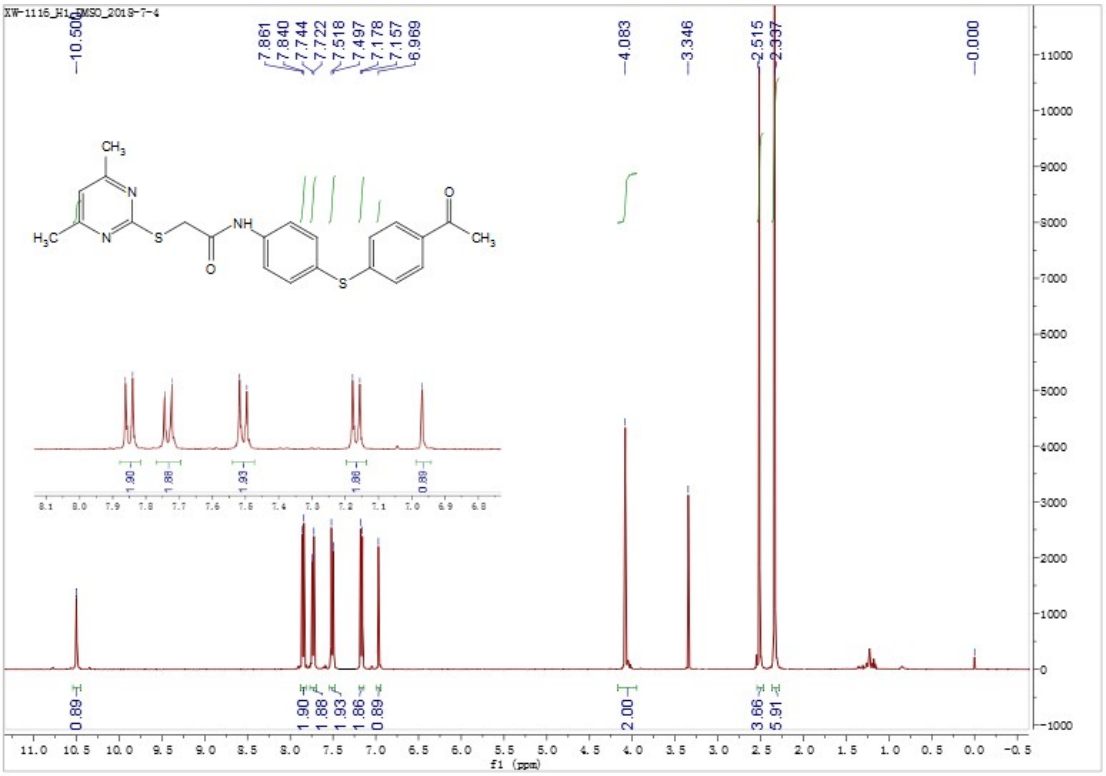
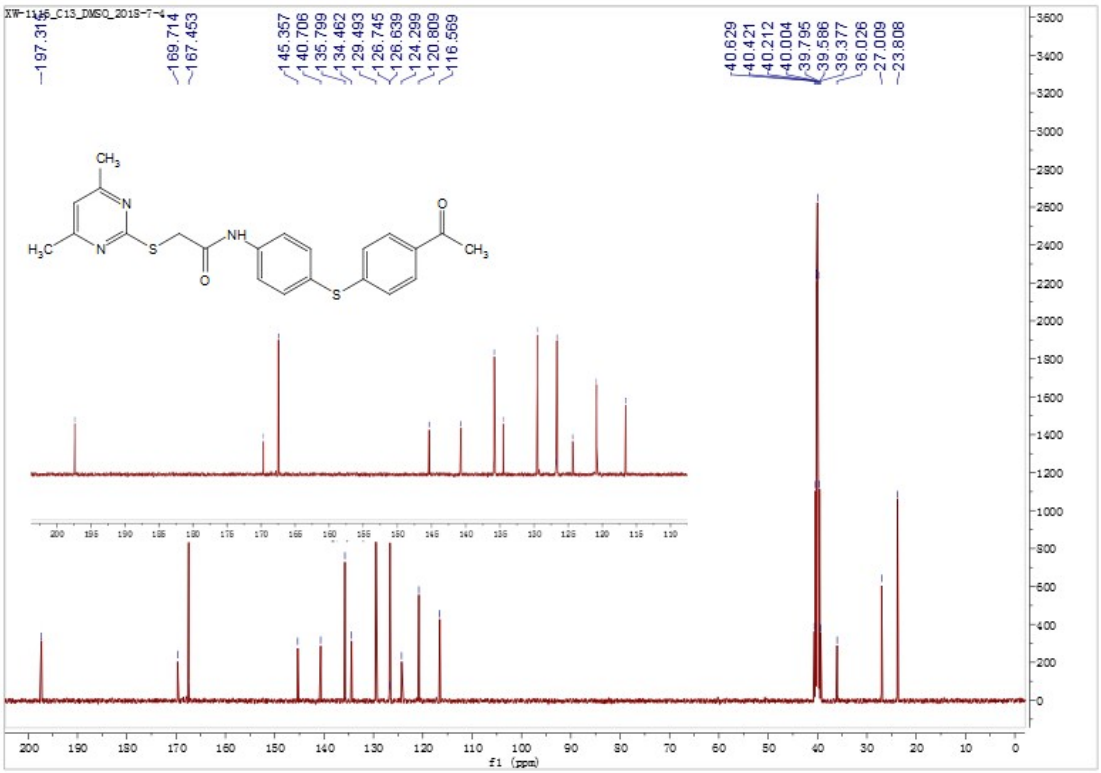


Fig. S27 ^{1}H, ^{13}C NMR, and mass spectra of **12k**.


10.506
7.861
7.840
7.744
7.722
7.518
7.497
7.178
7.157
6.969
4.083
3.346
2.515
2.337
0.000
0.89
1.90
1.88
1.93
1.86
0.89
2.00
3.66
5.91
f1 (ppm)


197.315
169.714
167.453
145.357
140.706
135.799
134.462
129.493
126.745
126.639
124.299
120.809
116.569
40.629
40.421
40.212
40.004
39.795
39.586
39.377
36.026
27.009
23.808
f1 (ppm)

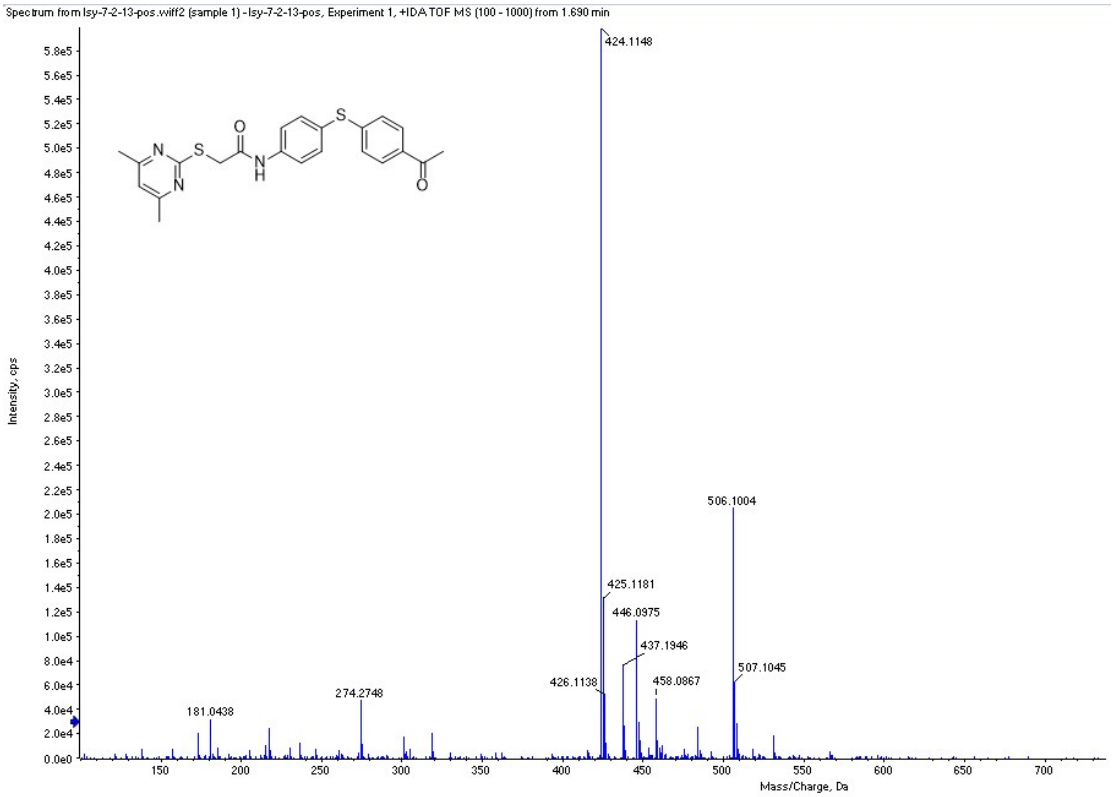


Fig. S28 ^{1}H, ^{13}C NMR, and mass spectra of **12l**.

Supplementary Tables

Table S1. Crystal growth conditions.

Sample	Method	Sample composition	Crystallization condition	Vapor diffusion condition
SIRT2:**1a**	Co-crystallization	13 mg/mL SIRT2 proteins, 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM Compound **1a**	0.1 M HEPES pH7.5, 20% PEG8000	Hanging drop, protein-to-well ratio, 1:1, 277K

Table S2. Data collection and refinement statistics.

Structure (PDB ID)	SIRT2:**1a** (5YQM)
Data Collection	
Space Group	P 1 21 1
Cell Dimensions: *a*, *b*, *c* (Å)	35.748, 73.952, 55.947
Cell Dimensions: α, β, γ (˚)	90.000, 94.963, 90.000
*Mol/ASU	1
Resolution Range (outer shell) (Å)	50-1.75 (1.79-1.75)
Number of Unique Reflections	29513
Completeness (%)	99.0
I/σ(I) (outer shell)	9.0 (2.5)
Wilson B Factor (Å^2)	18.6
Refinement	
Overall B Factor (Å^2)	25
Protein B Factor (Å^2)	21
Ligand B Factor (Å^2) (occupancy)	25 (1.00)
Water B Factor (Å^2)	52.5
‡RMSD from Ideal Bond Length (Å)	0.007
‡RMSD from Ideal Angles (˚)	1.306
R_{work} (%)	17.60
R_{free} (%)	21.48

*Mol/ASU = molecules per asymmetric unit; ‡RMSD = root mean square deviation.

Reference

1 L.-L. Yang, H.-L. Wang, L. Zhong, C. Yuan, S.-Y. Liu, Z.-J. Yu, S. Liu, Y.-H. Yan, C. Wu, Y. Wang, Z. Wang, Y. Yu, Q. Chen and G.-B. Li, *Eur. J. Med. Chem.*, 2018, **155**, 806-823.

2 A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J Appl. Crystallogr.*, 2007, **40**, 658-674.

3 P. D. Adams, P. V. Afonine, G. Bunkoczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart, *Acta Crystallogr. D Biol. Crystallogr.*, 2010, **66**, 213-221.

4 P. Emsley and K. Cowtan, *Acta Crystallogr. D Biol. Crystallogr.*, 2004, **60**, 2126-2132.

5 W. F. Hawse and C. Wolberger, *J. Biol. Chem.*, 2009, **284**, 33654-33661.

6 J. L. Feldman, K. E. Dittenhafer-Reed, N. Kudo, J. N. Thelen, A. Ito, M. Yoshida and J. M. Denu, *Biochemistry*, 2015, **54**, 3037-3050.

7 T. Rumpf, M. Schiedel, B. Karaman, C. Roessler, B. J. North, A. Lehotzky, J. Olah, K. I. Ladwein, K. Schmidtkunz, M. Gajer, M. Pannek, C. Steegborn, D. A. Sinclair, S. Gerhardt, J. Ovadi, M. Schutkowski, W. Sippl, O. Einsle and M. Jung, *Nat. Commun.*, 2015, **6**, 6263.

8 O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455-461.