Supporting Information

Optimization of Chromeno[2,3-c]pyrrol-9(2H)-ones as Highly Potent, Selective, and Orally Bioavailable PDE5 Inhibitors: Structure-Activity Relationship, X-ray Crystal Structure, and Pharmacodynamic Effect on Pulmonary Arterial Hypertension

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Crystallization, data collection and analysis.

PDE5A was concentrated to 10 mg /mL for crystallization trials. The storage buffer is 20 mM Tris 7.5, 50mM NaCl, 1 mM EDTA and 1 mM B-Me. Attempts to grow complex PDE5A crystals in the presence of compound **2** were unsuccessful. We therefore used a soaking method. The precipitant solution was made of 0.2 M $MgSO_4$, 0.1 M Cacodylate sodium salt, pH 6.5, 2.5% ethanol, and 18% PEG3350. and crystals appeared within three days at 25°C. The data collection, process and refinement were carried as we reported previously.[1] 3D structure of PDE5A in complex with **11b** and **17c** were built at the resolution of 2.6 Å and 2.8 Å, respectively. The coordinates and structure factors have been deposited in the Protein Data Bank with PDB ID 5ZZ2 and 6ACB. Data collection and refinement statistics for all structures are shown in Table S1.

Table S1. Diffraction data and structure refinement statistic for PDE5A-11b and PDE5A-17c structures

Data collection	PDE5A-**11b**	PDE5A-**17c**
Wavelength (Å)	1.5418	1.5418
Temperature (K)	100	100
Resolution (Å)	24.73-2.60	24.00-2.80
Space group	P 3_1 2 1	P 3_1 2 1
Unit Cell		
a, b, c (Å)	74.4739,74.4739,132.341	74.577,74.577,132.09
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00
No. reflections	102197(13486)	82853(10461)
Completeness (%)	99.29(98.12)	95.74(95.37)
R_{merge}	0.068(0.336)	0.132(0.441)
$<I/\sigma(I)>$	27.7(4.1)	13.96(3.94)
Redundancy	7.6(4.84)	7.84(8.06)
Structure refinement		
R-factor/R-free	0.21/0.26	0.19/0.27
RMS deviations		
Bond lengths	0.0078 Å	0.0113 Å
Bond angles	1.1578	1.5096
Average B-factor (Å^2)(atoms)		
Protein	47.7(2418)	43.1(2499)
Inhibitor	99.2(29)	59.8(40)
Zn	44.2(1)	30.76(1)
Mg	29.34(1)	23.08(1)
Waters	33.8(41)	26.0(45)

Ramachandran plot		
Preferred	96.59%	93.42%
Allowed	3.41%	4.61%

General Procedures for Bioassays.

Protein expression and purification. The expression and purification of PDE5A were carried out similarly to our previously published protocols.[2,3] In brief, the catalytic domain coding (535-860) of PDE5A was cloned to vector pET-15b and then the cDNA was transferred to *E. coli* strain BL21 (CodonPlus, Stratagene) for overexpreesion. When the cell carrying the plasmid was cultivated in LB medium at 37 °C until OD_{600} = 0.7, 0.1mM isopropyl b-D-thiogalactopyranoside (IPTG) was added to induce PDE5A expression for further 40h growth at 15 °C. PDE5A protein was purified through Ni-NTA column (ϕ=2.5 cm, 15 ml QIAGEN agarose beads), Q-column (ϕ 2.5 × 8 cm, GE Healthcare) and Superdex 200 column (ϕ 2.5 × 45 cm, GE Healthcare). A typical batch cell yielded over 10mg PDE5A protein from 2L LB medium, with a purity>95% shown by SDS-PAGE.

The catalytic domains of PDE1B (10-487), PDE2A (580-919), PDE3A (679-1087), PDE4D (86-413), PDE6C (1-858), PDE7A (130-482), PDE9A (181-506), PDE10A (449-770), and PDE11A (588-911) were purified by the similar protocol. PDE8A (480-820) was expressed and purified following the protocol published previously.[4-6]

Enzymatic assays. In our study, the enzymatic activities of PDE5A, PDE6A, PDE9A, PDE1B, and PDE2A were assayed by using ^{3}H-cGMP as substrates to test the selectivity index of compound **3** while those of PDE3A, PDE4D, PDE7A, PDE8A, PDE10A, and PDE11A using ^{3}H-cAMP as the substrates. Sildenafil or tadalafil worked as the reference compound. The assay buffer contains 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$ or 4 mM $MnCl_2$, 1mM DTT. ^{3}H-cGMP or ^{3}H-cAMP was diluted with the assay buffer to 20 000-30 000 cpm per assay (GE Healthcare). The reaction was carried out at room temperature for 15 min and then terminated by addition of 0.2 M $ZnSO_4$. The reaction product ^{3}H-GMP or ^{3}H-AMP was precipitated by 0.2 N $Ba(OH)_2$, while unreacted ^{3}H-cGMP or ^{3}H-cAMP remained in the supernatant. Radioactivity of the supernatant was measured in 1.7 ml Ultima Gold liquid scintillation cocktails (Perkin Elmer) using a PerkinElmer 2910 liquid

scintillation counter. For the measurement of IC_{50}, at least eight concentrations of inhibitors were used for the measurement. Each measurement was repeated at least three times. The IC_{50} values with the SD values were calculated by nonlinear regression.

Druglikeness Profile of Compound 3.

Cytochrome inhibition. Cytochrome P450s (CYPs) are the major enzymes involved in the metabolism of various xenobiotics. In this study, the inhibitory activities of compound **3** against five human hepatic CYP enzymes (Table S2) were tested. As a result, it has an IC_{50} of 0.135 μM against CYP2C9 and its IC_{50} values of other four CYPs (CYP1A2, 2B6, 2D6, and 3A4) were uniformly more than 10 μM. The results suggest that **3** showed very weak inhibitory effects on these CYP isoenzymes except CYP1A2. Thus, compound **3** is unlikely to exhibit significant pharmacokinetic interactions with drugs that are metabolized by the seven major CYP isoforms.

Table S2. Inhibition of Compound 3 against Seven Cytochrome P450s

	CYP1A2	CYP2B6	CYP2C9	CYP2D6	CYP3A4
IC_{50}	>10 μM	>10 μM	0.135 μM	>10 μM	>10 μM

hERG inhibition. Human ether-a-go-go related gene (hERG) forms the major portion of one of the ion channel proteins that conducts potassium ions out of the muscle cells of the heart, and this current is critical in correctly timing the return to the resting state of the cell membrane during the cardiac action potential, which has made hERG inhibition an important antitarget that must be avoided during drug development. In our study, compound **3** inhibited hERG with an IC_{50} more than 30 μM by using automated patch clamp electrophysiology measurement in CHO-hERG cells. The results suggest that **3** showed weak inhibitory effects on hERG and thus was appropriate to initiate further development of compound **3**.

Plasma protein binding (PPB) assay for 3. PPB of **3** was assayed with a Single-Use RED Plate (Thermo Scientific) and performed by Medicilon Company, Shanghai, China. The compound **3** was added into 500 μL blank plasma to make 1 μM concentrations. The plasma and buffer chambers were added respectively by 300 μL samples and 500 μL dialysis buffer (PBS, containing 100 mM sodium phosphate and 150 mM sodium chloride). The unit were sealed and incubated on an orbital shaker at 37°C for 4 hours. After dialysis, 50 μL sample from the buffer chamber was added by 50 μL plasma, while 50 μL PBS was added to the plasma sample. Then, 3-fold volume of methanol containing internal standard were added to precipitate the plasma protein. After centrifugation, the

supernatant was injected to a LC/MS/MS for analysis. The bound percentage was calculated by the ratio of peak area for the buffer chamber over that for the plasma chamber. Each measurement was repeated two times and the results are the mean of triplicate determinations. Propranolol and warfarin were used as the positive controls. The results indicated that **3** strongly interact with plasma proteins, as shown by the bound percentage of 97.74%

Table S3. Binding of 3 to human plasma proteins

Compounds	species	Concentration (μM)	Bound percentile	mean
3	Human	1 μM	97.45% 98.02%	97.74%
warfarin	Human	1 μM	99.36% 99.27%	99.31%

Biopharmaceutical Profiling (hERG inhibition and human CYP450 inhibition). The assays were performed at Medicilon Company, Shanghai, China. hERG inhibition was performed by using automated patch clamp electrophysiology measurement in CHO-hERG cells. It is well-known that several human hepatic CYP enzymes play a dominant role in the metabolism of drugs and other xenobiotics. CYP450 inhibition assay was tested by incubating compound **3** with human liver microsomes and NADPH in the presence of CYP450-isoform specific probe substrate. The IC_{50} values of compound **3** towards five CYP isoenzymes CYP1A2, CYP2B6, CYP2C9, CYP2D6, and CYP3A4 were tested.

Stability of compound 3 in the Rat Liver Microsomes. The assays were performed at Medicilon Company, Shanghai, China. The experimental procedures were similar to our previous work. Compound **3** was dissolved in 100% DMSO as 10 mM stock solution and diluted to a final concentration of 0.5 μM for the experiments. Sildenafil was used as the positive control.

Acute Toxicity of compound 3. The acute toxicity was tested following the similar protocols described in our previous study. Thirty KM mice (22 days, 18−20 g), purchased from the Laboratory Animal Center of Sun Yat-Sen University (Guangzhou, China), were used to evaluate the acute toxicity of **3**. Mice were randomly divided into three groups, each of which was given in single oral dose of 0, 1000, or 1500 mg/kg **3** on the first day of the experiment. Mice were maintained on a 12 h

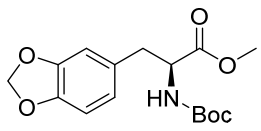
light/dark cycle (light from 7:00 to 19:00) at room temperature and 60−70% relative humidity. Sterile food and water were given according the institutional guidelines. Prior to each experiment, mice were fasted overnight and allowed free access to water. Compound **3** was dissolved in 0.5% CMC-Na solution and orally administrated. Mice were observed for any abnormal behavior, mortality, and weighed at the fourth hour and then the 24th hour for 14 days. Animals were sacrificed on the 14th day, and tissue samples of heart, liver, and kidney were macroscopically examined for possible damages.

Pharmacodynamics Effects of compound 3 against PAH in animals. All animal care and experimental protocols were in accordance with "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication, revised 1996, No.86-23, Bethesda, MD) and were approved by the Institutional Ethical Committee for Animal Research of Sun Yat-sen University. Forty Wister rats (7 weeks, 220−250 g), purchased from the Laboratory Animal Center of Southern Medical University (Guangzhou, China), were used to evaluate the pharmacodynamics effects of **3** on PAH. The rats were randomly divided into four groups: control group, model, compound **3** (1.25 mg/kg), and positive (sildenafil citrate, 10 mg/kg). Rats were maintained on a 12 h light/dark cycle (light from 7:00 to 19:00) at 24±1 °C and 60−70% relative humidity. Sterile food and water were given according to the institutional guidelines. Prior to each experiment, the rats were fasted overnight and allowed free access to water. All the rats were administrated with MCT 60 mg/kg except group control. Then, the rats were orally treated with drug vehicle (control and model groups), compound **3** (1.25 mg/kg) and sildenafil citrate (10 mg/kg) for 3 weeks, respectively. Compound **3** and sildenafil citrate were dissolved in 0.5% CMC-Na solution and orally administrated 0.4 mL per 100 g weight. The method of right cardiac catheter was applied to measure the pulmonary artery pressure and the mean pulmonary artery pressure (mPAP) was used to conduct statistics. Subsequently, the rats were killed and the hearts were dissected into right ventricle (RV) and left

ventricle and interventricular septum (LV+S); the 2 parts of the hearts were weighed with electronic scales, the value of RV/(LV+S) was used to conduct statistics.

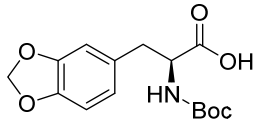
General Procedures for the Synthesis of Chromeno[2,3-c]pyrrol-9(2H)-ones.

*methyl (S)-3-(benzo[d][1,3]dioxol-5-yl)-2-((tert-butoxycarbonyl)amino)propanoate (**5**).*



To a solution of methyl (S)-2-((tert-butoxycarbonyl)amino)-3- (3,4-dihydroxyphenyl)propanoate (**4)** (31.1 g, 100 mmol) in acetonitrile (300 mL) was added potassium carbonate (41.4 g, 300 mmol) and diiodomethane (12.1 mL, 150 mmol). The mixture was stirred at 80 °C for 12 hours. After the solution had cooled to room temperature it was concentrated to remove the solvent and diluted with ethyl acetate (300 mL). The mixture was then filtered and washed with ethyl acetate, the filtrate was washed with water (3×150 mL). The organic layer was dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 10:1) to get the product **5** (23.3 g) as a colorless oil. Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (d, J = 7.9 Hz, 1H), 6.63 (d, J = 1.5 Hz, 1H), 6.59 (dd, J = 7.9, 1.6 Hz, 1H), 5.95 (d, J = 1.3 Hz, 2H), 5.00 (d, J = 6.3 Hz, 1H), 4.54 (d, J = 6.7 Hz, 1H), 3.74 (s, 3H), 3.02 (qd, J = 13.8, 5.7 Hz, 2H), 1.45 (s, 9H).

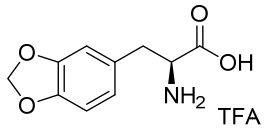
*(S)-3-(benzo[d][1,3]dioxol-5-yl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**6**).*



To a solution of **5** (23.3 g, 72 mmol) in methanol (250 mL) was added 1N aqueous NaOH (72 mL). The mixture was stirred at room temperature for 4 hours. Then it was evaporated to remove most of the solvent. The resulting mixture was diluted with water (150 mL) and acidified by the addition of 2 N aqueous HCl. The solution was then extracted with portions of ethyl acetate (3×150 mL). The combined organic extracts were washed with water (2×100 mL) and dried over anhydrous sodium

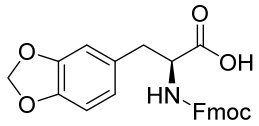
sulfate, and concentrated to afford the product **6** (22.0 g) as a colorless oil. Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (d, J = 7.9 Hz, 1H), 6.69 (s, 1H), 6.65 (d, J = 7.9 Hz, 1H), 5.95 (d, J = 1.8 Hz, 2H), 5.01 (d, J = 7.0 Hz, 1H), 4.57 (d, J = 5.8 Hz, 1H), 3.20-2.96 (m, 2H), 1.45 (s, 9H).

*(S)-2-amino-3-(benzo[d][1,3]dioxol-5-yl)propanoic acid 2,2,2-trifluoroacetaldehyde salt (**7**).*



To a solution of **6** (22.0 g, 71.1 mmol) in dichloromethane (90 mL) was added trifluoroacetic acid (30 mL). The mixture was stirred at room temperature for 2 h. Then it was evaporated to remove the solvent. The residue was placed in a solution of 20% ethyl acetate in petroleum ether under vigorous stirring until a gray precipitate was decanted. The mixture was then filtered to get the product **7** (20.7 g) as a gray solid. Yield: 95%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 8.22 (s, 2H), 6.88 (d, J = 7.9 Hz, 1H), 6.84 (d, J = 1.5 Hz, 1H), 6.72 (dd, J = 7.9, 1.6 Hz, 1H), 6.00 (s, 2H), 4.20 – 4.09 (m, 1H), 3.07 – 2.95 (m, 2H).

*(S)-2-(Fmoc-amino)-3-(benzo[d][1,3]dioxol-5-yl)propanoic acid (**8**).*



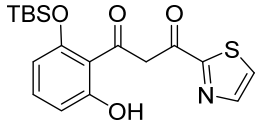
To a solution of **7** (20.7 g, 67.5 mmol) in a mixed solvent of dioxane (150 mL) and water (150 mL) was added sodium carbonate decahydrate (57.9 g, 202.5 mmol) at 0 °C. The mixture was stirred for 15 min and a solution of FmocCl (17.5 g, 67.5 mmol) in dioxane (150 mL) was added dropwise. The mixture was stirred for 1h at 0 °C and then for 2 h at room temperature. The reaction mixture was poured into water and washed with Et_2O (2×150 ml). The aqueous phase was acidified with concentrated aqueous HCl, and extracted with portions of ethyl acetate (3×150 mL). The combined organic extracts were washed with water (2×100 mL) and dried over anhydrous sodium sulfate, and

concentrated to afford the product **8** (25.0 g) as a white solid. Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 7.5 Hz, 2H), 7.39 (dd, J = 15.7, 7.9 Hz, 2H), 7.35 – 7.27 (m, 2H), 7.16 (dd, J = 12.3, 6.9 Hz, 2H), 6.68 – 6.56 (m, 2H), 6.52 (s, 1H), 6.50 – 6.39 (m, 1H), 5.71 (s, 2H), 5.63 (s, 1H), 4.47 (d, J = 5.0 Hz, 1H), 4.40 – 4.24 (m, 1H), 4.08 – 3.99 (m, 1H), 3.04 (dd, J = 18.3, 8.8 Hz, 1H), 2.88 (dd, J = 13.5, 7.4 Hz, 1H).

General Procedure for Synthesis of Compounds 10a-10m.

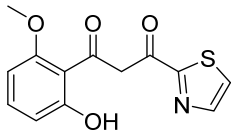
To a solution of 2'-hydroxyacetophenone derivatives (1.0 mmol) in toluene (5.0 mL) at 0°C was added sodium hydride (60% in mineral oil, 200 mg, 5.0 mmol), respectively. The mixture was stirred at room temperature for 15 min and ethyl thiazole-2-carboxylate (**9**) (1.2 mmol) was added and the mixture was stirred at 60°C for 2 h. After the solution had cooled to room temperature it was poured into a mixture of ice and water and acidified by the addition of 2 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (2×30 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 6:1 to 2:1) to get the product **10a-10m** as a yellow solid.

*1-(2-((tert-butyldimethylsilyl)oxy)-6-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10a**).*



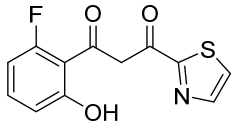
Yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.37 (br, 1H), 11.64 (s, 1H), 7.99 (d, J = 3.0 Hz, 1H), 7.72 (s, 1H), 7.63 (d, J = 3.0 Hz, 1H), 7.25 (t, J = 8.2 Hz, 1H), 6.61 (dd, J = 8.3, 0.8 Hz, 1H), 6.42 (dd, J = 8.1, 0.8 Hz, 1H), 1.00 (s, 9H), 0.32 (s, 6H).

*1-(2-hydroxy-6-methoxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10b**).*



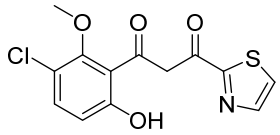
Yield: 39%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.92 (s, 1H), 8.06 (d, J = 3.0 Hz, 1H), 7.74 (d, J = 3.0 Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 6.62 (d, J = 8.4 Hz, 1H), 6.34 (d, J = 8.3 Hz, 1H), 4.80 (s, 2H), 3.58 (s, 3H).

*1-(2-fluoro-6-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10c**).*



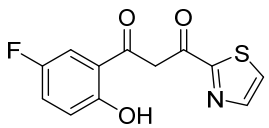
Yield: 61%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 10.87 (s, 1H), 8.24 (d, J = 3.0 Hz, 1H), 8.16 (d, J = 2.6 Hz, 1H), 7.83 (s, 1H), 7.38 (d, J = 6.8 Hz, 1H), 6.95 (s, 1H), 6.86 – 6.79 (m, 1H), 6.77 (d, J = 3.3 Hz, 1H).

*1-(3-chloro-6-hydroxy-2-methoxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10d**).*



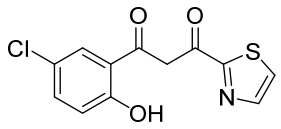
Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.21 (br, 1H), 11.84 (s, 1H), 8.04 (d, J = 3.0 Hz, 1H), 7.84 (s, 1H), 7.66 (d, J = 3.0 Hz, 1H), 7.44 (d, J = 9.0 Hz, 1H), 6.76 (d, J = 9.0 Hz, 1H), 3.91 (s, 3H).

*1-(5-fluoro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10e**).*



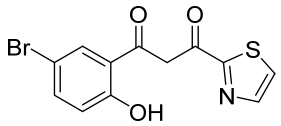
Yield: 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.13 (br, 1H), 11.65 (s, 1H), 8.07 (d, J = 3.1 Hz, 1H), 7.71 (d, J = 3.0 Hz, 1H), 7.56 (dd, J = 9.1, 3.1 Hz, 1H), 7.27 (s, 1H), 7.26 – 7.21 (m, 1H), 7.00 (dd, J = 9.1, 4.6 Hz, 1H).

*1-(5-chloro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10f**).*



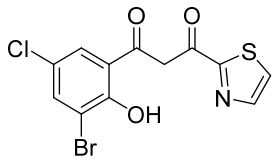
Yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.08 (br, 1H), 11.83 (s, 1H), 8.08 (d, J = 3.1 Hz, 1H), 7.86 (d, J = 2.5 Hz, 1H), 7.72 (d, J = 3.0 Hz, 1H), 7.45 (dd, J = 8.9, 2.5 Hz, 1H), 7.30 (s, 1H), 6.99 (d, J = 8.9 Hz, 1H).

*1-(5-bromo-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10g**).*



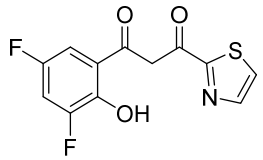
Yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.06 (br, 1H), 11.83 (s, 1H), 8.06 (d, J = 3.0 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 7.70 (d, J = 3.0 Hz, 1H), 7.56 (dd, J = 8.9, 2.3 Hz, 1H), 7.27 (s, 1H), 6.91 (d, J = 8.9 Hz, 1H).

*1-(3-bromo-5-chloro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10h**).*



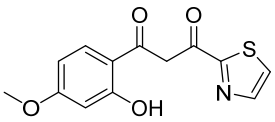
Yield: 63%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.88 (br, 1H), 12.55 (s, 1H), 8.09 (d, J = 2.9 Hz, 1H), 7.85 (d, J = 2.2 Hz, 1H), 7.77 (d, J = 2.2 Hz, 1H), 7.74 (d, J = 3.0 Hz, 1H), 7.31 (s, 1H).

*1-(3,5-difluoro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10i**).*



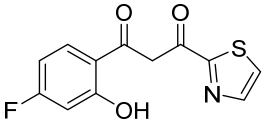
Yield: 59%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.03 (br, 1H), 11.62 (s, 1H), 8.06 (d, J = 2.9 Hz, 1H), 7.71 (d, J = 2.9 Hz, 1H), 7.37 (d, J = 8.7 Hz, 1H), 7.24 (s, 1H), 7.16 – 7.08 (m, 1H).

*1-(2-hydroxy-4-methoxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10j**).*



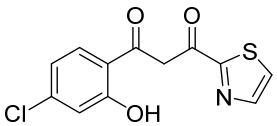
Yield: 46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.04 (br, 1H), 12.41 (s, 1H), 8.03 (d, J = 3.0 Hz, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 2.9 Hz, 1H), 7.24 (s, 1H), 6.53 – 6.45 (m, 2H), 3.88 (s, 3H).

*1-(4-fluoro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10k**).*



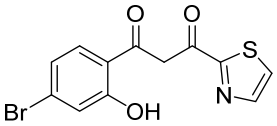
Yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.93 (br, 1H), 12.26 (s, 1H), 8.11 – 7.92 (m, 1H), 7.92 – 7.78 (m, 1H), 7.70 – 7.52 (m, 1H), 7.20 (s, 1H), 6.74 – 6.53 (m, 2H).

*1-(4-chloro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10l**).*



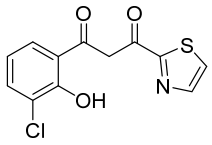
Yield: 61%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (br, 1H), 12.07 (s, 1H), 8.03 (d, J = 3.0 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 7.68 (d, J = 3.1 Hz, 1H), 7.28 (s, 1H), 7.03 (d, J = 2.0 Hz, 1H), 6.92 (dd, J = 8.7, 2.0 Hz, 1H).

*1-(4-bromo-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10m**).*



Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.00 (br, 1H), 12.01 (s, 1H), 8.03 (d, J = 3.0 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.68 (d, J = 3.0 Hz, 1H), 7.28 (s, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.07 (dd, J = 8.6, 1.7 Hz, 1H).

*1-(3-chloro-2-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**10n**).*

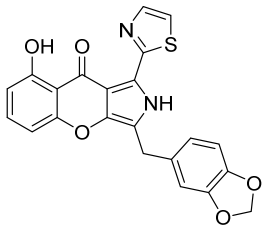


Yield: 54%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.93 (br, 1H), 12.46 (s, 1H), 8.04 (d, J = 3.0 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 3.0 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.35 (s, 1H), 6.90 (t, J = 8.0 Hz, 1H).

General Procedure for Synthesis of Compounds 2, and 11a-10m.

To a solution of **10a-10n** (0.5 mmol), (S)-2-(Fmoc-amino)-3-(benzo[d][1,3]dioxol-5-yl)propanoic acid (388 mg, 0.9 mmol), 4-dimethylpyridine (25 mg, 0.4 mmol) in pyridine (5.0 mL) was added DCC (206 mg, 1.0 mmol), respectively. The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h, and a major yellow spot could be observed by TLC. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (40 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 8:1 to 4:1) to afford **2**, and **11a-11m** as a yellow solid.

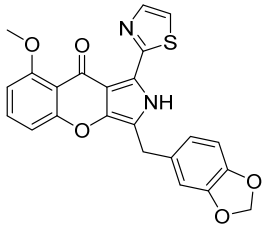
*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-hydroxy-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**2**).*



Yield: 38%; purity: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.97 (s, 1H), 11.00 (br, 1H), 7.76 (d, J = 2.8 Hz, 1H), 7.49 (t, J = 8.3 Hz, 1H), 7.40 (d, J = 2.9 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.61 (d, J = 7.8 Hz, 1H), 6.51 (s, 1H), 6.47 (d, J = 7.7 Hz, 1H), 5.77 (s, 2H), 3.99 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.95, 167.82, 162.65, 157.56, 157.45, 147.89, 146.40 , 142.22, 141.83, 135.72, 131.27, 121.03, 120.24, 117.91, 114.77 , 110.25, 108.55, 108.39, 106.88, 101.04,

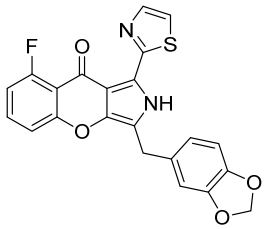
99.99, 29.53; HRMS (ESI) m/z calcd $C_{22}H_{15}N_2O_5S^+$ $[M+H]^+$ 419.0696, found 419.0693.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-methoxy-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11a**).*



Yield: 21%; purity: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (br, 1H), 7.72 (d, J = 2.4 Hz, 1H), 7.52 (t, J = 8.2 Hz, 1H), 7.32 (d, J = 2.3 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 7.9 Hz, 1H), 6.60 (s, 1H), 6.57 (d, J = 7.4 Hz, 1H), 5.84 (s, 2H), 4.04 (s, 3H), 4.02 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.88, 161.53, 159.35, 157.86, 147.91, 146.38, 141.59, 141.34, 133.94, 131.58, 121.17, 121.14, 119.79, 113.83, 112.92, 110.70, 110.03, 108.74, 108.33, 105.04 , 100.98, 56.47, 29.70; HRMS (ESI) m/z calcd $C_{23}H_{17}N_2O_5S^+$ $[M+H]^+$ 433.0853, found 433.0858.

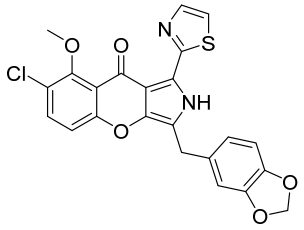
*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-fluoro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11b**).*



Yield: 38%; purity: >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.23 (br, 1H), 7.76 (d,0. J = 3.1 Hz, 1H), 7.57 (td, J = 8.3, 5.7 Hz, 1H), 7.39 (d, J = 3.2 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.00 (dd, J = 10.9, 8.3 Hz, 1H), 6.58 (d, J = 7.9 Hz, 1H), 6.50 (s, 1H), 6.43 (d, J = 7.9 Hz, 1H), 5.80 (s, 2H), 3.99 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.19, 163.78, 161.15, 158.25, 157.80, 147.83, 146.33, 141.54, 133.84, 133.73, 131.36, 121.41, 120.91, 120.10, 114.51, 113.51, 110.52, 110.30, 108.46, 108.25, 101.00, 29.52; HRMS (ESI) m/z calcd $C_{22}H_{14}FN_2O_4S^+$ $[M+H]^+$ 421.0653, found 421.0657.

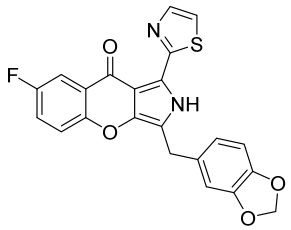
3-(benzo[d][1,3]dioxol-5-ylmethyl)-7-chloro-8-methoxy-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H

*)-one (**11c**).*



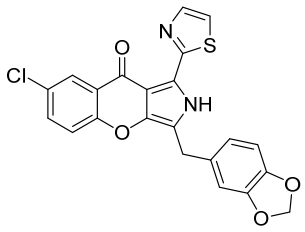
Yield: 34%; purity: 97%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.73 (br, 1H), 7.76 (d, J = 3.2 Hz, 1H), 7.63 (d, J = 9.1 Hz, 1H), 7.38 (d, J = 3.2 Hz, 1H), 7.17 (d, J = 9.1 Hz, 1H), 6.66 (d, J = 7.9 Hz, 1H), 6.59 (d, J = 1.5 Hz, 1H), 6.54 (dd, J = 7.9, 1.7 Hz, 1H), 5.84 (s, 2H), 4.09 (s, 3H), 4.03 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.21, 157.80, 157.26, 156.70, 147.81, 146.30, 141.55, 141.42, 134.18, 131.42, 123.29, 121.33, 120.98, 119.95, 118.28 , 114.48, 114.27, 110.10, 108.53, 108.27, 100.97, 61.73, 29.53; HRMS (ESI) m/z calcd $C_{23}H_{16}ClN_2O_5S^+$ $[M+H]^+$ 467.0463, found 467.0464.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-7-fluoro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11d**).*



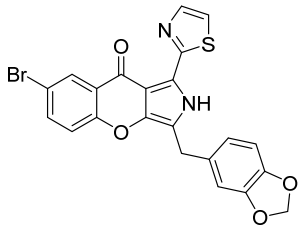
Yield: 37%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.12 (br, 1H), 7.93 (d, J = 2.9 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.75 (d, J = 2.9 Hz, 1H), 7.70 – 7.57 (m, 2H), 6.94 (s, 1H), 6.90 – 6.76 (m, 2H), 5.95 (s, 2H), 4.10 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.79, 159.34, 157.19, 153.11, 147.74, 146.11, 142.86, 141.75, 133.39, 122.77, 122.52, 121.59, 121.04, 120.59, 120.34, 116.48, 111.38, 111.14, 109.22, 108.69, 101.22, 29.37; HRMS (ESI) m/z calcd $C_{22}H_{14}FN_2O_4S^+$ $[M+H]^+$ 421.0653, found 421.0658.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-7-chloro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11e**).*



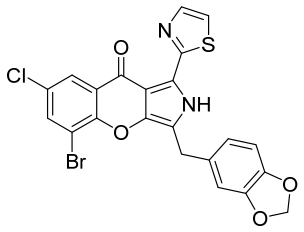
Yield: 41%; purity: 95%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.13 (br, 1H), 8.09 (d, J = 2.7 Hz, 1H), 7.94 (d, J = 3.2 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.58 (d, J = 8.9 Hz, 1H), 6.94 (s, 1H), 6.86 – 6.78 (m, 2H), 5.94 (s, 2H), 4.10 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.40, 157.12, 155.33, 147.74, 146.12, 142.87, 141.55, 134.56, 133.33, 127.89, 125.61, 123.39, 121.61, 121.13, 120.65, 120.63, 116.64, 109.23, 108.68, 108.65, 101.22, 29.36; HRMS (ESI) m/z calcd $C_{22}H_{14}ClN_2O_4S^+$ $[M+H]^+$ 437.0357, found 437.0364.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-7-bromo-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11f**).*



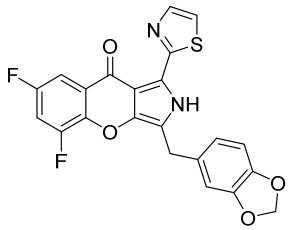
Yield: 37%; purity: >99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.15 (br, 1H), 8.23 (d, J = 1.2 Hz, 1H), 7.94 (d, J = 1.7 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 1.9 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 6.94 (s, 1H), 6.84 – 6.79 (m, 2H), 5.95 (s, 2H), 4.09 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.32, 157.14, 155.77, 147.76, 146.14, 142.91, 141.52, 137.32, 133.34, 128.74, 123.86, 121.63, 121.18, 120.94, 120.71, 116.69, 115.62, 109.25, 108.71, 108.67, 101.24, 29.37; HRMS (ESI) m/z calcd $C_{22}H_{14}BrN_2O_4S^+$ $[M+H]^+$ 480.9852, found 480.9852.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-5-bromo-7-chloro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11g**).*



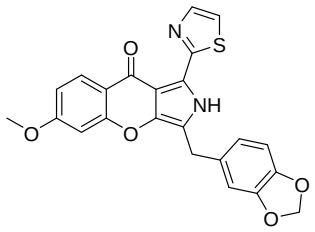
Yield: 34%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.23 (br, 1H), 8.18 (s, 1H), 8.07 (s, 1H), 7.95 (s, 1H), 7.77 (s, 1H), 7.01 (s, 1H), 6.89 (d, J = 7.5 Hz, 1H), 6.83 (d, J = 7.8 Hz, 1H), 5.94 (s, 2H), 4.09 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 172.66, 156.95, 151.94, 147.78, 146.23, 142.98, 141.04, 136.93, 133.06, 128.04, 125.52, 124.28, 121.84, 121.32, 120.77, 117.29, 112.44, 109.38, 108.76, 101.25, 99.99, 29.70; HRMS (ESI) m/z calcd $C_{22}H_{13}BrClN_2O_4S^+$ $[M+H]^+$ 514.9462, found 514.9453.

3-(benzo[d][1,3]dioxol-5-ylmethyl)-5,7-difluoro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(11h)***.



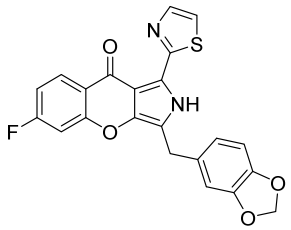
Yield: 32%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.29 (br, 1H), 8.01 (d, J = 2.2 Hz, 1H), 7.98 – 7.89 (m, 1H), 7.83 (d, J = 2.5 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 6.98 (s, 1H), 6.89 (d, J = 7.5 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 6.00 (s, 2H), 4.16 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 172.50, 162.41, 161.86, 151.93, 147.77, 146.17, 143.01, 136.96, 133.14, 131.62, 130.12, 126.34, 121.61, 121.33, 120.70, 116.89, 110.55, 109.31, 109.22, 108.74, 101.26, 21.52; HRMS (ESI) m/z calcd $C_{22}H_{13}F_2N_2O_4S^+$ $[M+H]^+$ 439.0559, found 439.0559.

3-(benzo[d][1,3]dioxol-5-ylmethyl)-6-methoxy-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(11i)***.



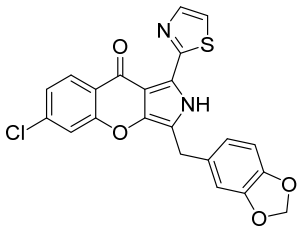
Yield: 35%; purity: >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (br, 1H), 8.30 (d, J = 8.9 Hz, 1H), 7.77 (d, J = 3.2 Hz, 1H), 7.37 (d, J = 3.2 Hz, 1H), 6.91 (dd, J = 8.9, 2.3 Hz, 1H), 6.85 (d, J = 2.3 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 6.67 (s, 1H), 6.64 (d, J = 7.9 Hz, 1H), 5.88 (s, 2H), 4.09 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.02, 167.81, 164.55, 158.93, 157.78, 148.02, 146.49, 142.50, 141.77, 131.48, 128.28, 121.23, 120.96, 119.78, 116.40, 114.43, 111.87, 108.78, 108.43, 101.03, 100.45, 55.75, 29.76; HRMS (ESI) m/z calcd $C_{23}H_{17}N_2O_5S^+$ $[M+H]^+$ 433.0853, found 433.0858.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-6-fluoro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11j**).*



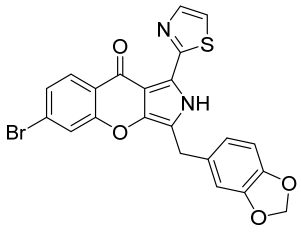
Yield: 36%; purity: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (br, 1H), 8.40 (dd, J = 8.7, 6.6 Hz, 1H), 7.78 (d, J = 3.2 Hz, 1H), 7.40 (d, J = 3.1 Hz, 1H), 7.15 – 7.02 (m, 2H), 6.71 (d, J = 7.8 Hz, 1H), 6.65 (s, 1H), 6.64 (d, J = 7.9 Hz, 1H), 5.88 (s, 2H), 4.09 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.49, 167.32, 164.78, 157.90, 147.78, 146.29, 141.56, 131.35, 129.16, 121.16, 120.94, 119.98, 119.39, 115.02, 111.69, 111.46, 108.46, 108.25, 104.43, 104.18, 100.94, 29.53; HRMS (ESI) m/z calcd $C_{22}H_{14}FN_2O_4S^+$ $[M+H]^+$ 421.0653, found 421.0647.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-6-chloro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11k**).*



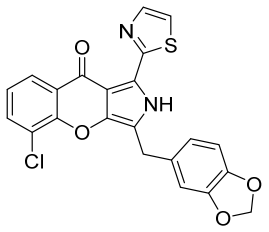
Yield: 39%; purity: 96%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.22 (br, 1H), 8.32 (d, J = 8.5 Hz, 1H), 7.76 (d, J = 3.2 Hz, 1H), 7.44 (d, J = 1.8 Hz, 1H), 7.40 (d, J = 3.2 Hz, 1H), 7.31 (dd, J = 8.5, 1.9 Hz, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.51 (s, 1H), 6.46 (d, J = 7.9 Hz, 1H), 5.78 (s, 2H), 4.00 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.57, 157.78, 157.17, 147.82, 146.34, 142.17, 141.63, 139.88, 131.30, 128.12, 123.83, 121.21, 121.11, 120.99, 120.01, 117.69, 115.09, 109.38, 108.50, 108.29, 100.97, 29.58; HRMS (ESI) m/z calcd $C_{22}H_{14}ClN_2O_4S^+$ $[M+H]^+$ 437.0357, found 437.0358.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-6-bromo-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11l**).*



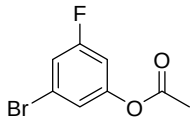
Yield: 36%; purity: 96%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.19 (br, 1H), 8.24 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 3.2 Hz, 1H), 7.62 (d, J = 1.5 Hz, 1H), 7.46 (dd, J = 8.5, 1.5 Hz, 1H), 7.40 (d, J = 3.2 Hz, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.50 (s, 1H), 6.45 (d, J = 7.9 Hz, 1H), 5.77 (s, 2H), 3.99 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.69, 157.76, 157.11, 147.85, 146.37, 142.10, 141.66, 131.28, 128.20×2, 126.67, 121.49, 121.25, 120.99, 120.75, 120.04, 115.09, 109.39, 108.50, 108.31, 100.99, 29.58; HRMS (ESI) m/z calcd $C_{22}H_{14}BrN_2O_4S^+$ $[M+H]^+$ 480.9852, found 480.9848.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-5-chloro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**11m**).*



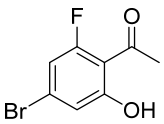
Yield: 35%; purity: >99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.16 (br, 1H), 8.12 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 3.1 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.75 (d, J = 3.1 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.00 (s, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 5.94 (s, 2H), 4.10 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.81, 157.11, 152.04, 147.74, 146.17, 142.88, 141.03, 134.80, 133.15, 125.71, 123.92, 123.77, 121.79, 121.75, 121.13, 120.54, 117.10, 109.37, 108.72, 108.56, 101.23, 29.71; HRMS (ESI) m/z calcd $C_{22}H_{14}ClN_2O_4S^+$ $[M+H]^+$ 437.0357, found 437.0354.

*3-bromo-5-fluorophenyl acetate (**13**)*



3-Bromo-5-fluorophenol (19.1 g, 100.0 mol) was added to a mixture of Ac_2O (100.0 mL, 300.0 mol) and anhydrous NaOAc (18.0 g, 220.0 mol) in a round-bottom flask. The mixture was stirred at 110°C for 2 h. After the solution had cooled to room temperature it was poured into ice-water (400 mL) and diluted with CH_2Cl_2 (200 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic layers were washed with water (200 mL) and sat. aq $NaHCO_3$ solution (100 mL). The organic layer was dried over anhydrous sodium sulfate, and evaporated in vacuo to give the **13** (19.7 g) as a white solid. Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 – 7.11 (m, 1H), 7.10 (s, 1H), 6.84 (dt, J = 9.1, 2.1 Hz, 1H), 2.29 (s, 3H).

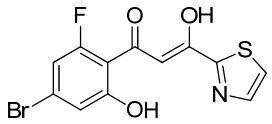
*1-(4-bromo-2-fluoro-6-hydroxyphenyl)ethan-1-one (**14**)*



3-bromo-5-fluorophenyl acetate (**13**) was transferred into a three-neck flask and $AlCl_3$ (34.0 g,

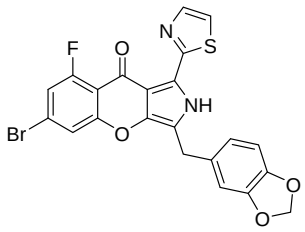
256.0mmol) was added in small portions. The mixture was heated and stirred at 140 °C for 3 h. After the solution had cooled to 80 °C, ice (200 g) and 10% HCl solution (100 mL) were added to the mixture slowly. Then the mixture was extracted with portions of ethyl acetate (3×100 mL). The combined organic layers were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 10:1) to get the product **14** (15.3 g) as a white solid. Yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.87 (s, 1H), 6.99 (s, 1H), 6.81 (d, J = 11.1 Hz, 1H), 2.67 (d, J = 7.2 Hz, 3H).

*1-(4-bromo-2-fluoro-6-hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**15**)*



To a solution of 1-(4-bromo-2-fluoro-6-hydroxyphenyl)ethan-1-one (**14**) (2.7 g, 20.0 mmol) in toluene (80 mL) at 0°C was added sodium hydride (60% in mineral oil, 4.0 g, 100 mmol). The mixture was stirred at room temperature for 15 min and ethyl thiazole-2-carboxylate (4.0 mL, 30.0 mmol) was added, and the mixture was stirred at 60°C for 2 h. After the solution had cooled to room temperature it was poured into a mixture of ice and water and acidified by the addition of 2 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (2×100 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1) to get the product 15 (3.2 g) as a yellow solid. Yield: 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.10 (br s, 1H), 12.39 (s, 1H), 8.05 (d, J = 2.9 Hz, 1H), 7.68 (d, J = 3.0 Hz, 1H), 7.44 (s, 1H), 7.02 (s, 1H), 6.86 (d, J = 11.6 Hz, 1H).

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-6-bromo-8-fluoro-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**16**)*

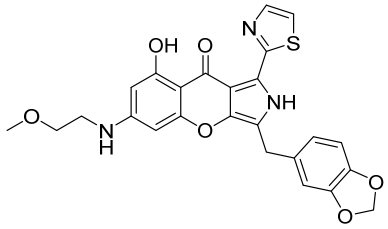


To a solution of **15** (3.4g g, 10.0 mmol), (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(benzo[d][1,3]dioxol-5-yl)propanoic acid (6.4 g, 15.0 mmol), 4-dimethylpyridine (610 mg, 5.0 mmol) in pyridine (25 mL) was added DCC (2.4 g, 12.0 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was then raised to 50°C for 6 h. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (150 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford **16** (2.7 g) as a yellow solid. Yield: 56%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.16 (s, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 1.9 Hz, 1H), 7.17 (s, 1H), 6.93 (s, 1H), 6.90 (s, 1H), 6.86 – 6.74 (m, 2H), 5.94 (s, 2H), 4.04 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 180.22, 162.74, 157.37, 156.73, 147.76, 146.17, 143.11, 141.13, 133.14, 129.29, 121.70×2, 120.96, 116.77, 113.42, 110.84, 109.29, 108.73, 107.63, 107.40, 101.26, 29.26; HRMS (ESI) m/z calcd $C_{22}H_{13}BrFN_2O_4S^+$ $[M+H]^+$ 498.9758, found 498.9745.

General Procedure for Synthesis of Compounds 3 and 17a-c. To a solution of **16** (500 mg, 1.0 mmol), amine (3.0 mmol), 2,2'-bis(diphenylphosphino)-1,1'-bisnaphthyl (124 mg, 0.2 mmol,) in DMSO (15 mL) was added 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (82 mg, 0.1 mmol) and sodium tert-butoxide (192 mg, 2.0mmol). The mixture was then heated to 100 °C for 24 h under an argon atmosphere. After the solution had cooled to room temperature it was diluted with ethyl acetate (150 mL) and washed with brine (3 × 100 mL).

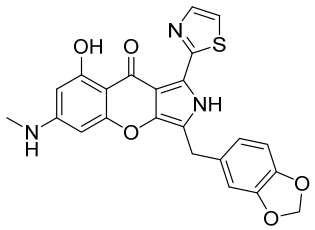
The organic layer was dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 4:1) to get the product **3** and **17a-c** as a yellow solid.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-hydroxy-6-((2-methoxyethyl)amino)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**3**).*



Yield: 37%; purity: 99%; ^{1}HNMR (500 M, Acetone – d_6) δ 13.25 (s, 1H), 7.82 (d, J = 3.1 Hz, 1H), 7.59 (d, J = 3.1 Hz, 1H), 6.89 (s, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 7.9 Hz, 1H), 6.17 (s, 1H), 6.01 (s, 1H), 5.94 (s, 2H), 4.18 (s, 2H), 3.60 (t, J = 5.3 Hz, 2H), 3.43 (dd, J = 10.2, 5.1 Hz, 2H), 3.35 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ178.23, 163.32, 159.06, 157.56, 155.90, 147.73, 146.11, 141.75, 141.50, 133.35, 121.56, 121.20, 119.26, 116.83, 109.20, 108.70, 108.10, 101.26, 99.81, 93.76, 89.75, 70.80, 58.52, 42.49, 29.32; HRMS (ESI) m/z calcd $C_{25}H_{22}N_3O_6S^+$ $[M+H]^+$ 492.1224, found 492.1223.

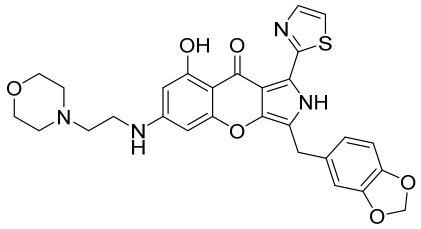
*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-hydroxy-6-(methylamino)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17a**).*



Yield: 32%; purity: 99%; ^{1}H NMR (500 MHz, DMSO – d_6) δ 13.20 (s, 1H), 12.86 (s, 1H), 7.91 (d, J = 3.2 Hz, 1H), 7.73 (d, J = 3.2 Hz, 1H), 6.91 (d, J = 1.5 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.77 (dd, J = 8.0, 1.6 Hz, 1H), 6.00 (d, J = 2.0 Hz, 1H), 5.95 (s, 2H), 5.86 (d, J = 2.0 Hz, 1H), 4.03 (s, 2H),

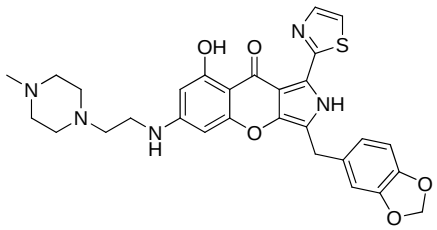
2.77 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) 178.49, 163.41, 159.09, 157.37, 156.64, 147.73, 146.09, 142.73, 141.33, 133.54, 121.53, 121.12, 119.95, 116.11, 109.19, 108.70, 107.74, 101.24, 99.75, 93.39, 89.46, 29.60, 29.31; HRMS (ESI) m/z calcd $C_{23}H_{18}N_3O_5S^+$ $[M+H]^+$ 448.0962, found 448.0960.

*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-hydroxy-6-((2-morpholinoethyl)amino)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17b**).*



Yield: 34%; purity: 98%; ^{1}HNMR (500 M, Acetone – d_6) δ 13.25 (s, 1H), 7.80 (s, 1H), 7.58 (s, 1H), 6.88 (s, 1H), 6.82 (d, J = 6.9 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 6.14 (s, 1H), 5.96 (s, 1H), 5.93 (s, 2H), 4.17 (s, 2H), 3.70 (s, 4H), 3.40 (s, 2H), 2.75 (s, 2H), 2.60 (s, 4H); ^{13}C NMR (101 MHz, Acetone – d_6) δ 178.74, 159.30, 157.21, 156.68, 155.36, 147.85, 146.22, 142.26, 141.70, 133.06, 129.68, 121.24, 120.40, 120.00, 115.31, 108.74, 108.06, 100.99, 100.33, 93.47, 89.69, 66.10×2, 56.67, 53.26×3, 29.27; HRMS (ESI) m/z calcd $C_{28}H_{27}N_4O_6S^+$ $[M+H]^+$ 547.1646, found 547.1644.

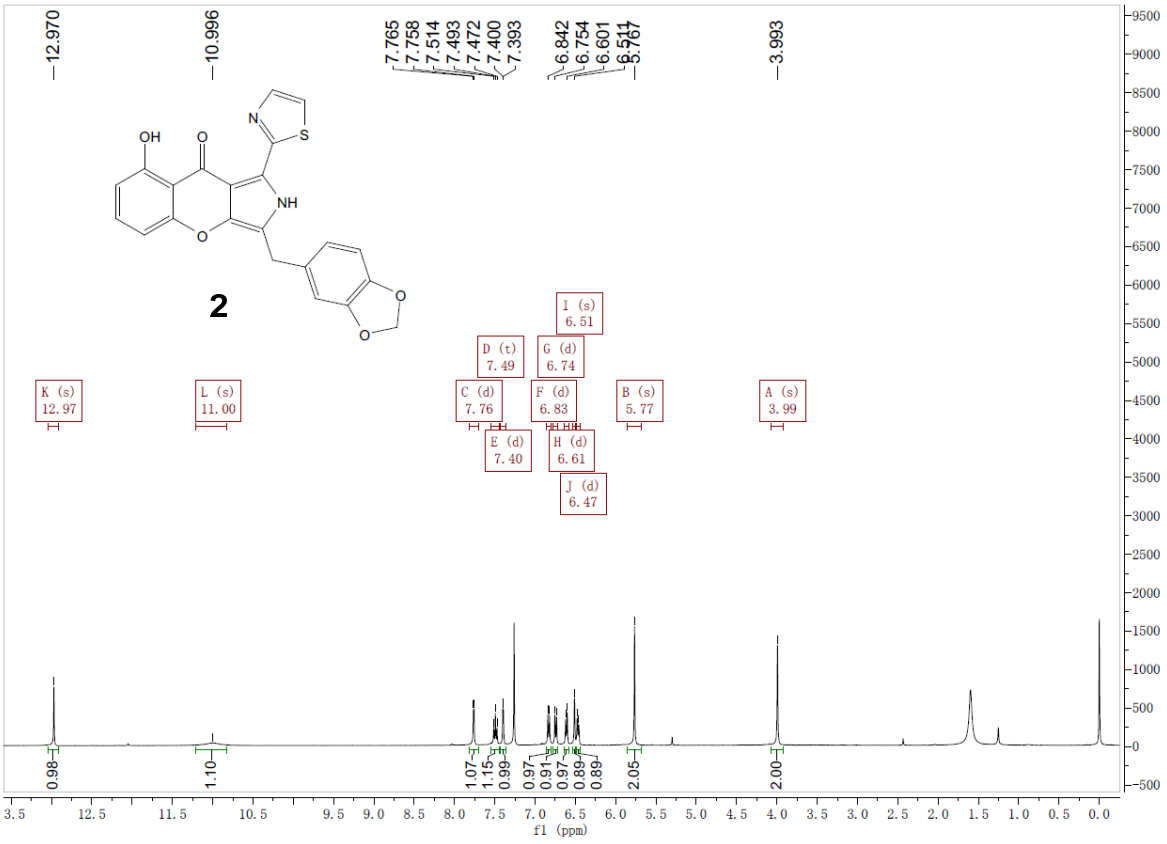
*3-(benzo[d][1,3]dioxol-5-ylmethyl)-8-hydroxy-6-((2-(4-methylpiperazin-1-yl)ethyl)amino)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17c**).*

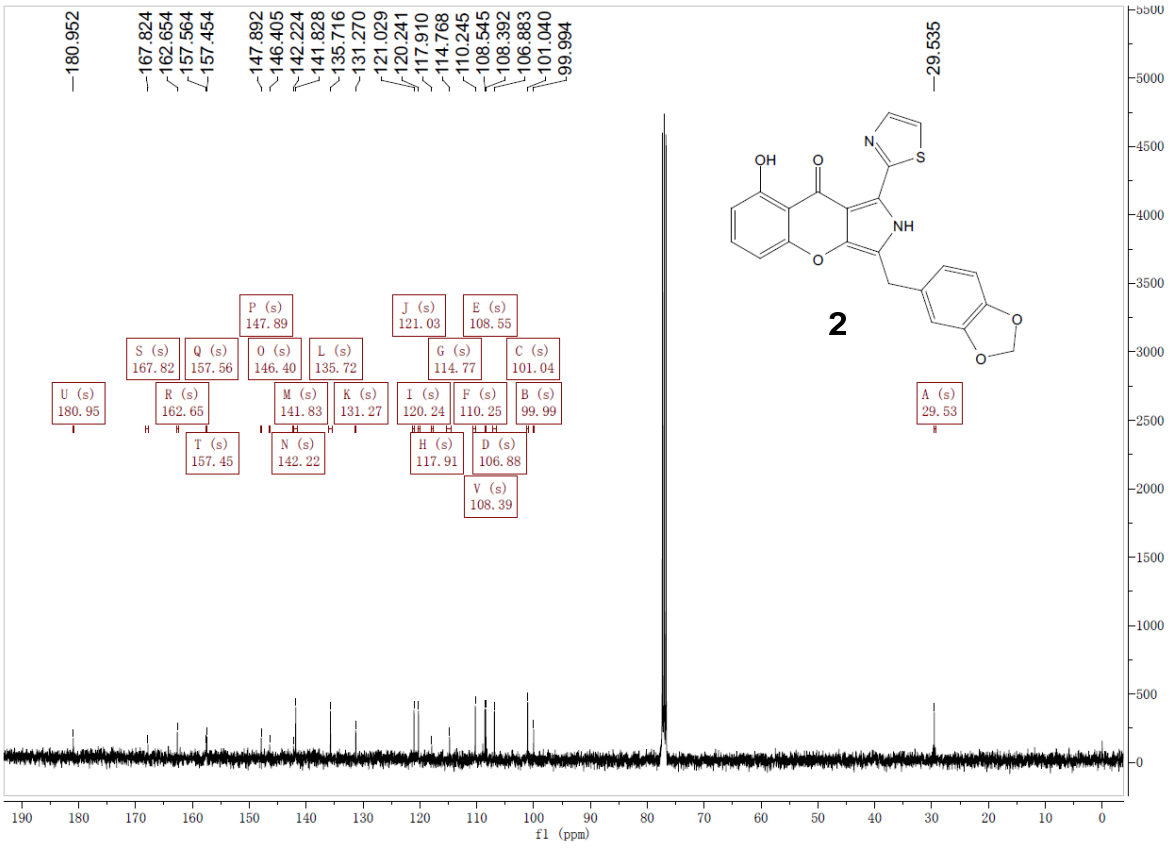


Yield: 36%; purity: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.19 (s, 1H), 7.75 (d, J = 3.2 Hz, 1H), 7.36 (d, J = 3.2 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 6.65 (s, 1H), 6.62 (d, J = 7.9 Hz, 1H), 6.02 (d, J = 2.0 Hz, 1H), 5.96 (d, J = 2.0 Hz, 1H), 5.89 (s, 2H), 5.07 (s, 1H), 4.03 (s, 2H), 3.27 (dd, J = 10.3, 5.4

Hz, 2H), 2.75 – 2.68 (m, 2H), 2.60 (s, 6H), 2.38 (s, 3H), 2.13 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.26, 163.93, 159.30, 154.50, 151.25, 147.90, 144.86, 141.67, 134.19, 131.58, 130.34, 121.11, 119.90, 114.47, 108.67, 108.37, 101.32, 101.06, 99.99, 94.09, 90.14, 55.84, 54.73×2, 52.12×2, 45.58, 39.27, 29.61; HRMS (ESI) m/z calcd $C_{29}H_{30}N_5O_5S^+$ $[M+H]^+$ 560.1962, found 560.1957.

^{1}H NMR, ^{13}C NMR and HRMS data for the tested compounds





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N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
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Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

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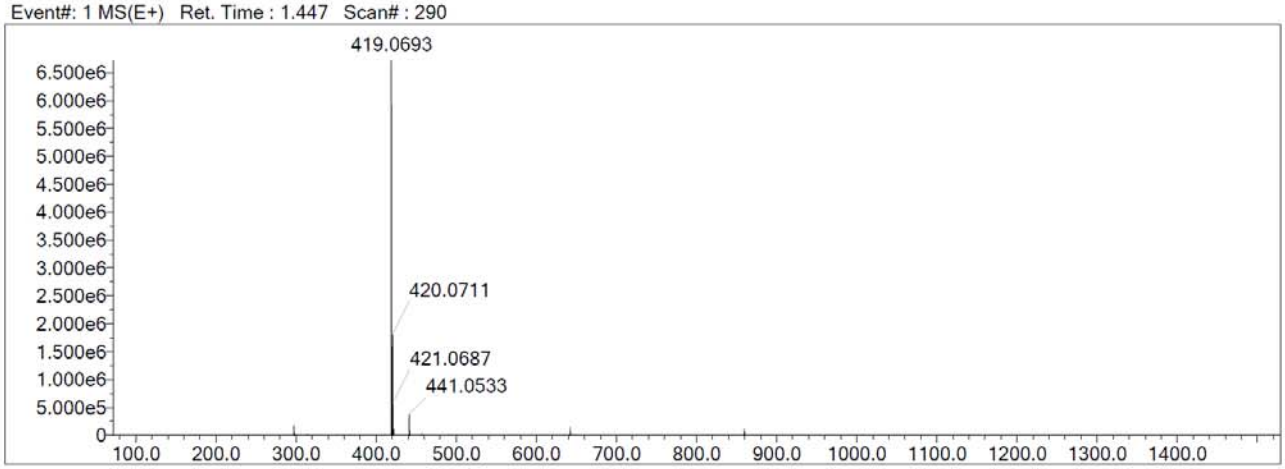
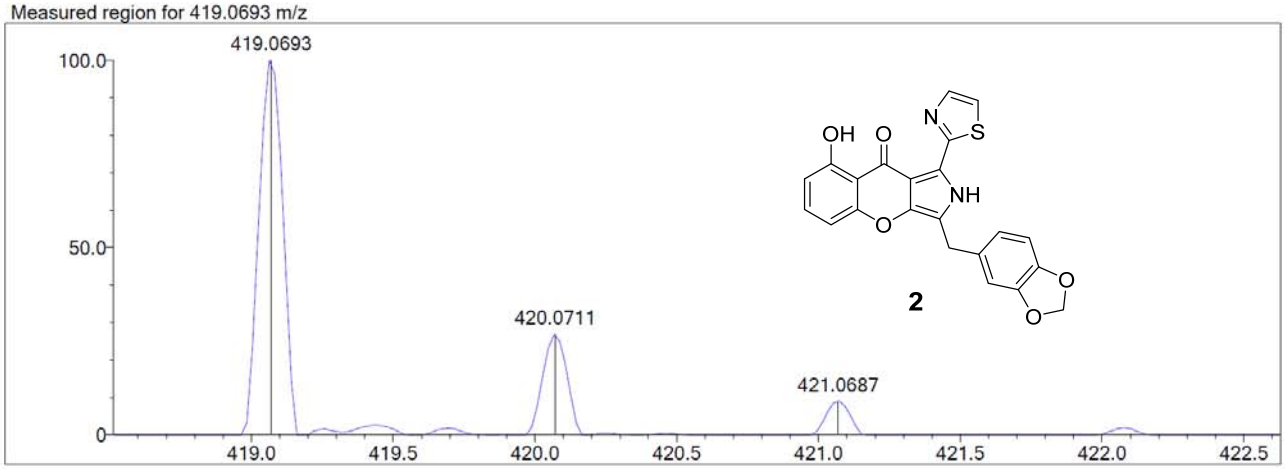
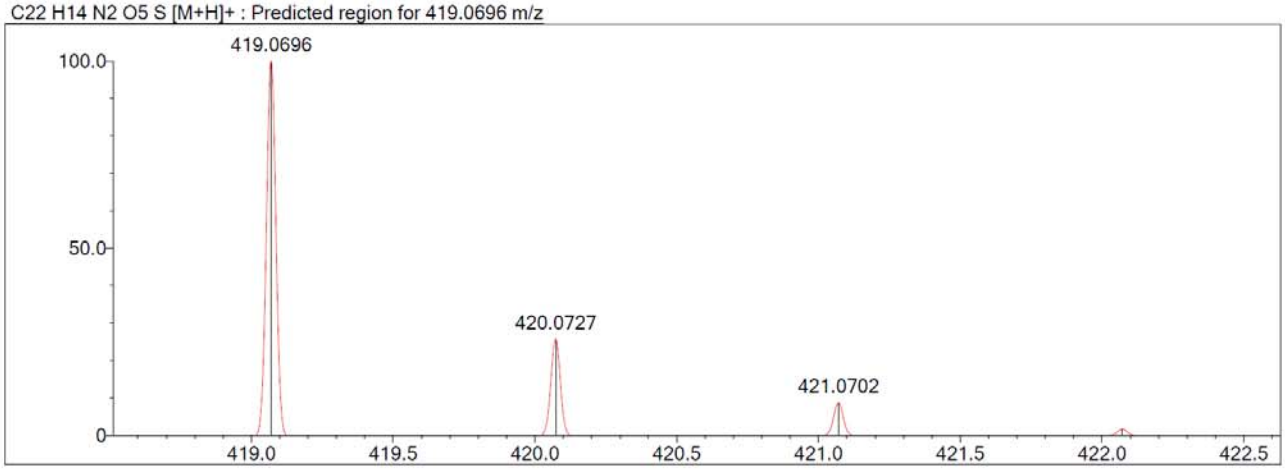


Measured region for 419.0693 m/z



C22 H14 N2 O5 S [M+H]+ : Predicted region for 419.0696 m/z



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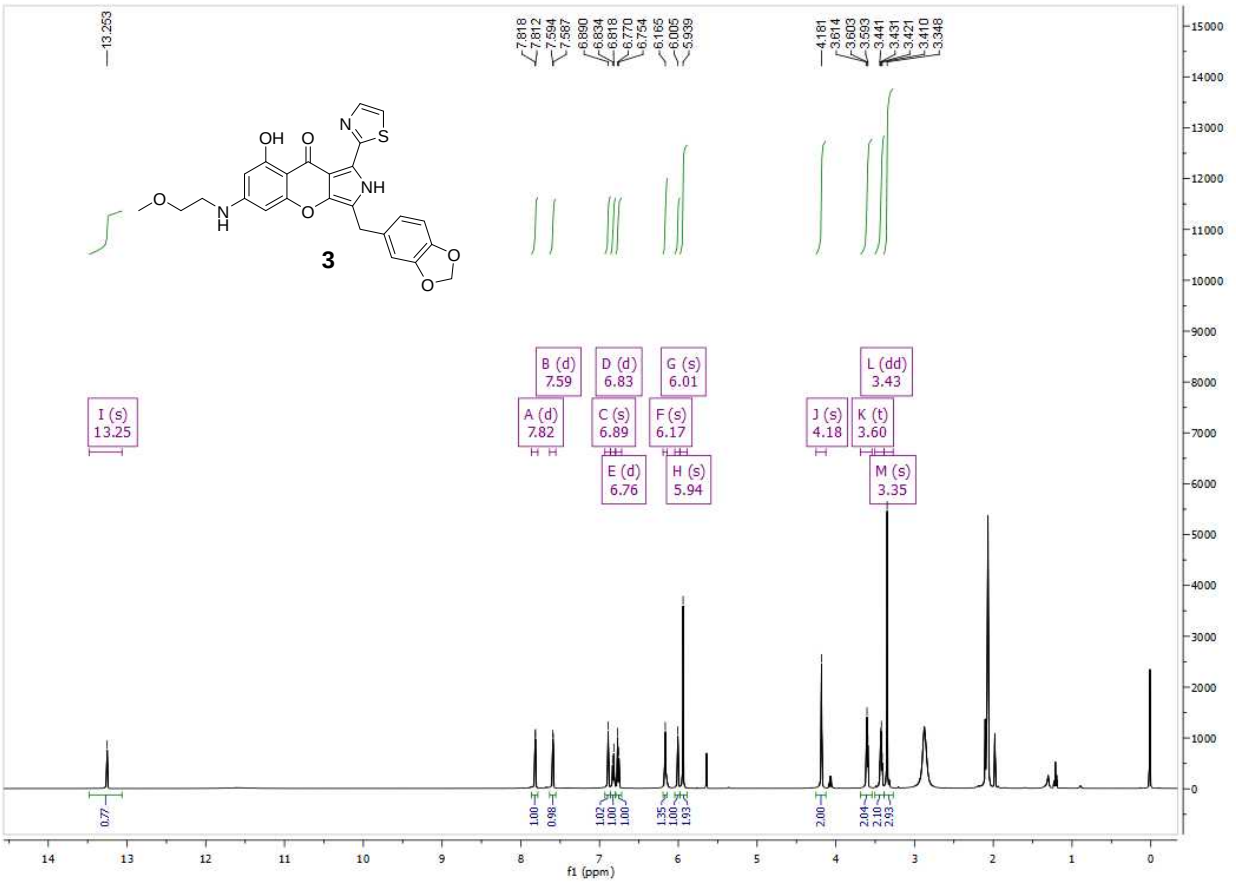
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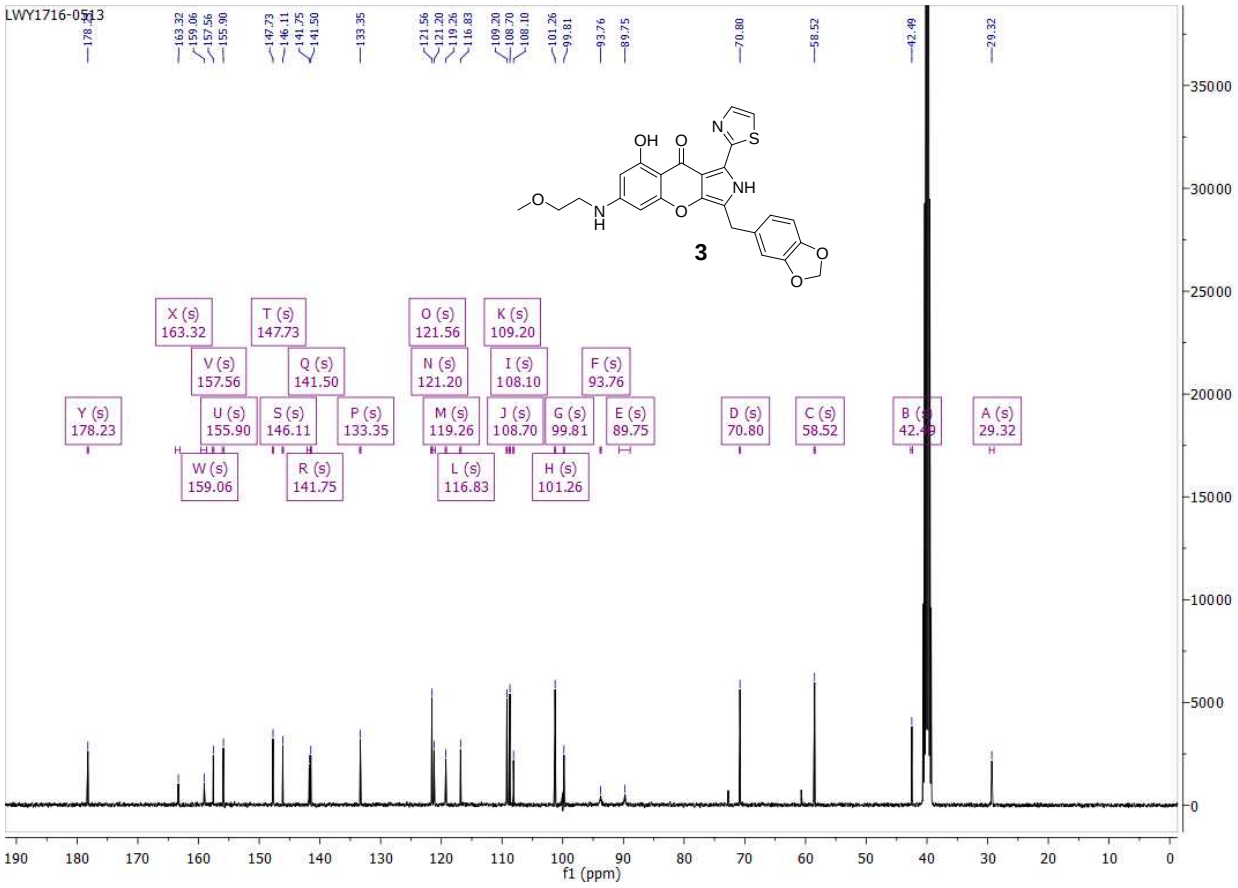
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89.75
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I (s) 108.10
F (s) 93.76
Y (s) 178.23
U (s) 155.90
S (s) 146.11
P (s) 133.35
M (s) 119.26
J (s) 108.70
G (s) 99.81
E (s) 89.75
D (s) 70.80
C (s) 58.52
B (s) 42.49
A (s) 29.32
W (s) 159.06
R (s) 141.75
L (s) 116.83
H (s) 101.26
f1 (ppm)

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
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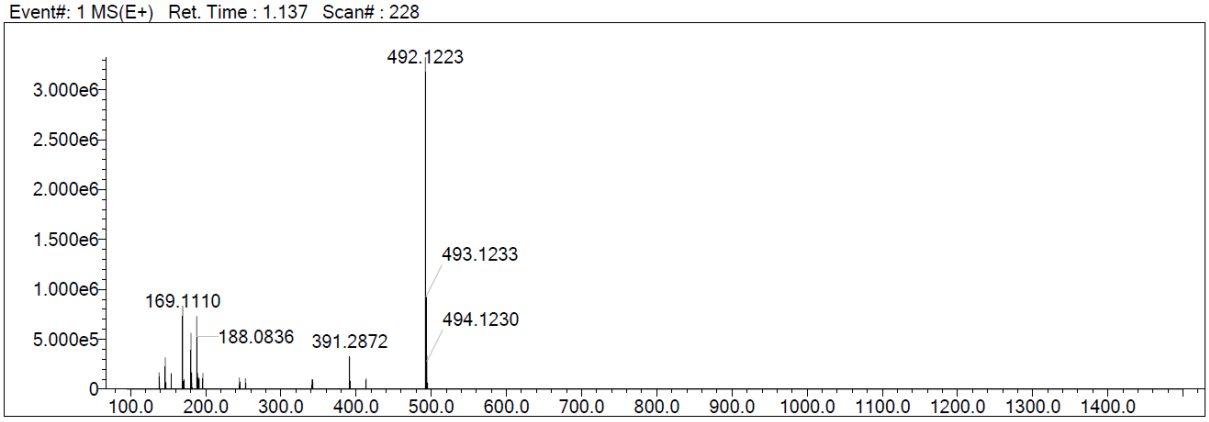
2H	1	0	0	F	1	0	1	Fe	2	0	0	Pt	2	0	0	Na
3H	1	0	0	Si	4	0	0	Cu	2	0	0	Au	1	0	0	
B	3	0	0	P	3	0	0	Se	2	0	0					
C	4	0	26	S	2	0	1	Br	1	0	0					
N	3	0	3	Cl	1	0	0	Pd	2	0	0					

Error Margin (ppm): 20
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

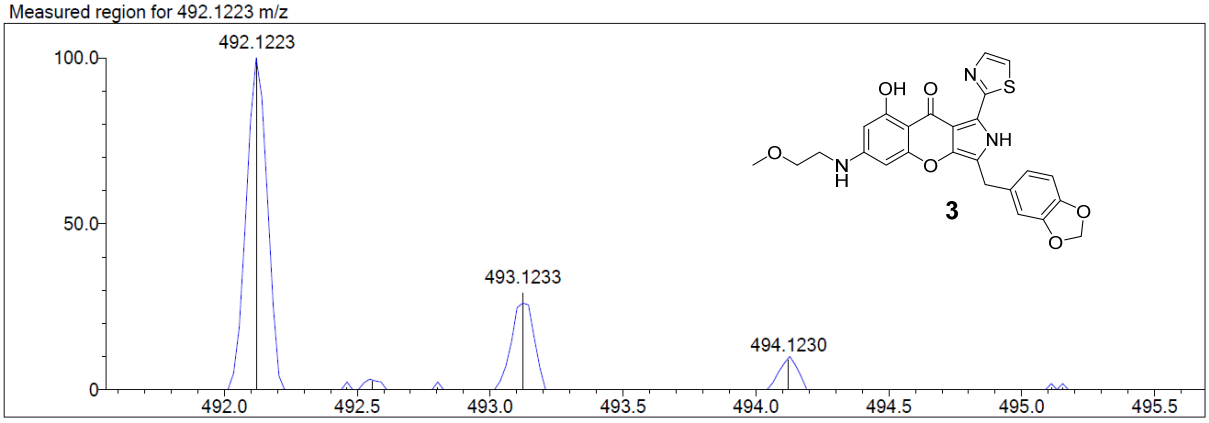
DBE Range: -50.0 - 50.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

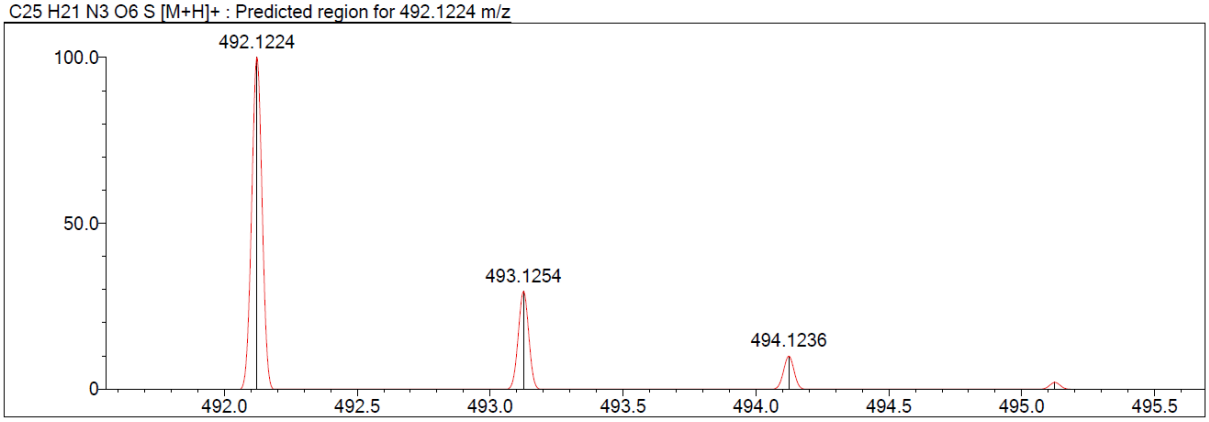
Event#: 1 MS(E+) Ret. Time : 1.137 Scan# : 228



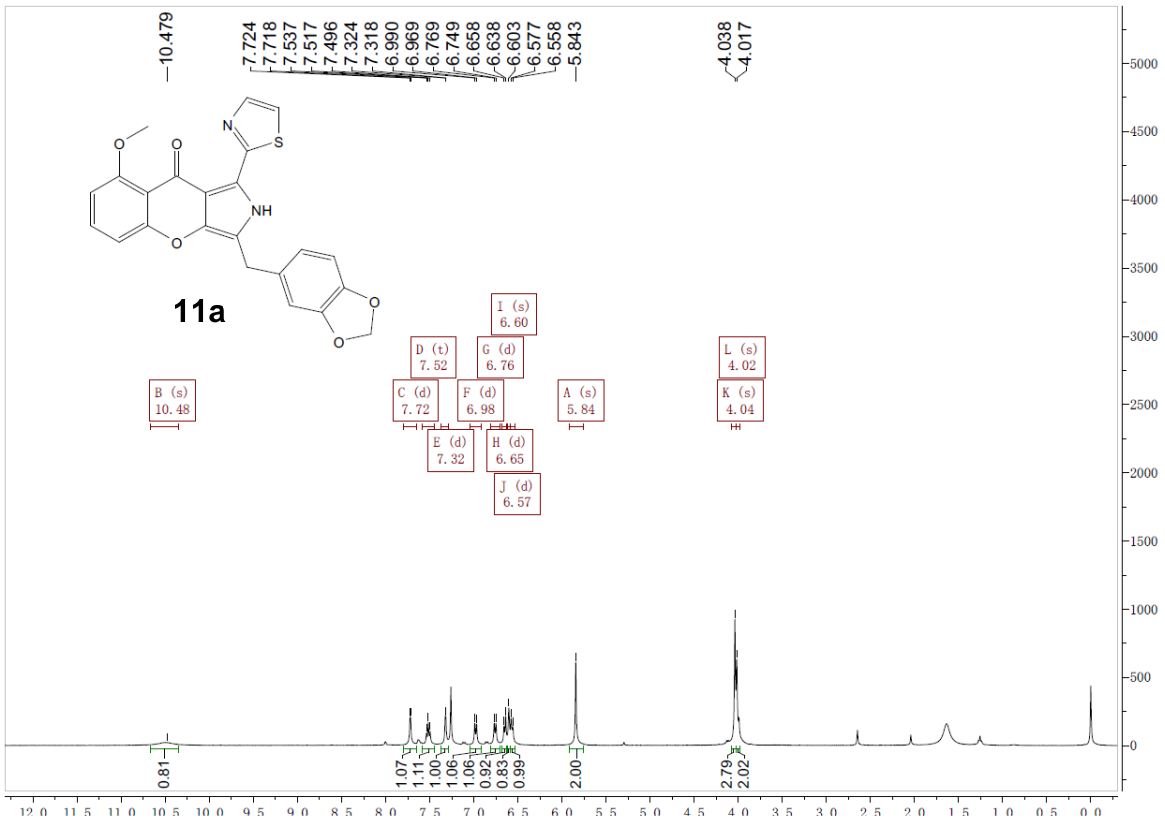
Measured region for 492.1223 m/z

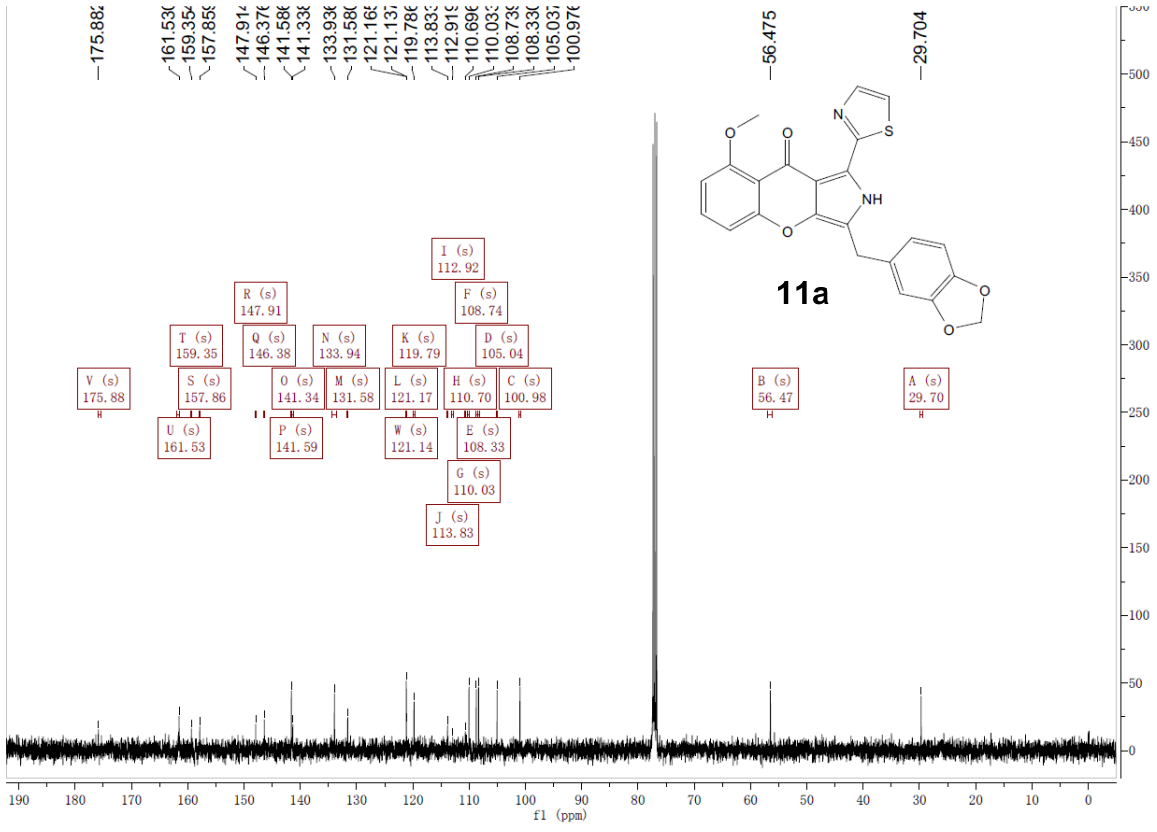


C25 H21 N3 O6 S [M+H]+ : Predicted region for 492.1224 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	73.38	C25 H21 N3 O6 S	[M+H]+	492.1223	492.1224	-0.1	-0.20	73.38	17.0


11a
10.479
7.724
7.718
7.537
7.517
7.496
7.324
7.318
6.990
6.969
6.769
6.749
6.658
6.638
6.603
6.577
6.558
5.843
4.038
4.017
B (s) 10.48
C (d) 7.72
D (t) 7.52
E (d) 7.32
F (d) 6.98
G (d) 6.76
H (d) 6.65
I (s) 6.60
J (d) 6.57
A (s) 5.84
L (s) 4.02
K (s) 4.04


11a
175.88
161.53
159.35
157.85
147.91
146.37
141.58
141.33
133.93
131.58
121.16
121.13
119.78
113.83
112.91
110.69
110.03
108.73
108.33
105.03
100.97
56.475
29.704
V (s) 175.88
S (s) 157.86
T (s) 159.35
U (s) 161.53
R (s) 147.91
Q (s) 146.38
O (s) 141.34
P (s) 141.59
N (s) 133.94
M (s) 131.58
L (s) 121.17
W (s) 121.14
K (s) 119.79
H (s) 110.70
I (s) 112.92
F (s) 108.74
D (s) 105.04
C (s) 100.98
E (s) 108.33
G (s) 110.03
J (s) 113.83
B (s) 56.47
A (s) 29.70
f1 (ppm)

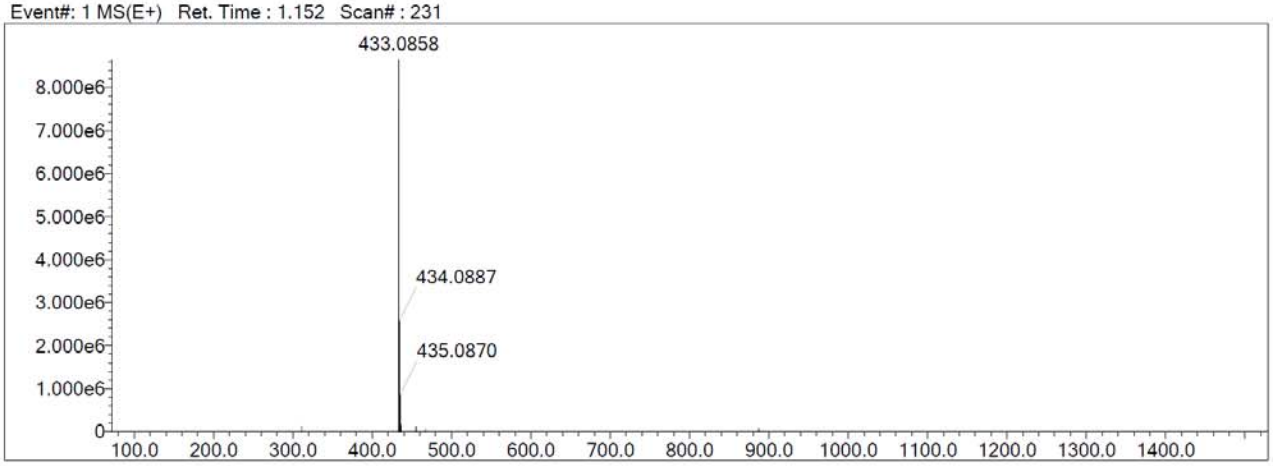
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

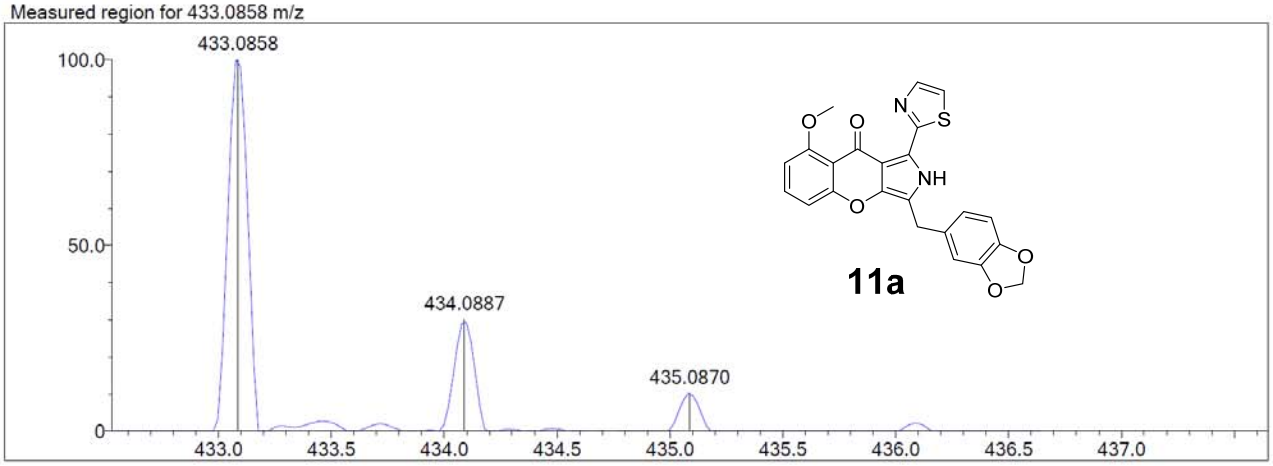
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.152 Scan# : 231

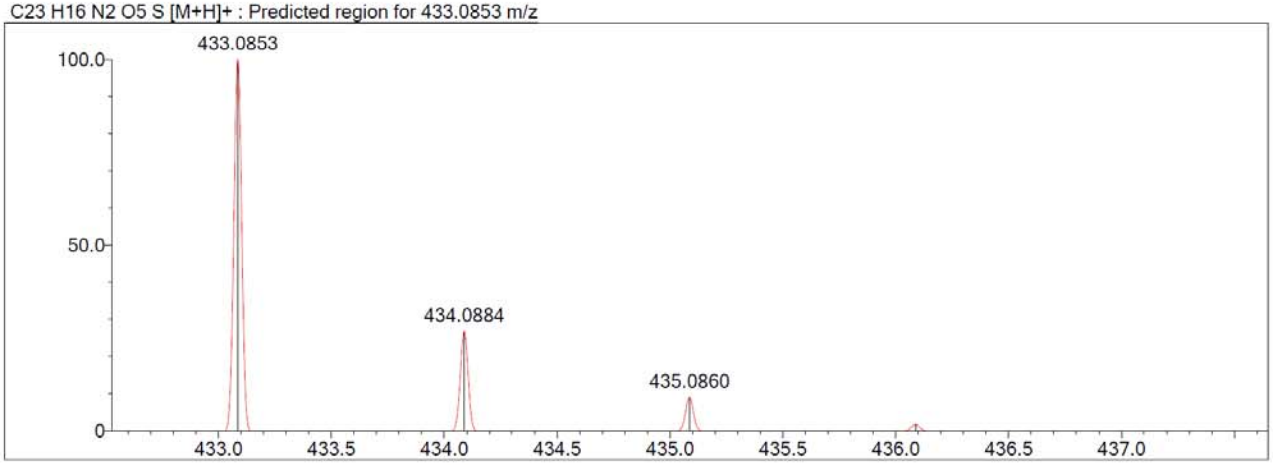


Measured region for 433.0858 m/z

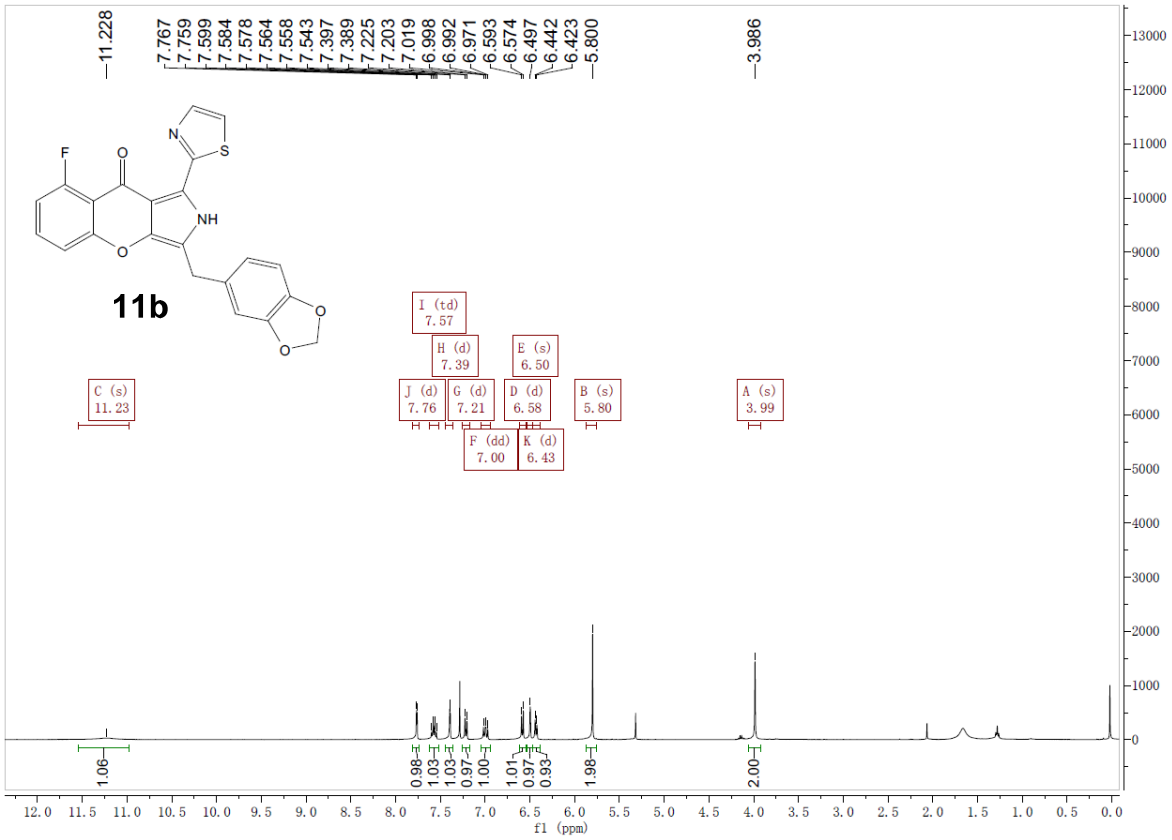


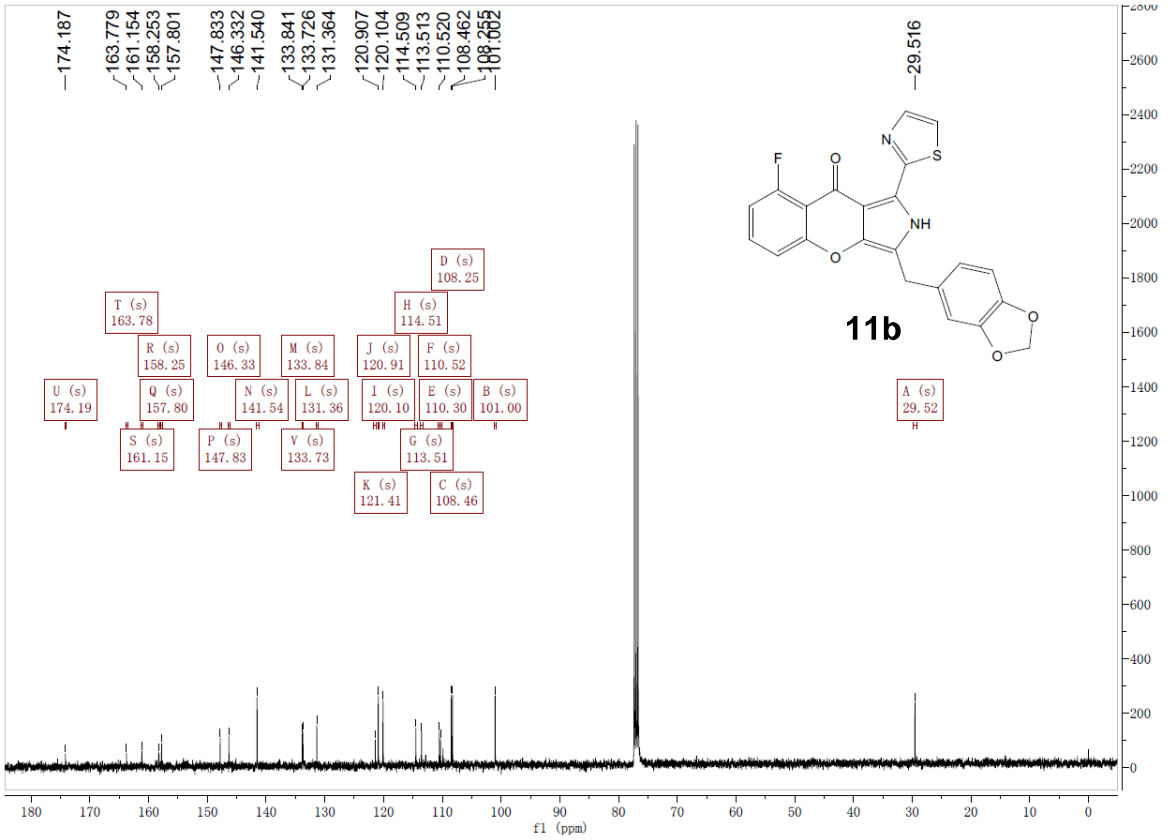
11a

C23 H16 N2 O5 S [M+H]+ : Predicted region for 433.0853 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	84.40	C23 H16 N2 O5 S	[M+H]+	433.0858	433.0853	0.5	1.15	84.72	17.0

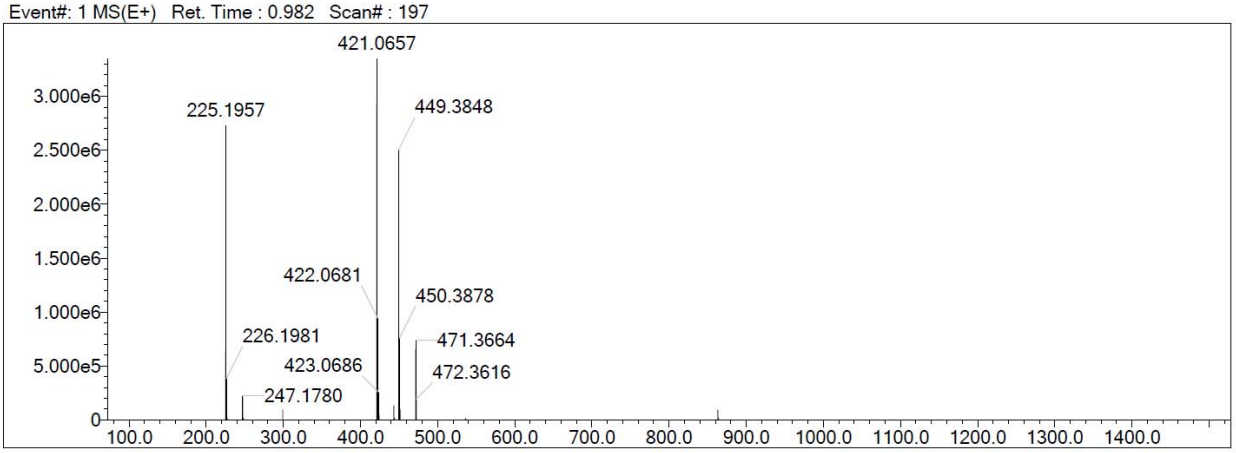

11.228
7.767
7.759
7.599
7.584
7.578
7.564
7.558
7.543
7.397
7.389
7.225
7.203
7.019
6.998
6.992
6.971
6.593
6.574
6.497
6.442
6.423
5.800
3.986
11b
I (td) 7.57
H (d) 7.39
E (s) 6.50
C (s) 11.23
J (d) 7.76
G (d) 7.21
D (d) 6.58
B (s) 5.80
A (s) 3.99
F (dd) 7.00
K (d) 6.43
1.06
0.98
1.03
1.03
0.97
1.00
1.01
0.97
0.93
1.98
2.00
f1 (ppm)


174.187
163.779
161.154
158.253
157.801
147.833
146.332
141.540
133.841
133.726
131.364
120.907
120.104
114.509
113.513
110.520
108.462
101.602
29.516
11b
D (s) 108.25
T (s) 163.78
H (s) 114.51
R (s) 158.25
O (s) 146.33
M (s) 133.84
J (s) 120.91
F (s) 110.52
U (s) 174.19
Q (s) 157.80
N (s) 141.54
L (s) 131.36
I (s) 120.10
E (s) 110.30
B (s) 101.00
A (s) 29.52
S (s) 161.15
P (s) 147.83
V (s) 133.73
G (s) 113.51
K (s) 121.41
C (s) 108.46
f1 (ppm)

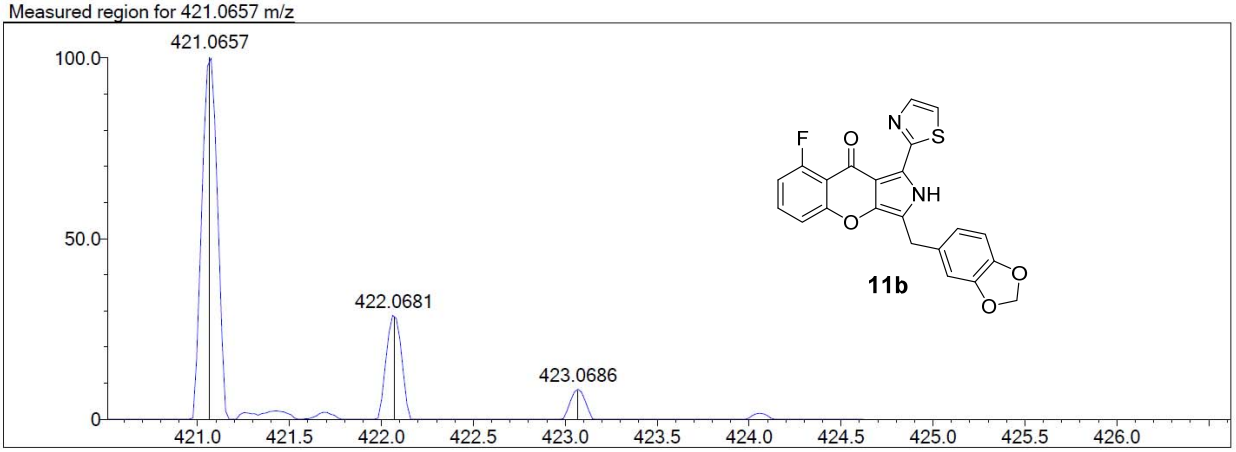
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	no	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

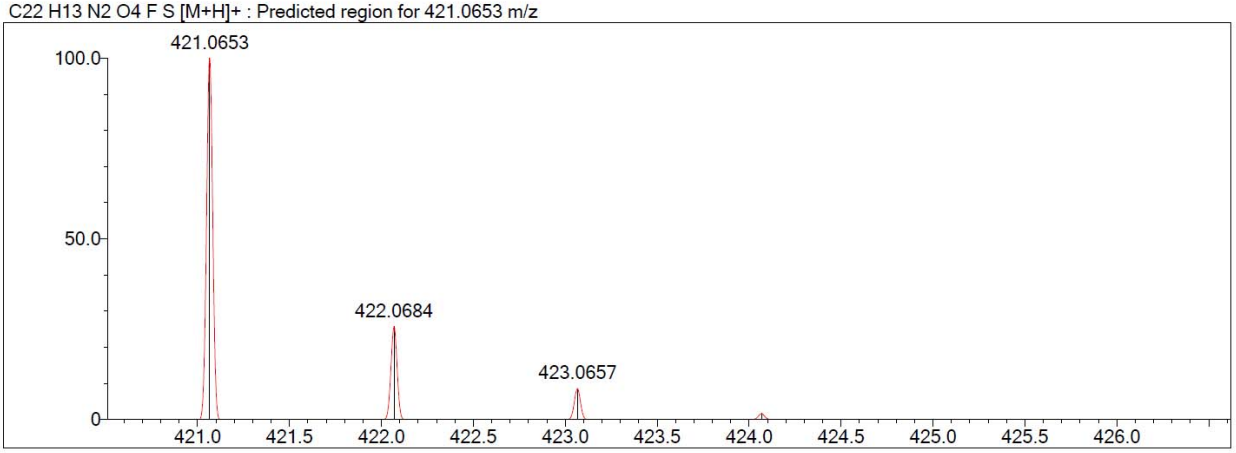
Event#: 1 MS(E+) Ret. Time : 0.982 Scan# : 197



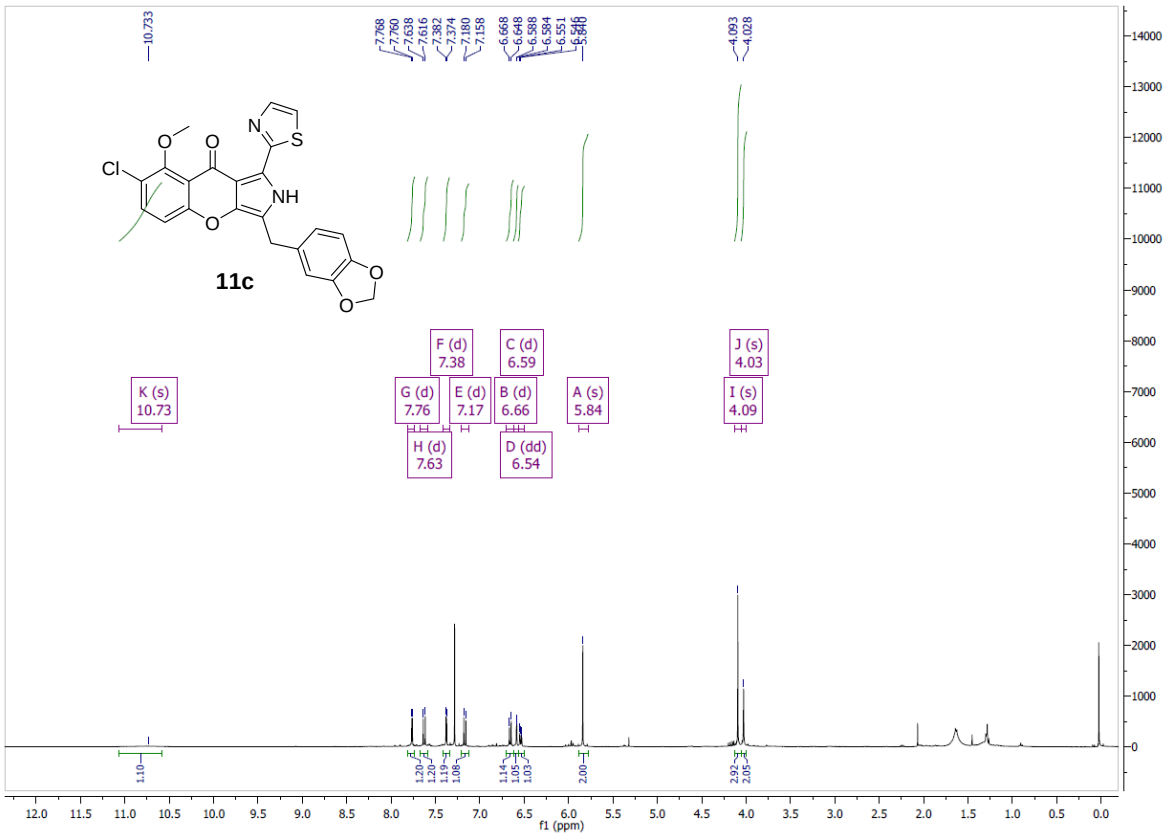
Measured region for 421.0657 m/z

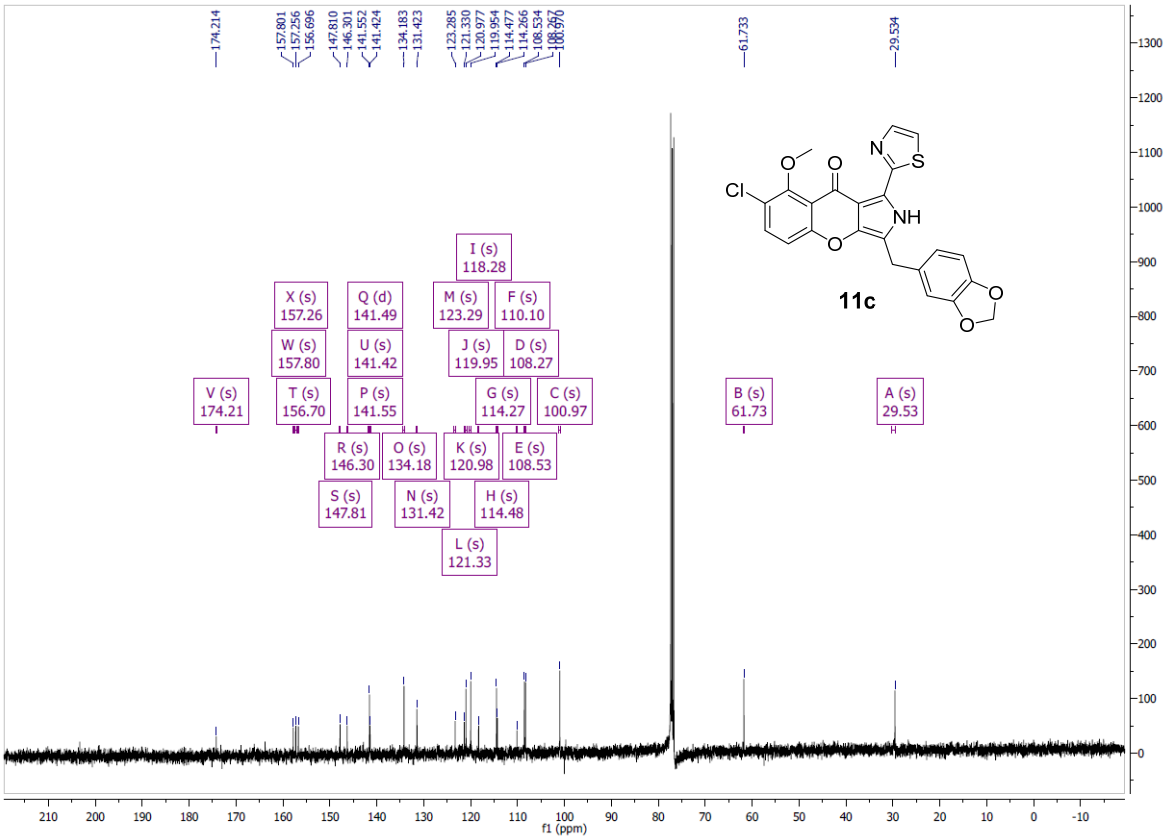


C22 H13 N2 O4 F S [M+H]+ : Predicted region for 421.0653 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	85.76	C22 H13 N2 O4 F S	[M+H]+	421.0657	421.0653	0.4	0.95	85.76	17.0


11c
K (s) 10.73
F (d) 7.38
C (d) 6.59
J (s) 4.03
G (d) 7.76
E (d) 7.17
B (d) 6.66
A (s) 5.84
I (s) 4.09
H (d) 7.63
D (dd) 6.54
f1 (ppm)


11c
I (s) 118.28
X (s) 157.26
Q (d) 141.49
M (s) 123.29
F (s) 110.10
W (s) 157.80
U (s) 141.42
J (s) 119.95
D (s) 108.27
V (s) 174.21
T (s) 156.70
P (s) 141.55
G (s) 114.27
C (s) 100.97
R (s) 146.30
O (s) 134.18
K (s) 120.98
E (s) 108.53
S (s) 147.81
N (s) 131.42
H (s) 114.48
L (s) 121.33
B (s) 61.73
A (s) 29.53
f1 (ppm)

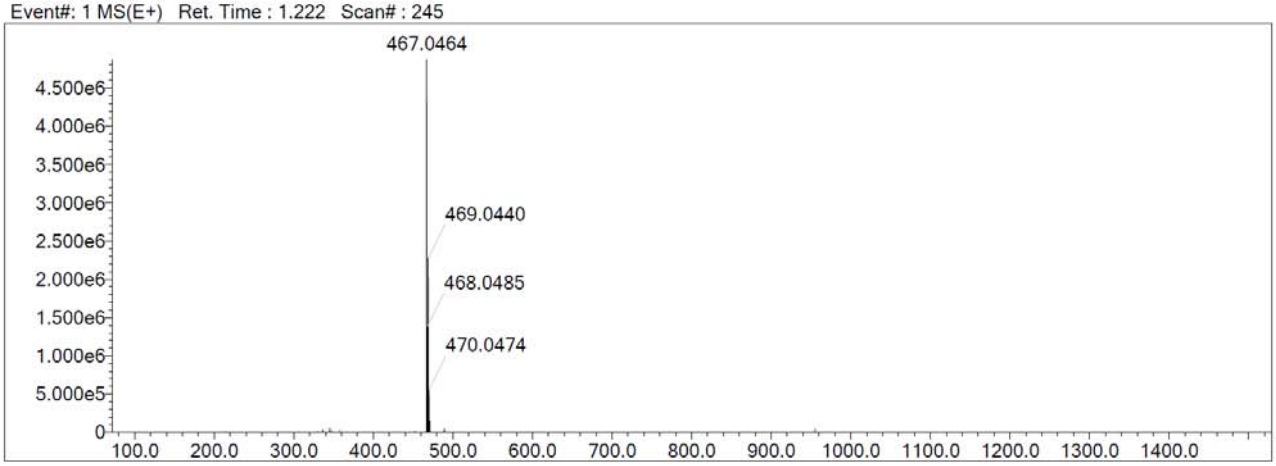
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

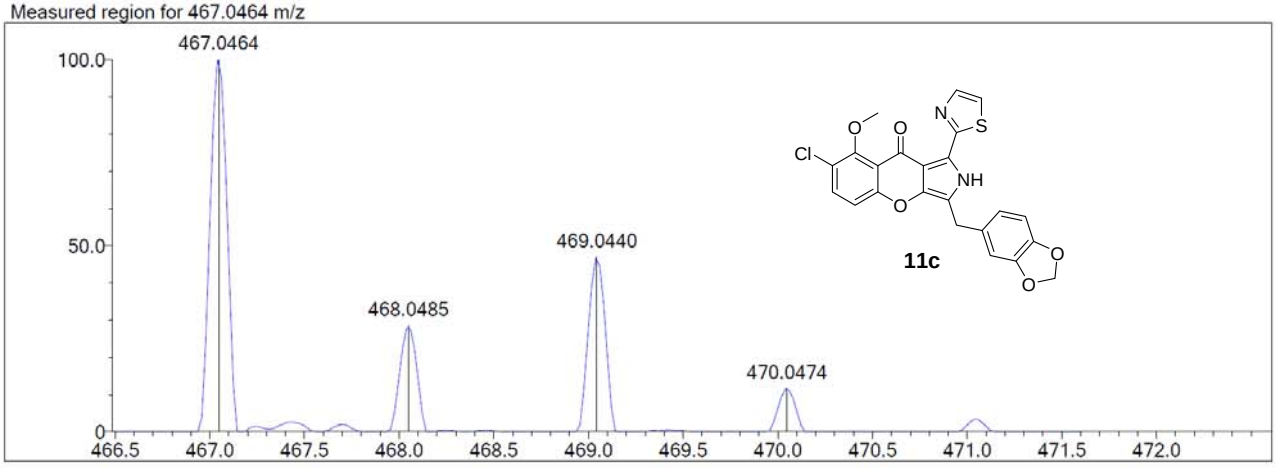
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.222 Scan# : 245

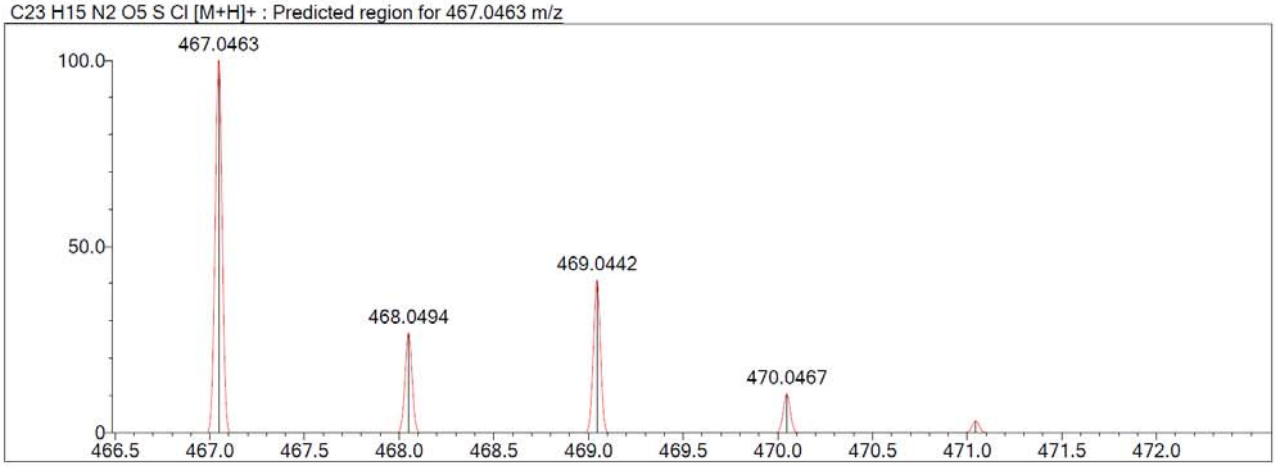


Measured region for 467.0464 m/z

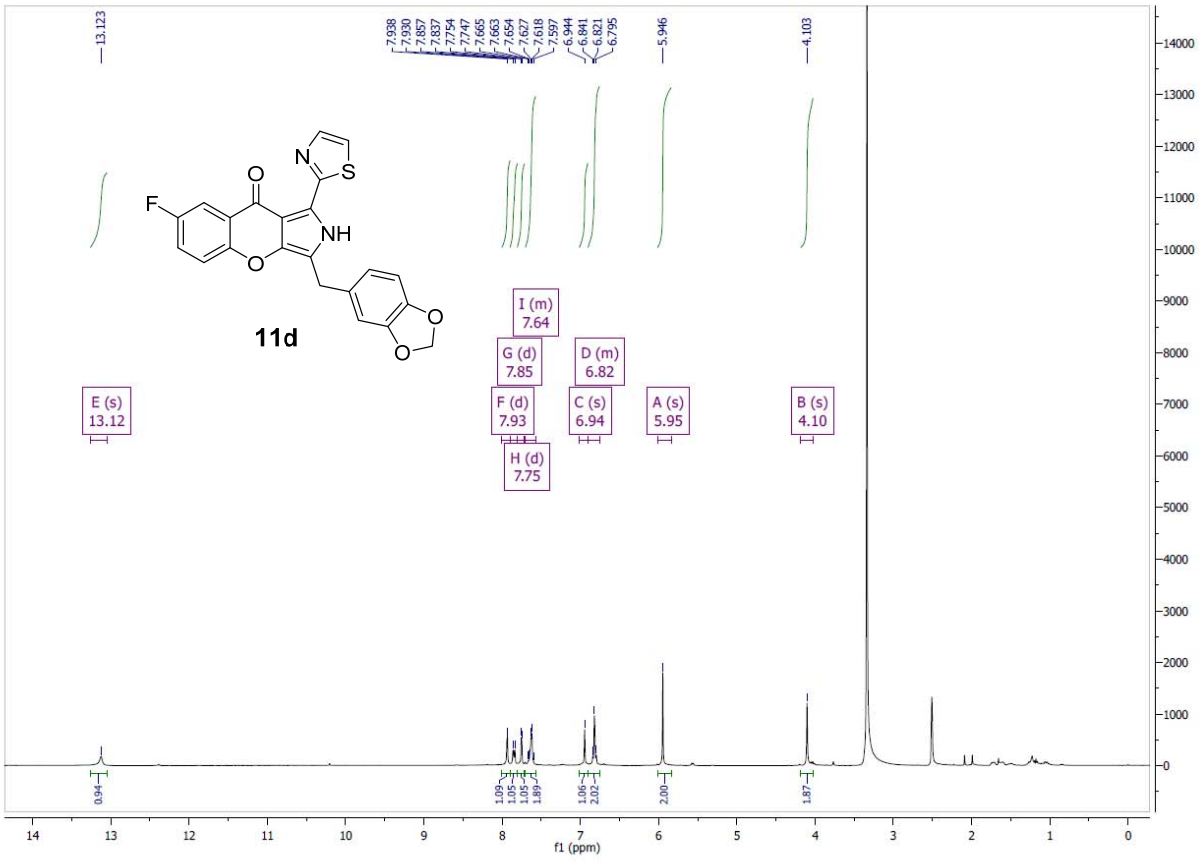


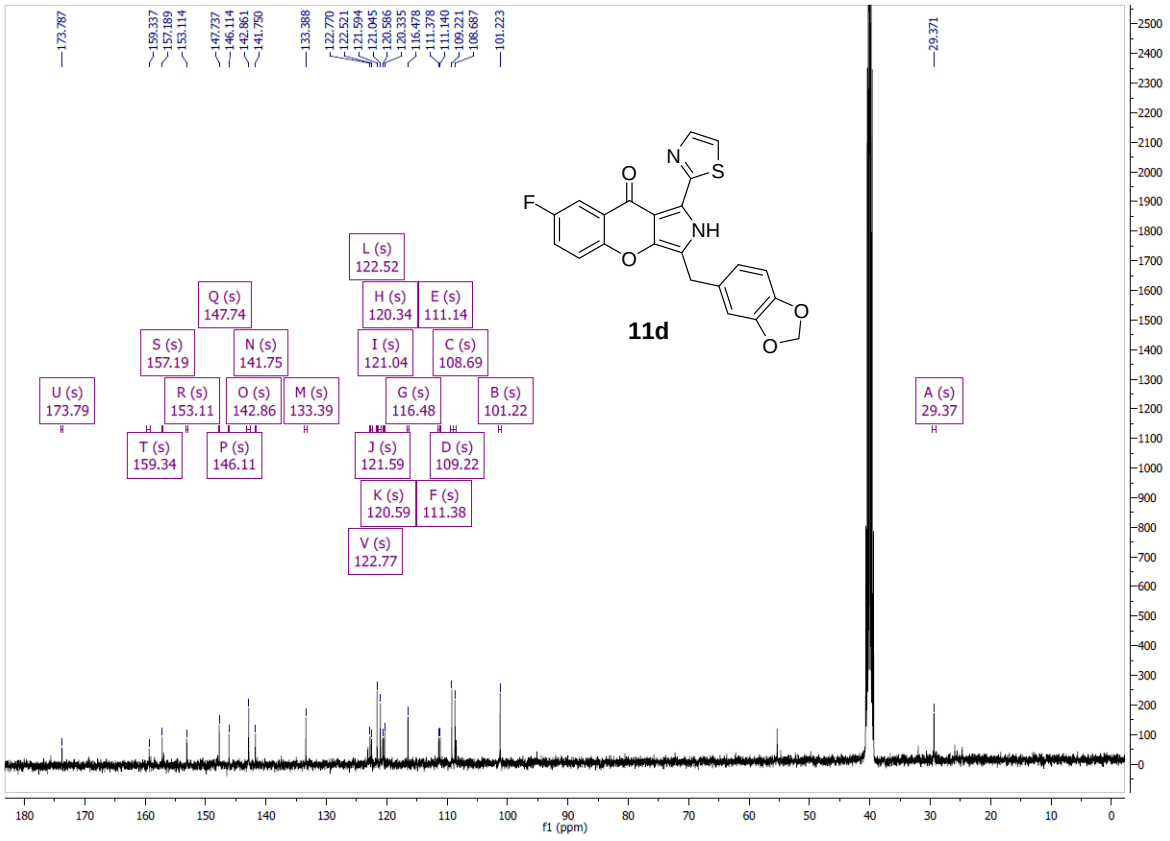
11c

C23 H15 N2 O5 S Cl [M+H]+ : Predicted region for 467.0463 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	94.70	C23 H15 N2 O5 S Cl	[M+H]+	467.0464	467.0463	0.1	0.21	94.70	17.0


13.123
7.938
7.930
7.857
7.837
7.754
7.747
7.665
7.663
7.654
7.627
7.618
7.597
6.944
6.841
6.821
6.795
5.946
4.103
11d
E (s) 13.12
I (m) 7.64
G (d) 7.85
F (d) 7.93
H (d) 7.75
D (m) 6.82
C (s) 6.94
A (s) 5.95
B (s) 4.10
f1 (ppm)


173.787
159.337
157.189
153.114
147.737
146.114
142.861
141.750
133.388
122.770
122.521
121.594
121.045
120.586
120.335
116.478
111.378
111.140
109.221
108.687
101.223
29.371
11d
L (s) 122.52
Q (s) 147.74
H (s) 120.34
E (s) 111.14
S (s) 157.19
N (s) 141.75
I (s) 121.04
C (s) 108.69
U (s) 173.79
R (s) 153.11
O (s) 142.86
M (s) 133.39
G (s) 116.48
B (s) 101.22
A (s) 29.37
T (s) 159.34
P (s) 146.11
J (s) 121.59
D (s) 109.22
K (s) 120.59
F (s) 111.38
V (s) 122.77
f1 (ppm)

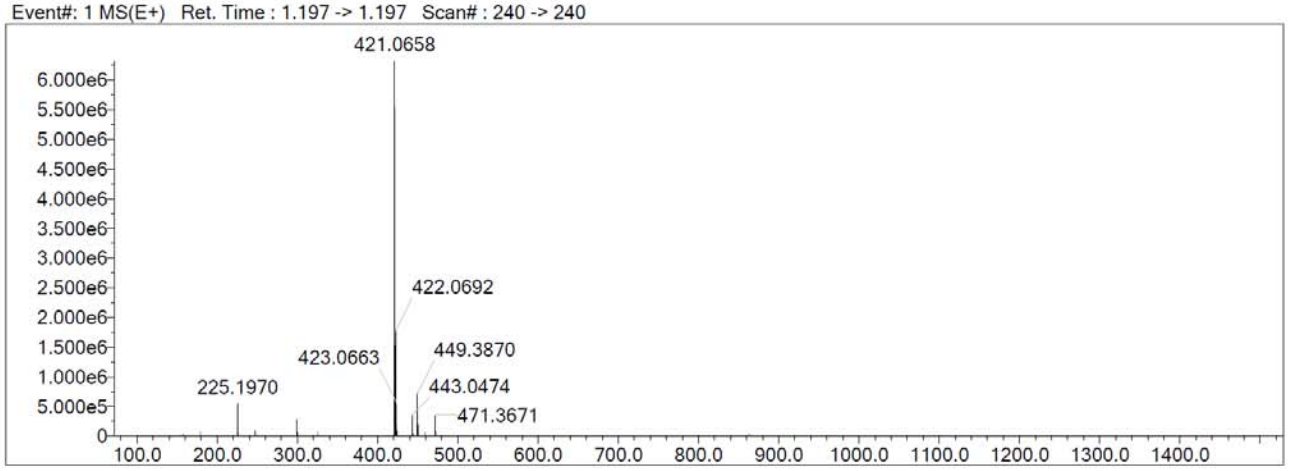
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

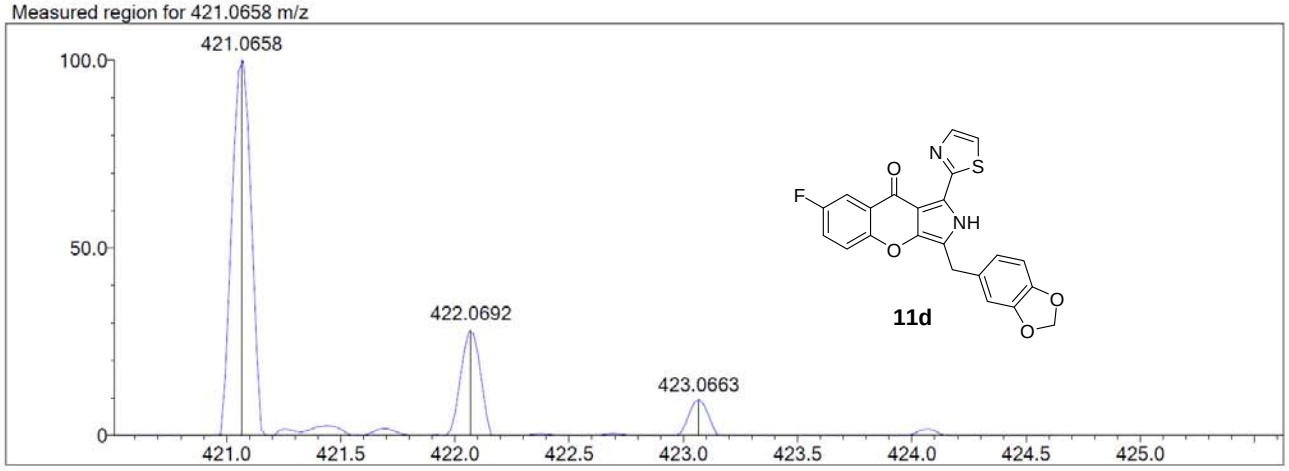
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

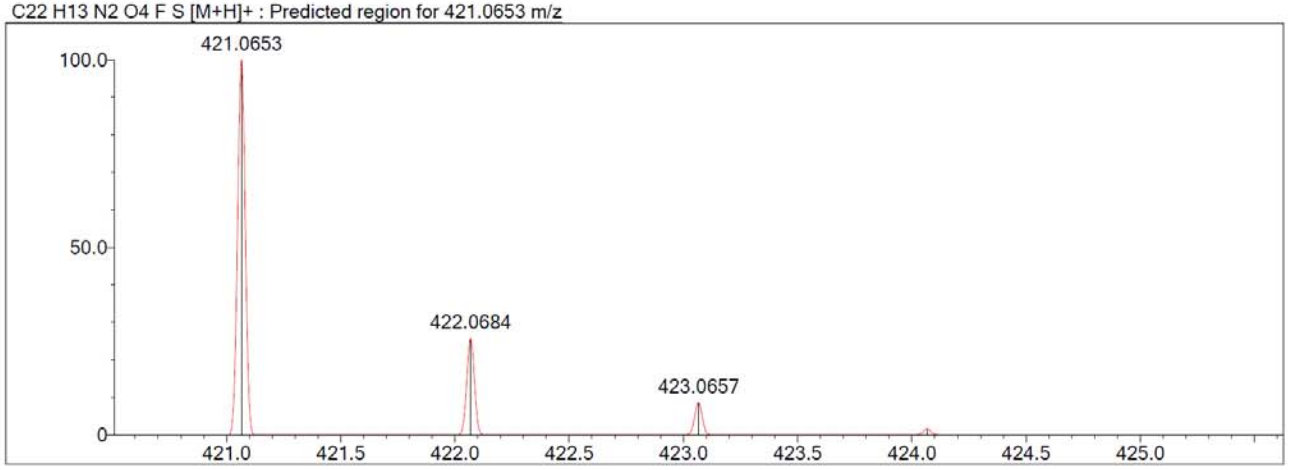
Event#: 1 MS(E+) Ret. Time : 1.197 -> 1.197 Scan# : 240 -> 240



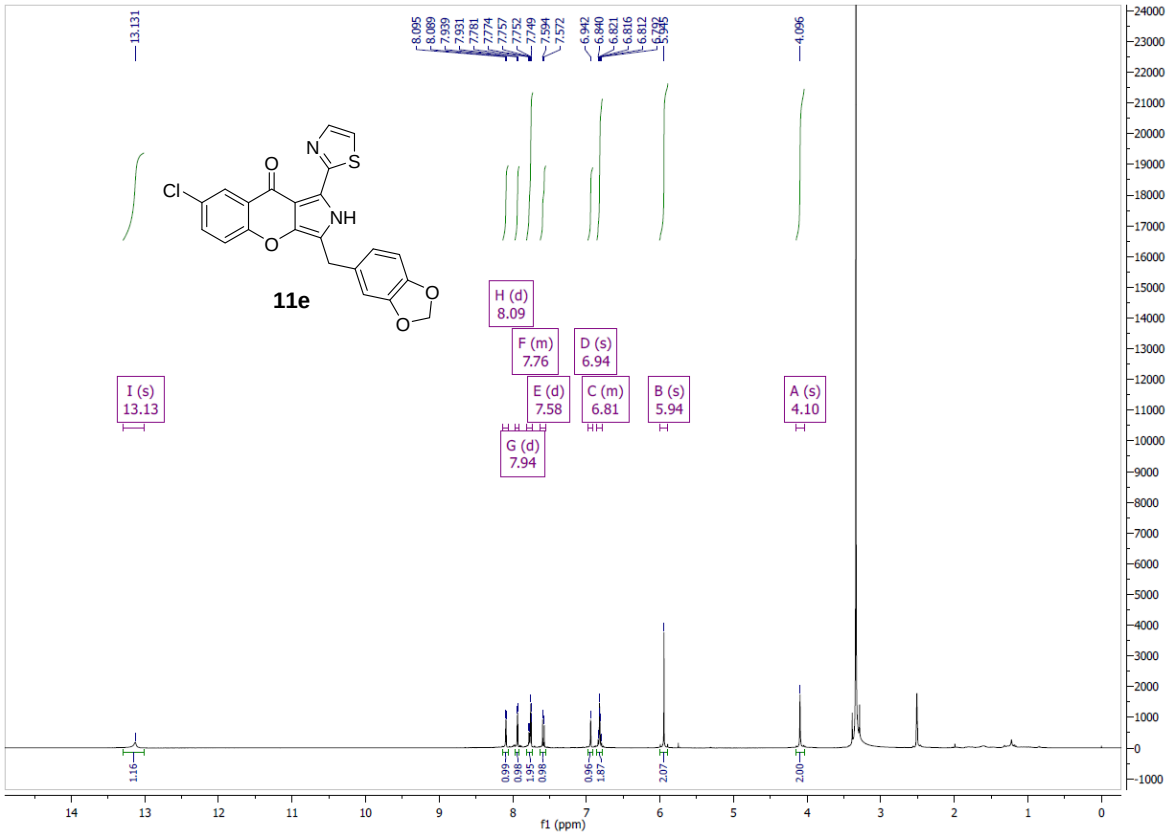
Measured region for 421.0658 m/z

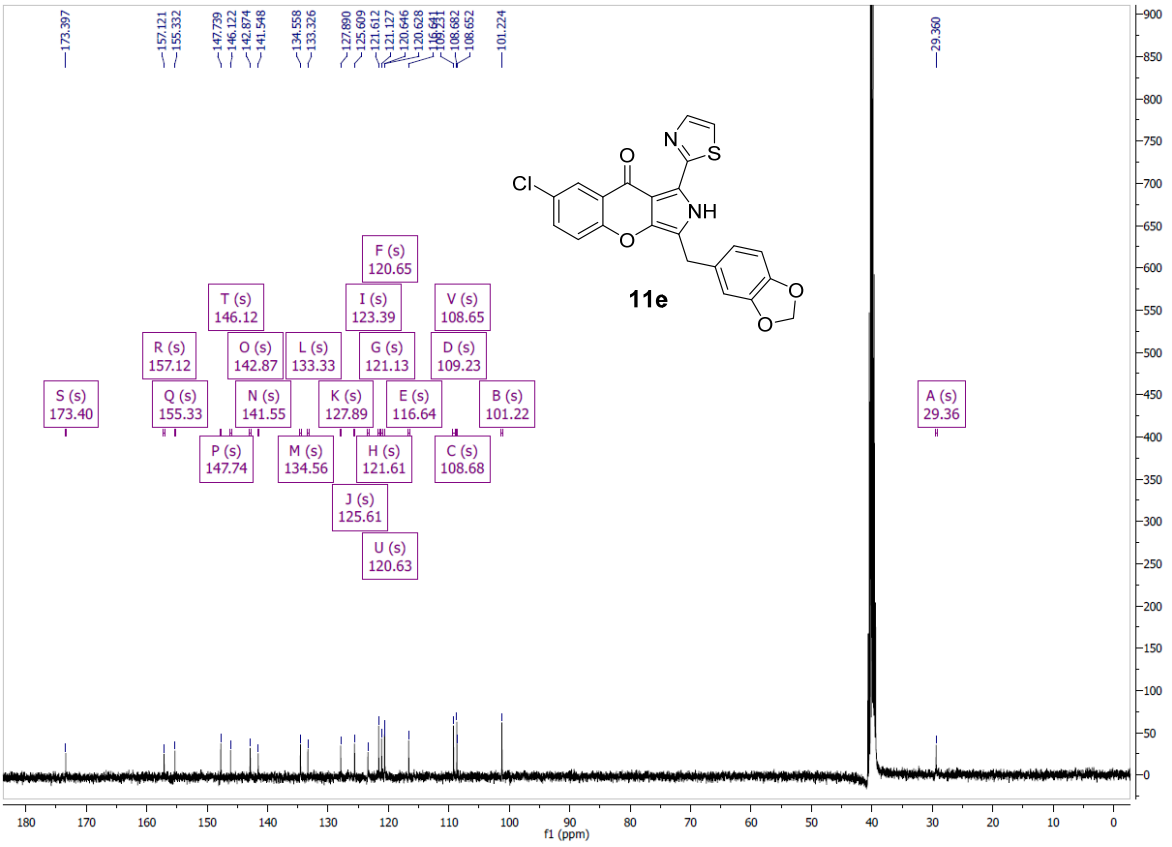


C22 H13 N2 O4 F S [M+H]+ : Predicted region for 421.0653 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	91.57	C22 H13 N2 O4 F S	[M+H]+	421.0658	421.0653	0.5	1.19	92.01	17.0


11e
H (d) 8.09
F (m) 7.76
D (s) 6.94
E (d) 7.58
C (m) 6.81
B (s) 5.94
A (s) 4.10
I (s) 13.13
G (d) 7.94
f1 (ppm)


11e
F (s) 120.65
T (s) 146.12
I (s) 123.39
V (s) 108.65
R (s) 157.12
O (s) 142.87
L (s) 133.33
G (s) 121.13
D (s) 109.23
S (s) 173.40
Q (s) 155.33
N (s) 141.55
K (s) 127.89
E (s) 116.64
B (s) 101.22
A (s) 29.36
P (s) 147.74
M (s) 134.56
H (s) 121.61
C (s) 108.68
J (s) 125.61
U (s) 120.63
f1 (ppm)

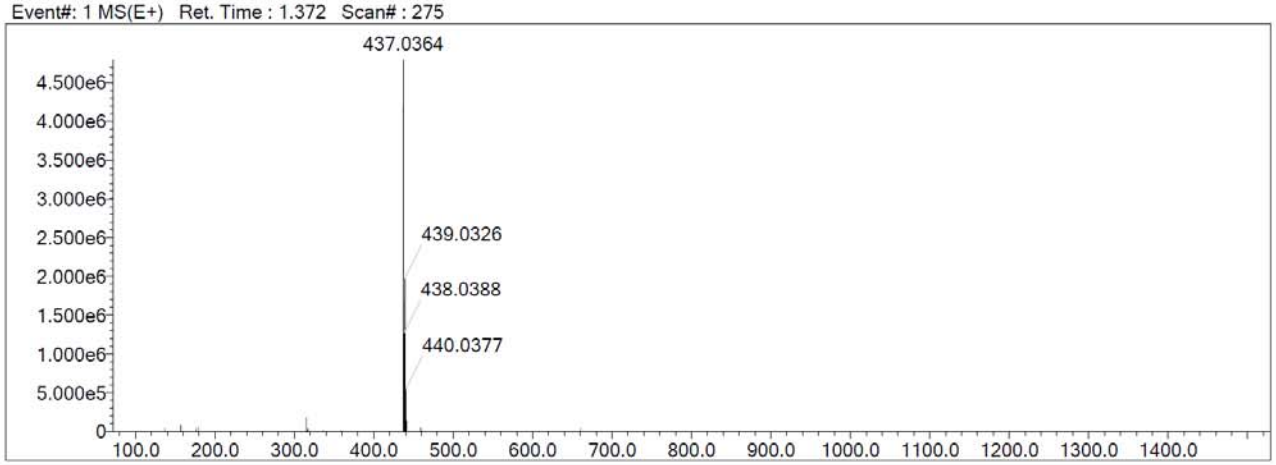
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

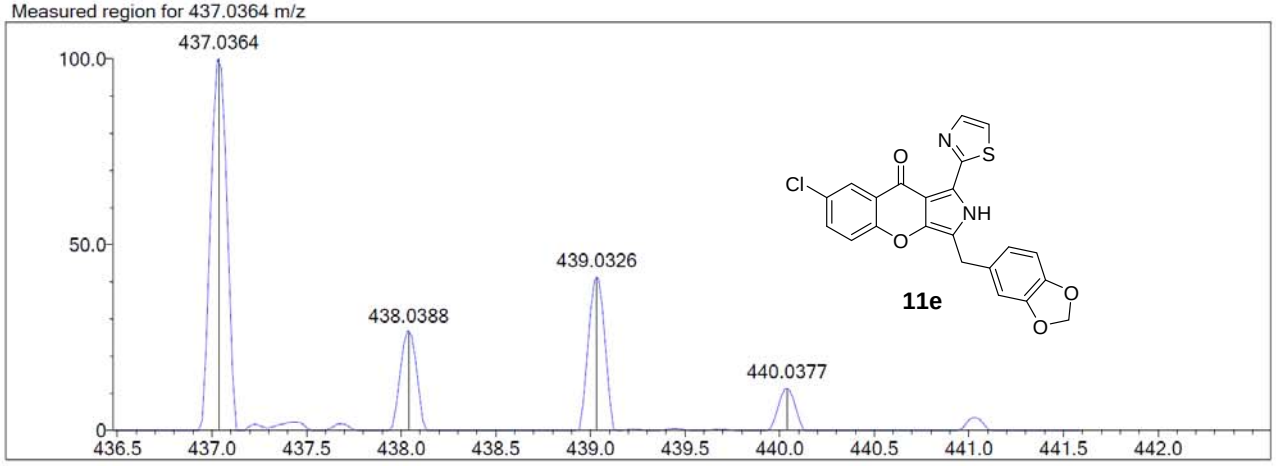
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.372 Scan# : 275

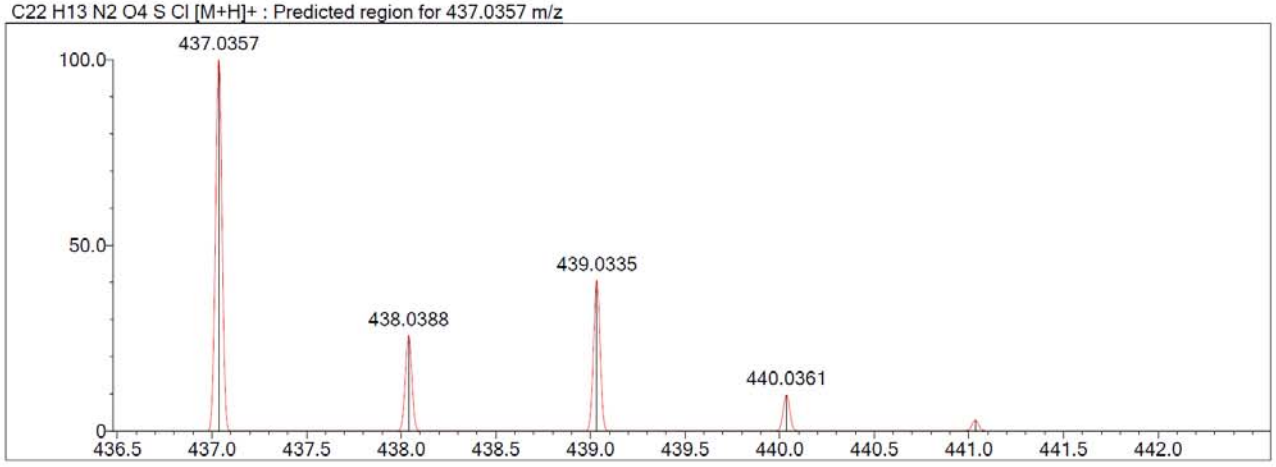


Measured region for 437.0364 m/z

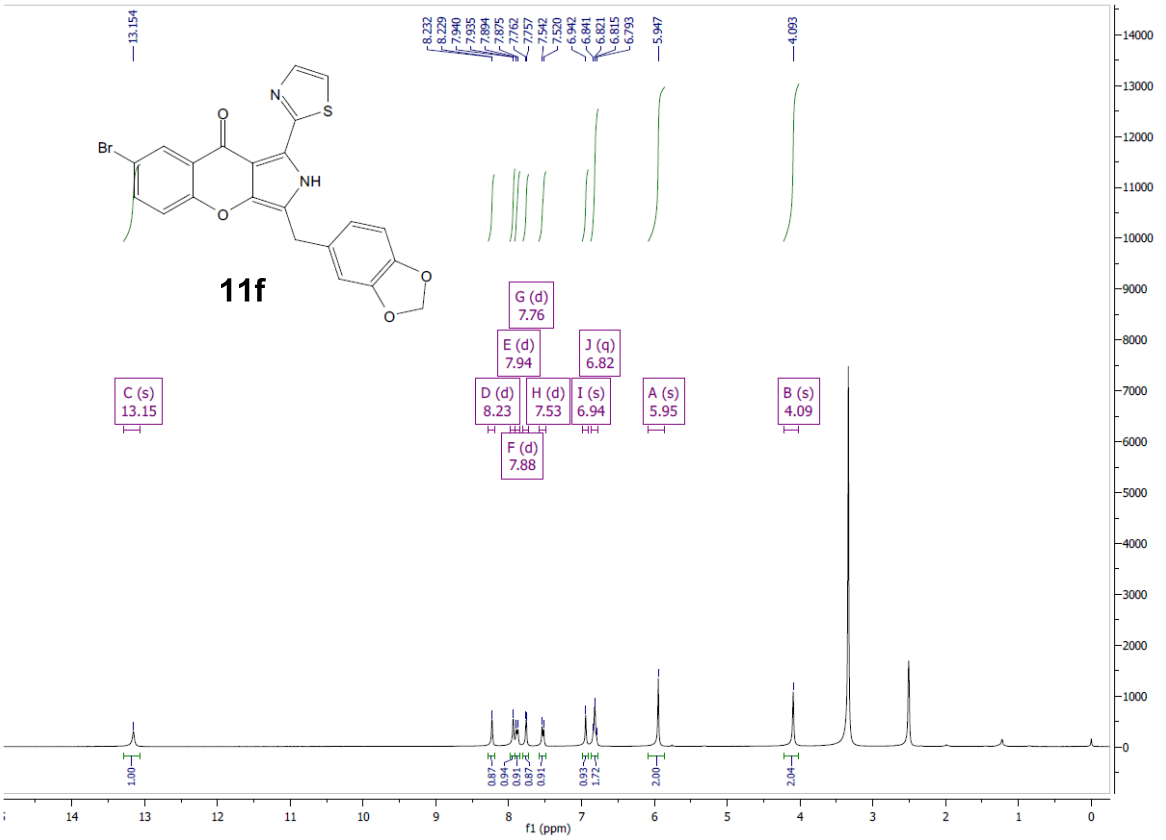


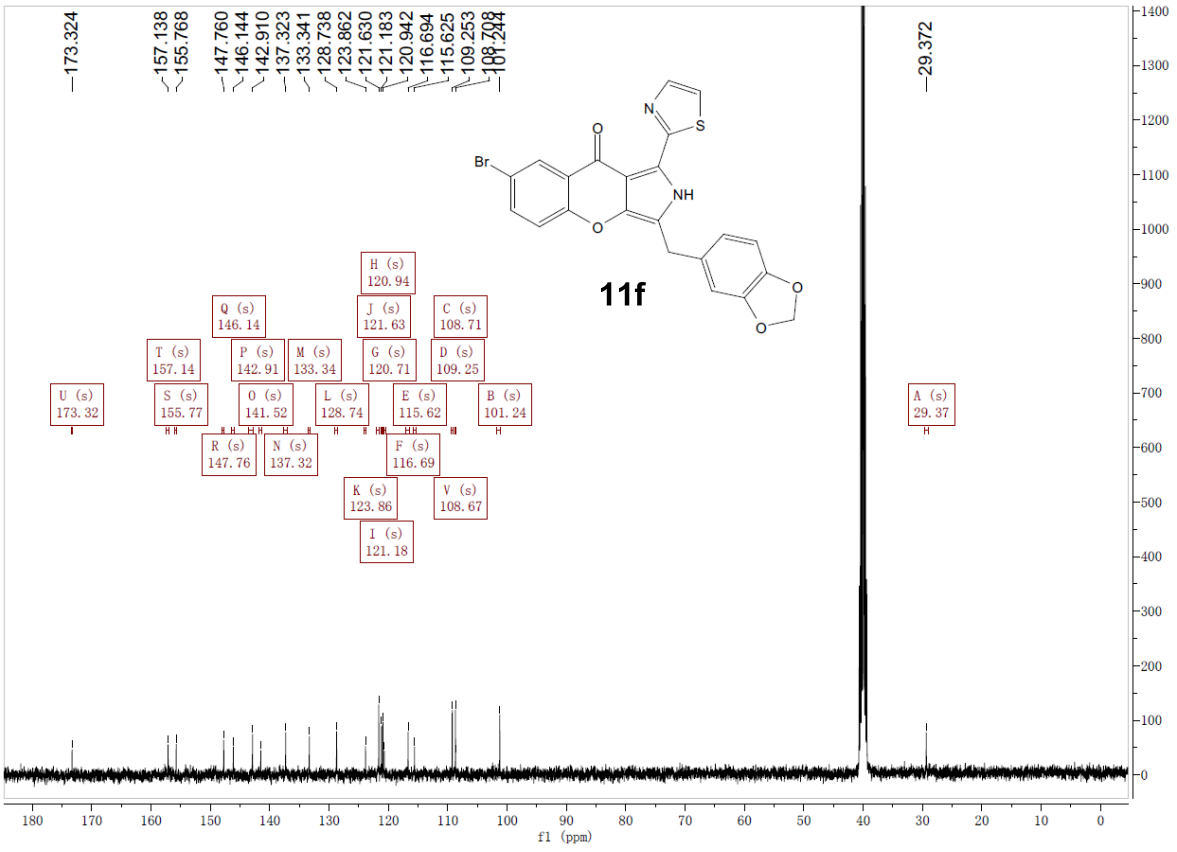
11e

C22 H13 N2 O4 S Cl [M+H]+ : Predicted region for 437.0357 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	90.15	C22 H13 N2 O4 S Cl	[M+H]+	437.0364	437.0357	0.7	1.60	91.52	17.0


11f
C (s) 13.15
G (d) 7.76
E (d) 7.94
J (q) 6.82
D (d) 8.23
H (d) 7.53
I (s) 6.94
A (s) 5.95
B (s) 4.09
F (d) 7.88
f1 (ppm)


11f
f1 (ppm)

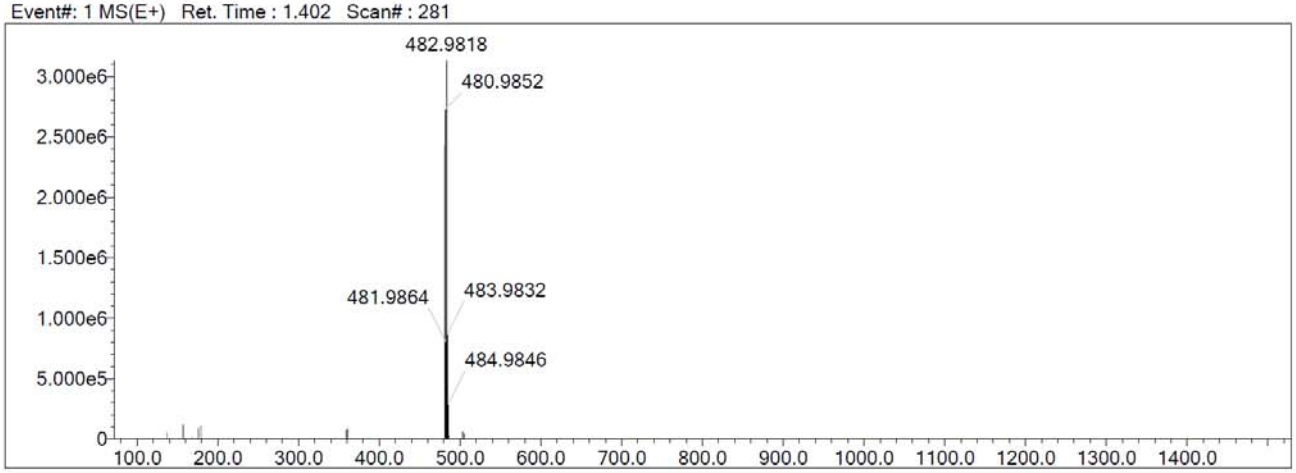
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	2	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

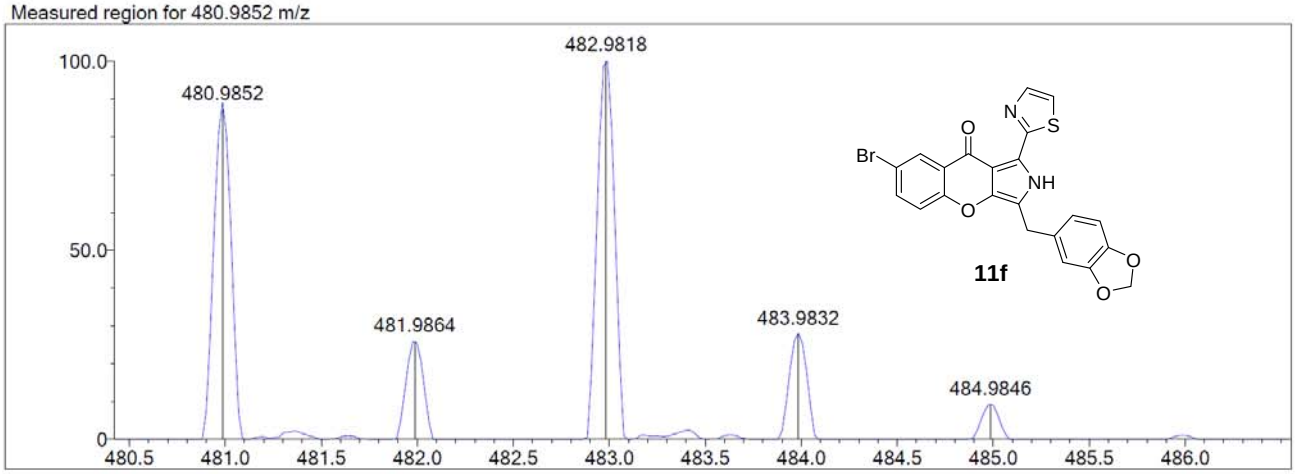
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.402 Scan# : 281

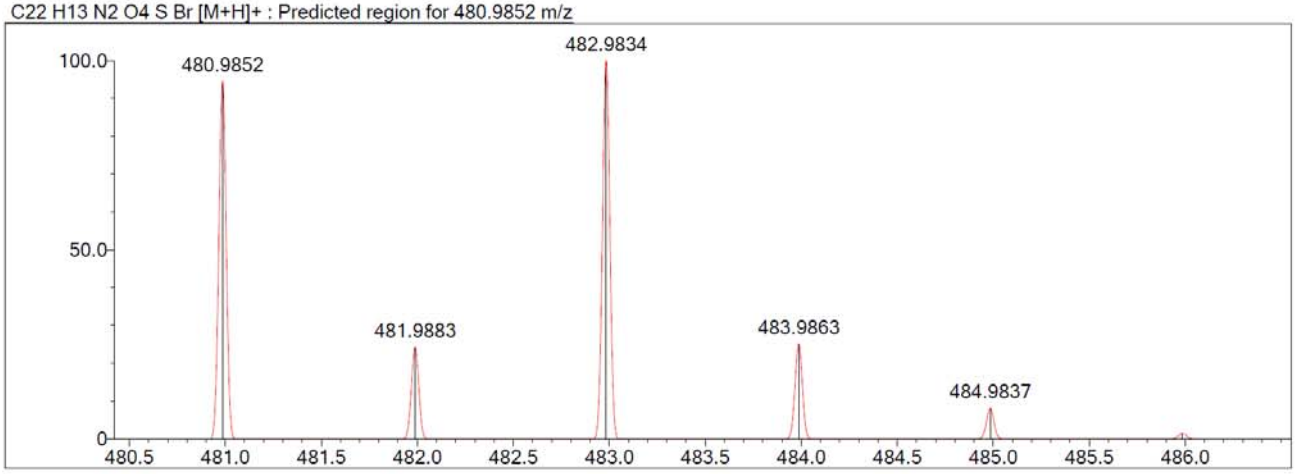


Measured region for 480.9852 m/z

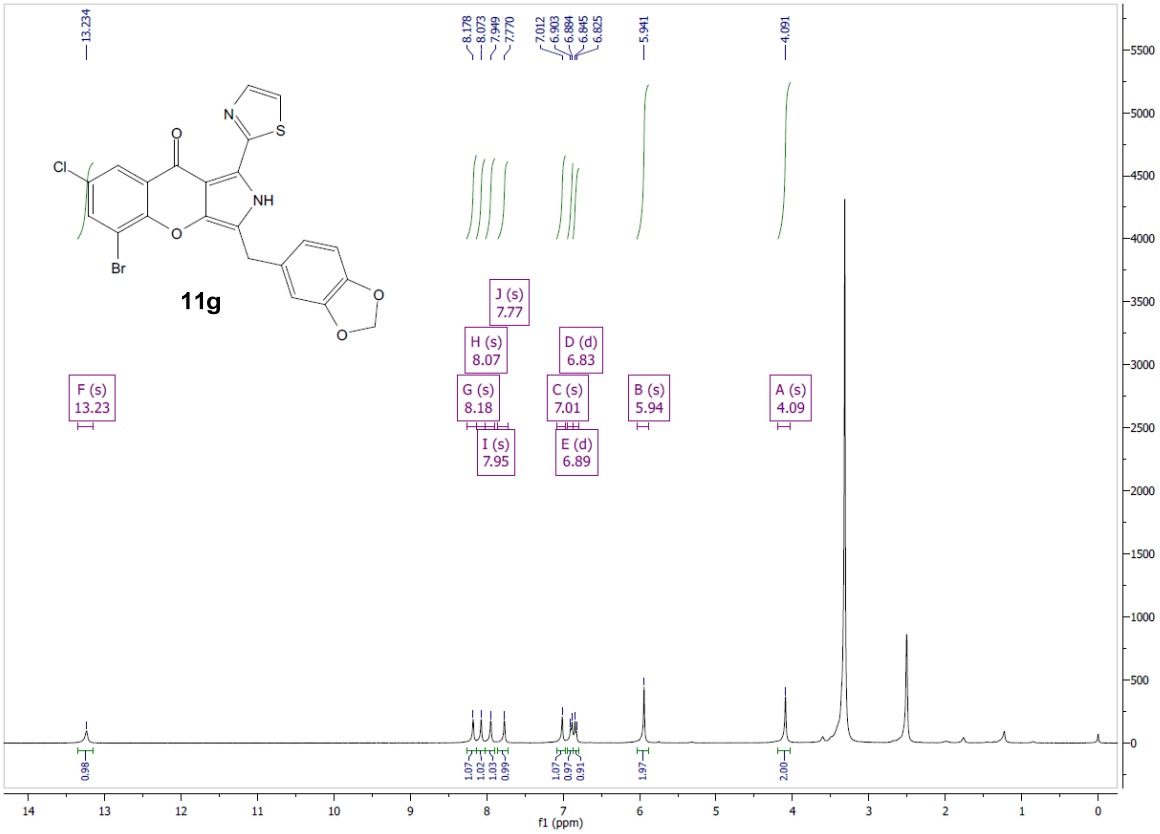


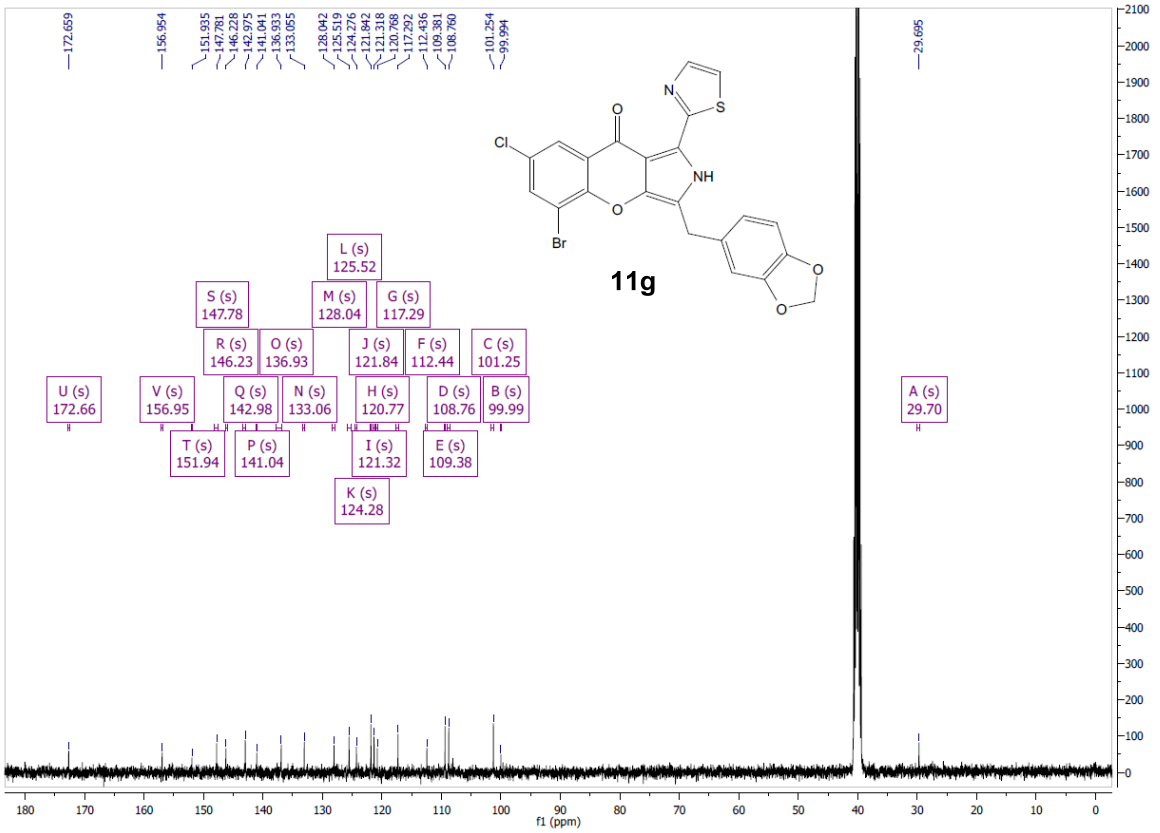
11f

C22 H13 N2 O4 S Br [M+H]+ : Predicted region for 480.9852 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	96.86	C22 H13 N2 O4 S Br	[M+H]+	480.9852	480.9852	-0.0	0.00	96.86	17.0

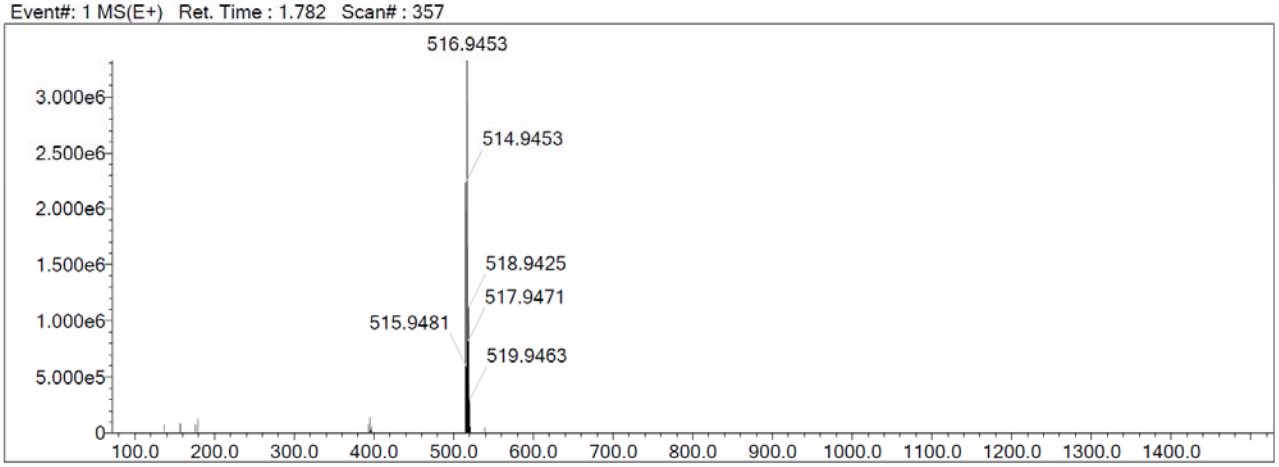

11g
F (s) 13.23
G (s) 8.18
H (s) 8.07
I (s) 7.95
J (s) 7.77
C (s) 7.01
D (d) 6.83
E (d) 6.89
B (s) 5.94
A (s) 4.09
f1 (ppm)


11g
L (s) 125.52
S (s) 147.78
M (s) 128.04
G (s) 117.29
R (s) 146.23
O (s) 136.93
J (s) 121.84
F (s) 112.44
C (s) 101.25
U (s) 172.66
V (s) 156.95
Q (s) 142.98
N (s) 133.06
H (s) 120.77
D (s) 108.76
B (s) 99.99
T (s) 151.94
P (s) 141.04
I (s) 121.32
E (s) 109.38
K (s) 124.28
A (s) 29.70
f1 (ppm)

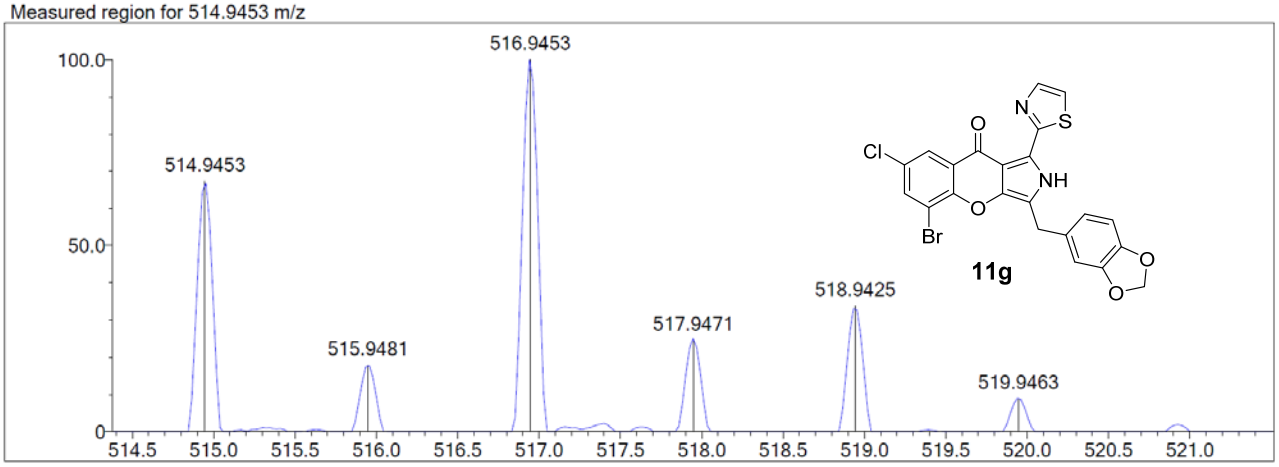
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	2	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	no	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

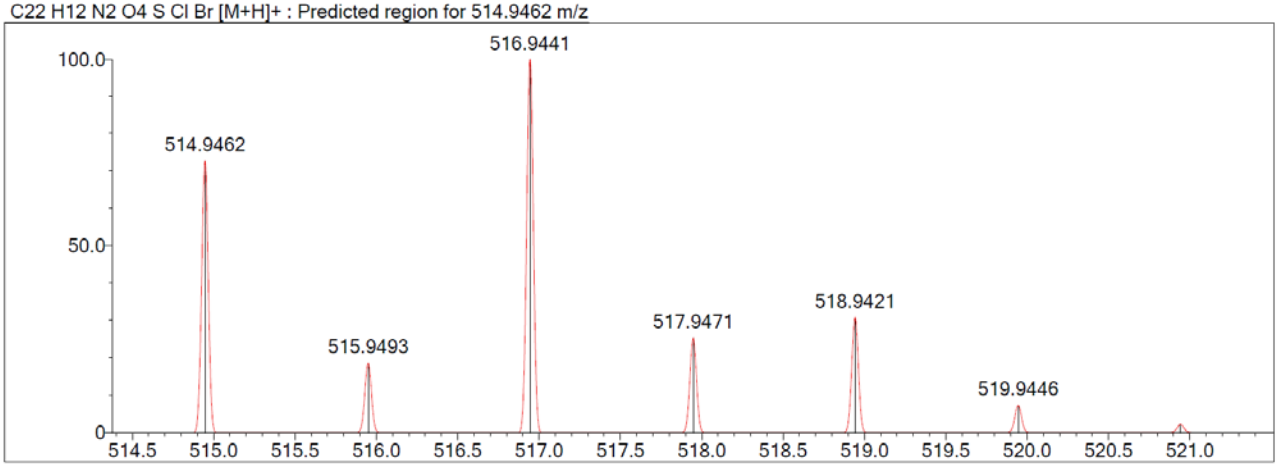
Event#: 1 MS(E+) Ret. Time : 1.782 Scan# : 357



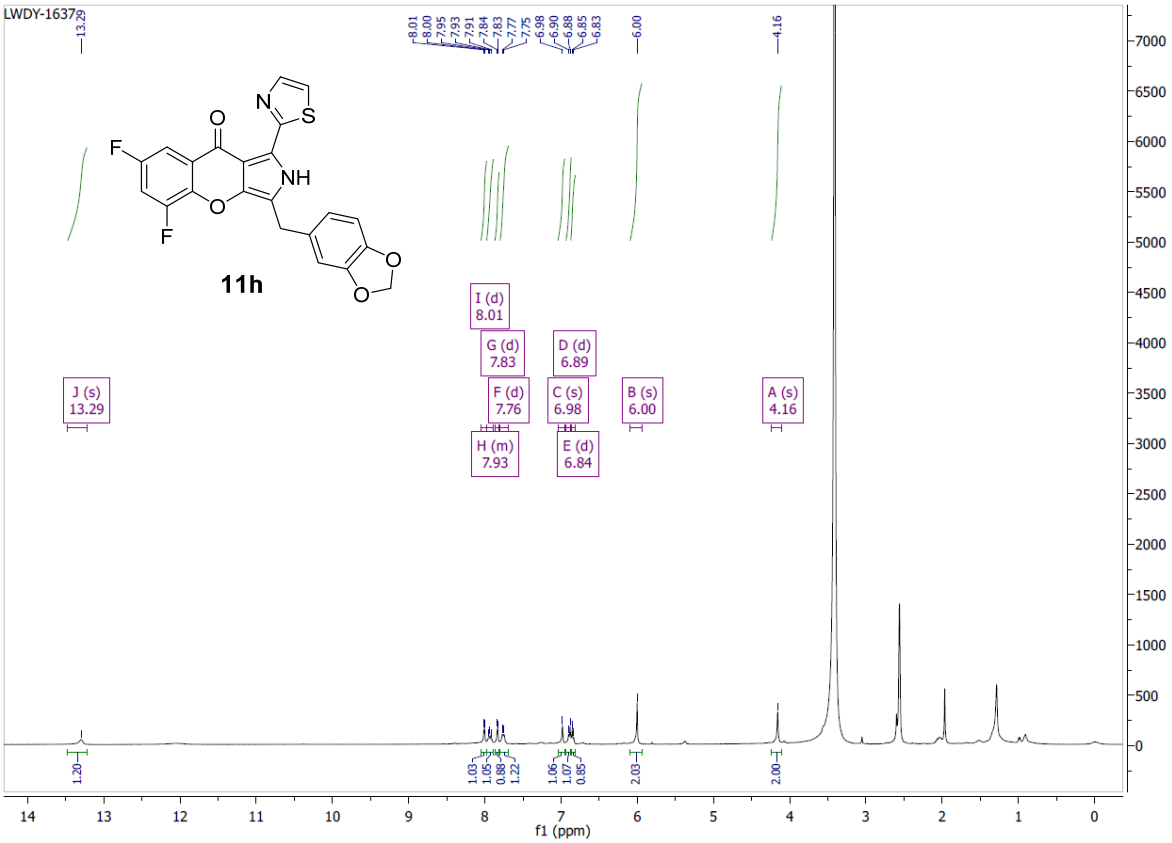
Measured region for 514.9453 m/z

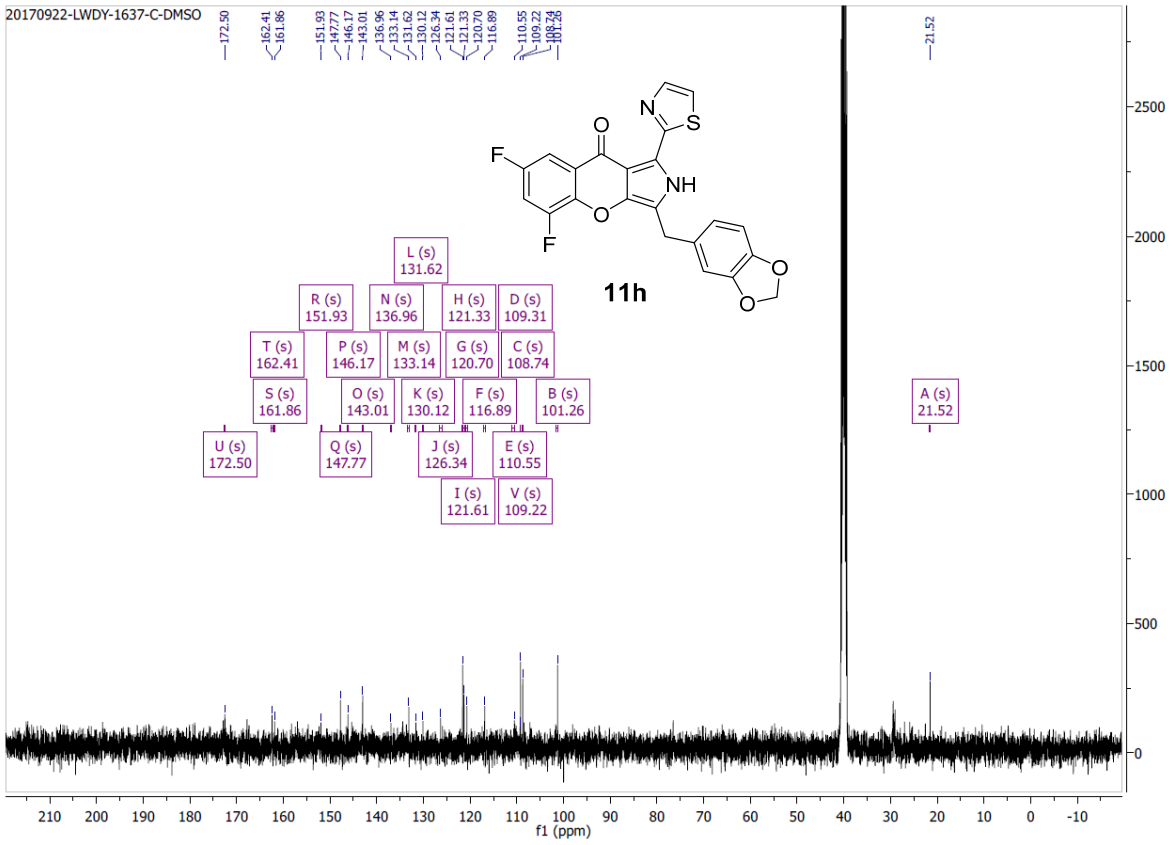


C22 H12 N2 O4 S Cl Br [M+H]+ : Predicted region for 514.9462 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.11	C22 H12 N2 O4 S Cl Br	[M+H]+	514.9453	514.9462	-0.9	-1.75	92.85	17.0

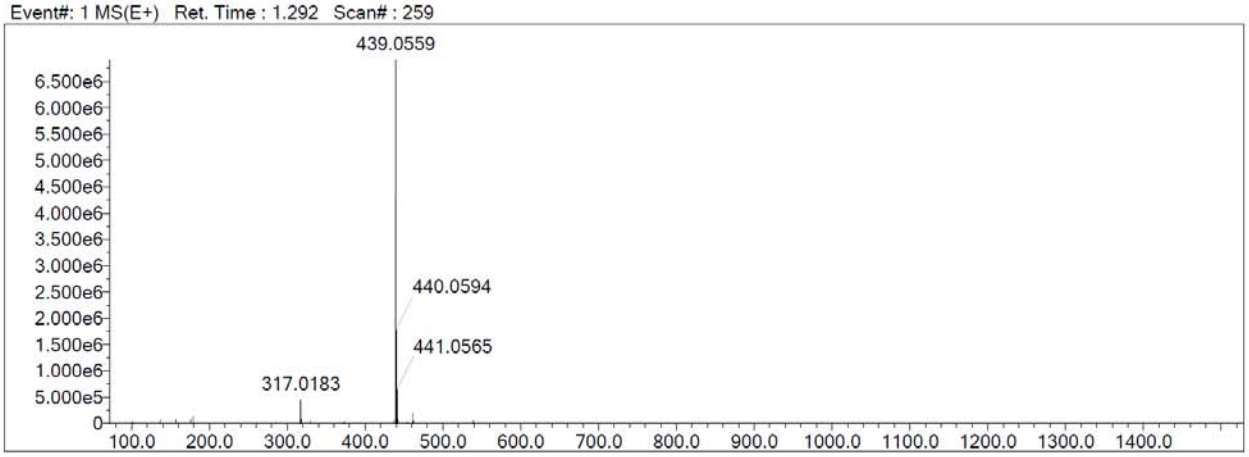

LWDY-1637
11h
J (s) 13.29
I (d) 8.01
G (d) 7.83
F (d) 7.76
H (m) 7.93
D (d) 6.89
C (s) 6.98
E (d) 6.84
B (s) 6.00
A (s) 4.16
f1 (ppm)


20170922-LWDY-1637-C-DMSO
11h
L (s) 131.62
R (s) 151.93
N (s) 136.96
H (s) 121.33
D (s) 109.31
T (s) 162.41
P (s) 146.17
M (s) 133.14
G (s) 120.70
C (s) 108.74
S (s) 161.86
O (s) 143.01
K (s) 130.12
F (s) 116.89
B (s) 101.26
U (s) 172.50
Q (s) 147.77
J (s) 126.34
E (s) 110.55
I (s) 121.61
V (s) 109.22
A (s) 21.52
f1 (ppm)

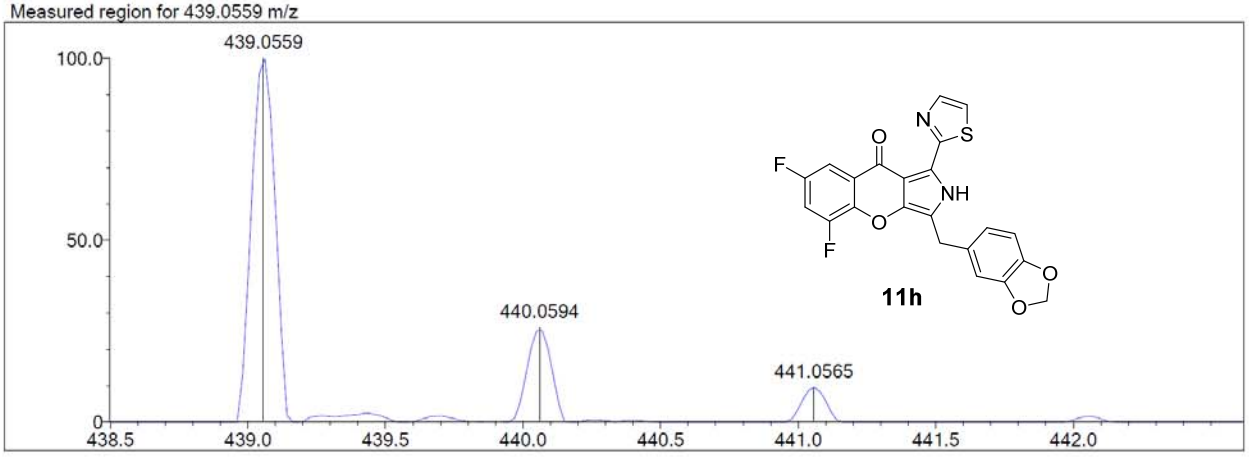
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	2	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	no	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

Event#: 1 MS(E+) Ret. Time : 1.292 Scan# : 259

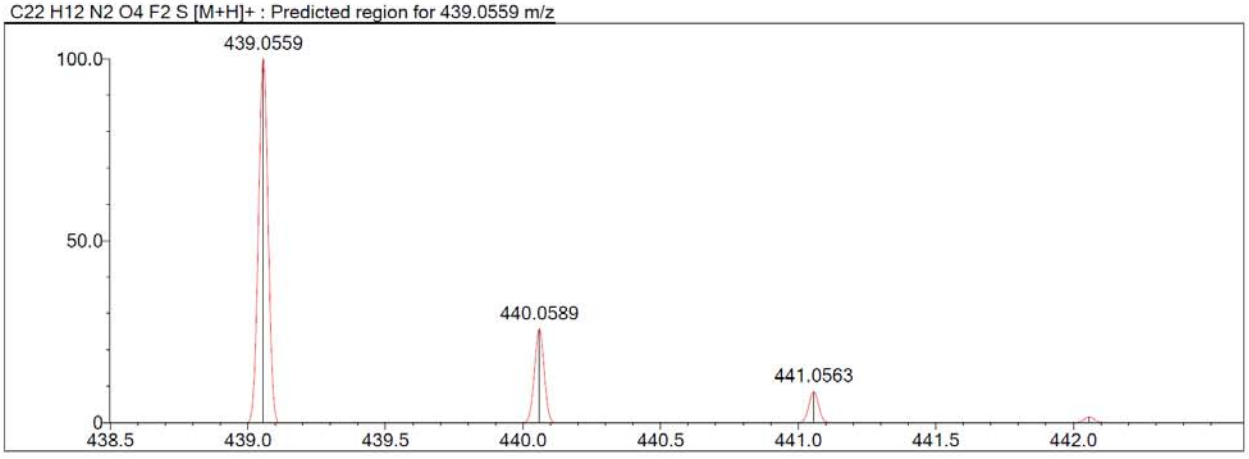


Measured region for 439.0559 m/z

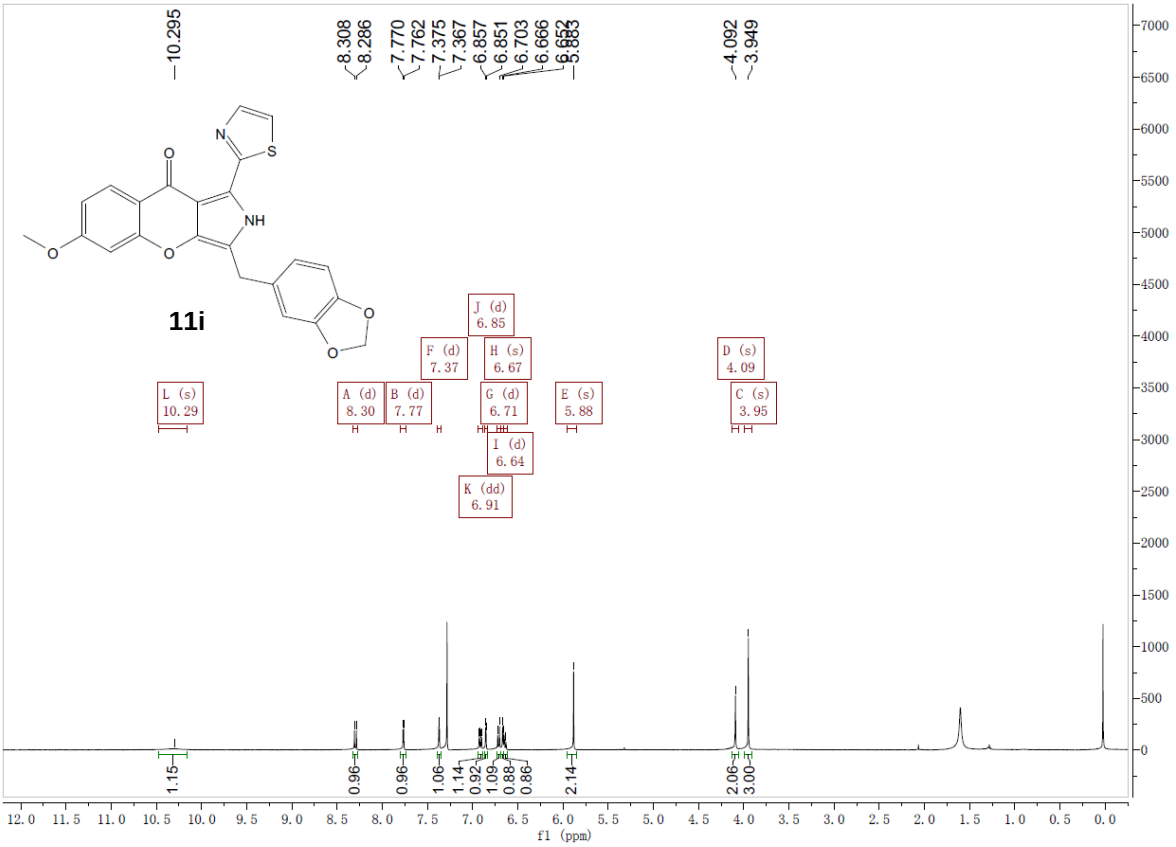


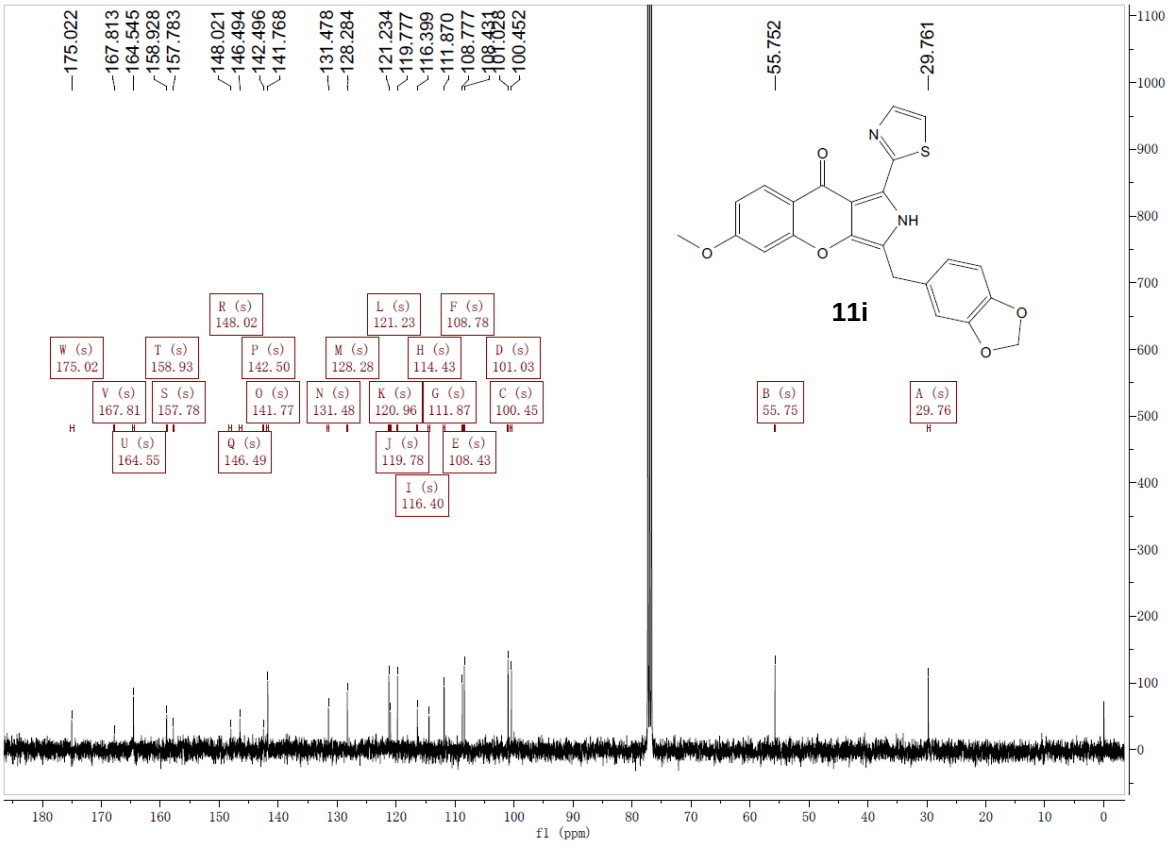
11h

C22 H12 N2 O4 F2 S [M+H]+ : Predicted region for 439.0559 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	91.57	C22 H12 N2 O4 F2 S	[M+H]+	439.0559	439.0559	0.0	0.00	91.57	17.0

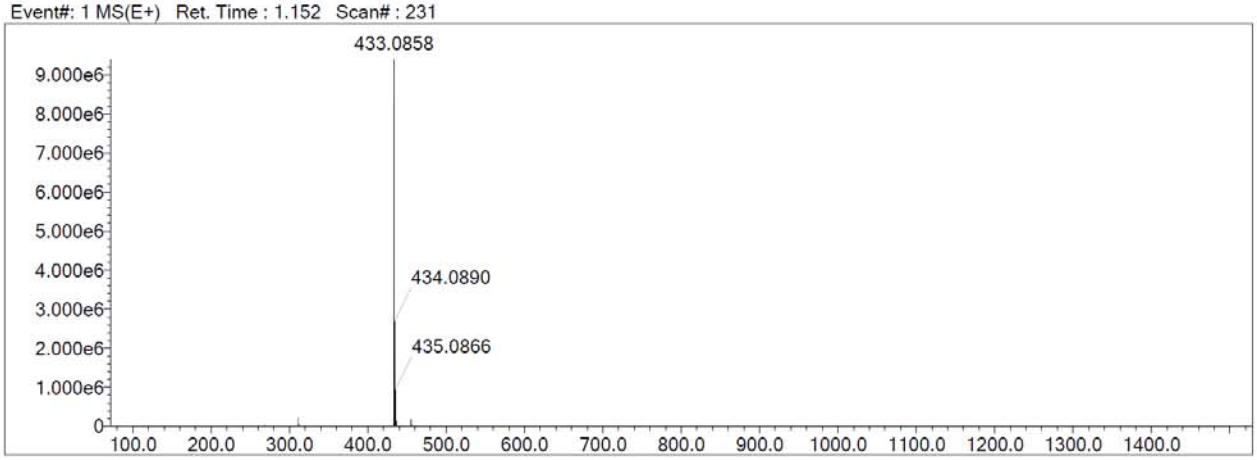

10.295
8.308
8.286
7.770
7.762
7.375
7.367
6.857
6.851
6.703
6.666
6.652
5.883
4.092
3.949
11i
J (d) 6.85
F (d) 7.37
H (s) 6.67
D (s) 4.09
L (s) 10.29
A (d) 8.30
B (d) 7.77
G (d) 6.71
E (s) 5.88
C (s) 3.95
I (d) 6.64
K (dd) 6.91
1.15
0.96
0.96
1.06
1.14
0.92
1.09
0.88
0.86
2.14
2.06
3.00
f1 (ppm)


175.022
167.813
164.545
158.928
157.783
148.021
146.494
142.496
141.768
131.478
128.284
121.234
119.777
116.399
111.870
108.777
108.431
101.028
100.452
55.752
29.761
11i
R (s) 148.02
L (s) 121.23
F (s) 108.78
W (s) 175.02
T (s) 158.93
P (s) 142.50
M (s) 128.28
H (s) 114.43
D (s) 101.03
V (s) 167.81
S (s) 157.78
O (s) 141.77
N (s) 131.48
K (s) 120.96
G (s) 111.87
C (s) 100.45
U (s) 164.55
Q (s) 146.49
J (s) 119.78
E (s) 108.43
I (s) 116.40
B (s) 55.75
A (s) 29.76
f1 (ppm)

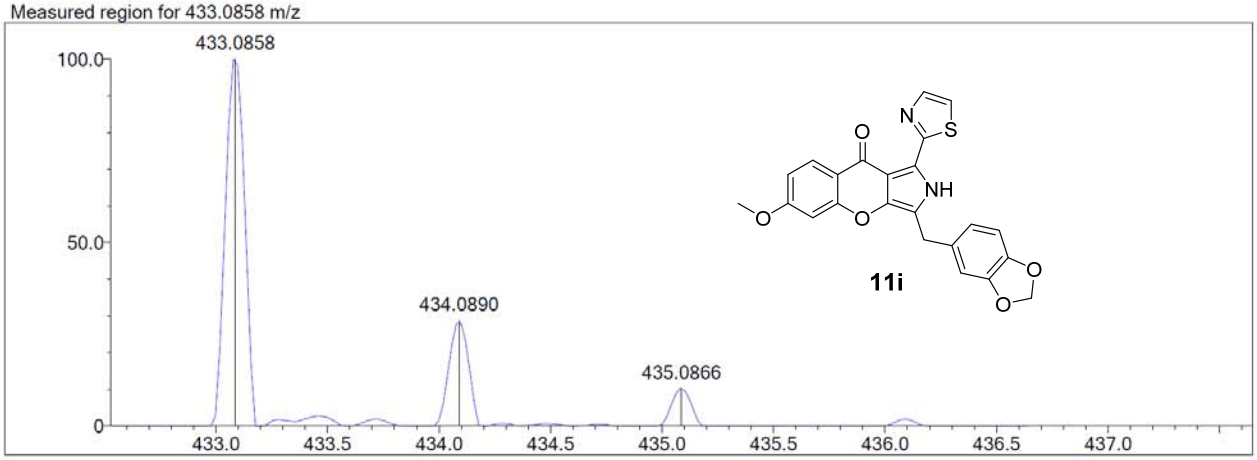
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	2	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	no	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

Event#: 1 MS(E+) Ret. Time : 1.152 Scan# : 231

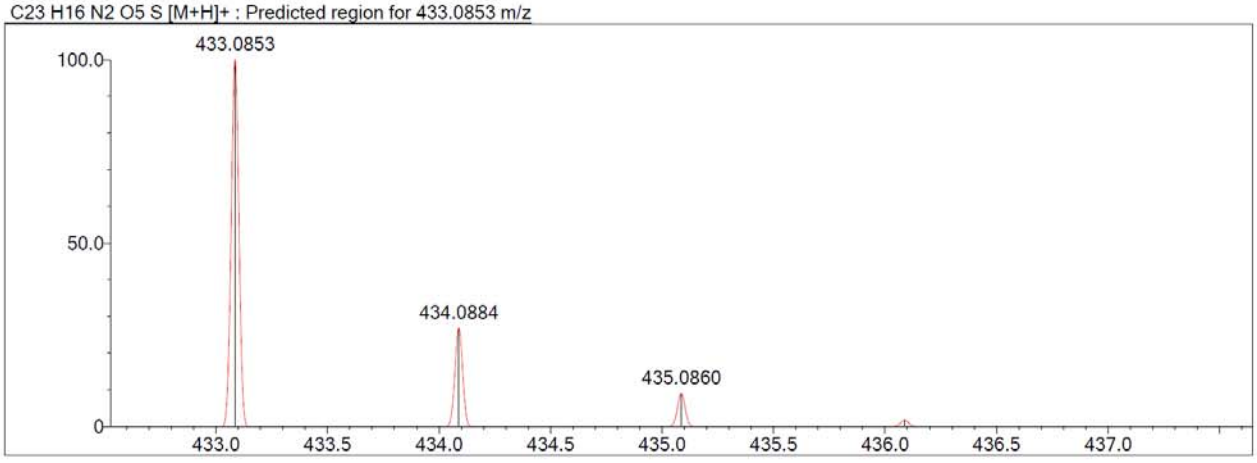


Measured region for 433.0858 m/z

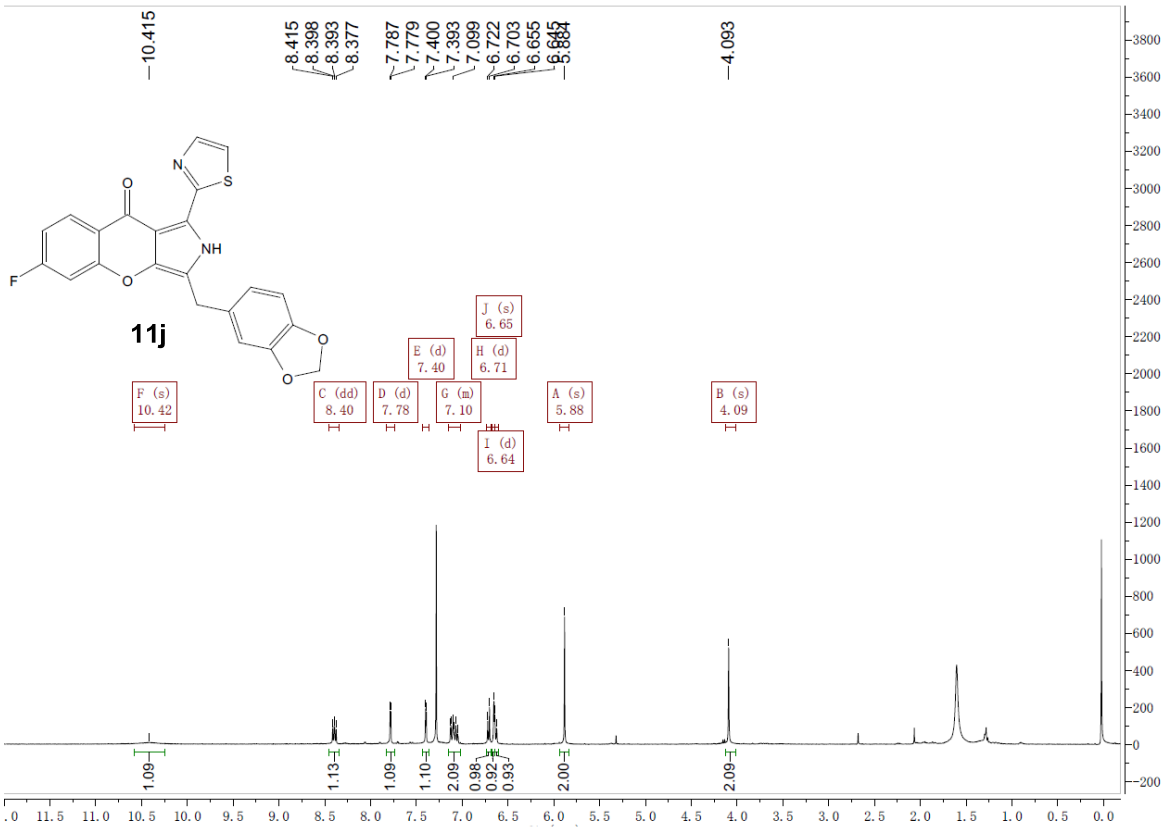


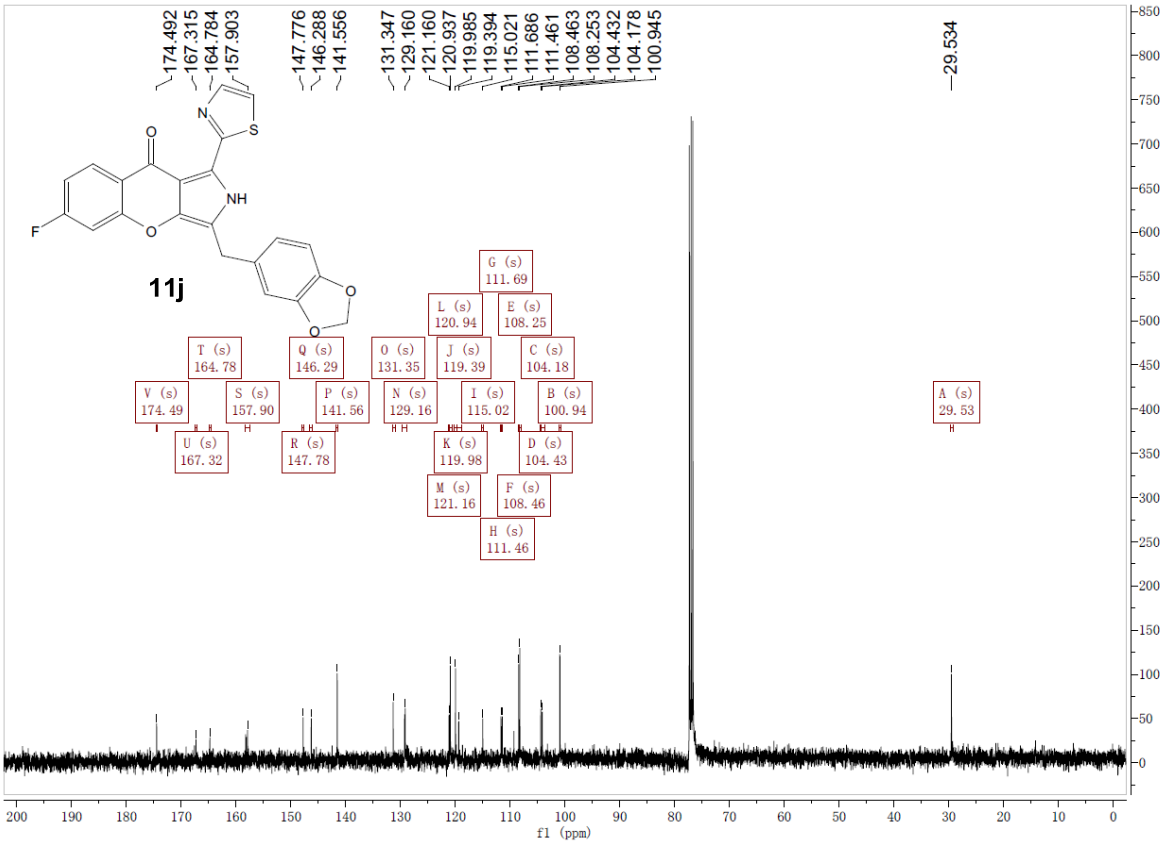
11i

C23 H16 N2 O5 S [M+H]+ : Predicted region for 433.0853 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	83.17	C23 H16 N2 O5 S	[M+H]+	433.0858	433.0853	0.5	1.15	83.48	17.0


10.415
8.415
8.398
8.393
8.377
7.787
7.779
7.400
7.393
7.099
6.722
6.703
6.655
5.884
4.093
11j
F (s) 10.42
C (dd) 8.40
D (d) 7.78
E (d) 7.40
G (m) 7.10
I (d) 6.64
H (d) 6.71
J (s) 6.65
A (s) 5.88
B (s) 4.09


174.492
167.315
164.784
157.903
147.776
146.288
141.556
131.347
129.160
121.160
120.937
119.985
119.394
115.021
111.686
111.461
108.463
108.253
104.432
104.178
100.945
29.534
11j
T (s) 164.78
V (s) 174.49
U (s) 167.32
S (s) 157.90
Q (s) 146.29
P (s) 141.56
R (s) 147.78
O (s) 131.35
N (s) 129.16
L (s) 120.94
J (s) 119.39
K (s) 119.98
M (s) 121.16
I (s) 115.02
G (s) 111.69
E (s) 108.25
C (s) 104.18
B (s) 100.94
D (s) 104.43
F (s) 108.46
H (s) 111.46
A (s) 29.53
f1 (ppm)

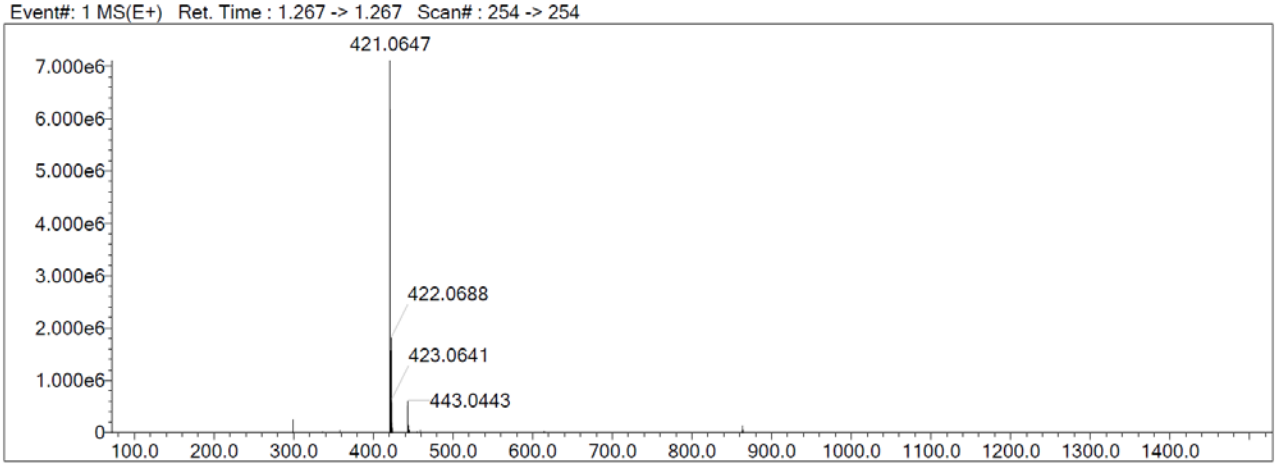
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

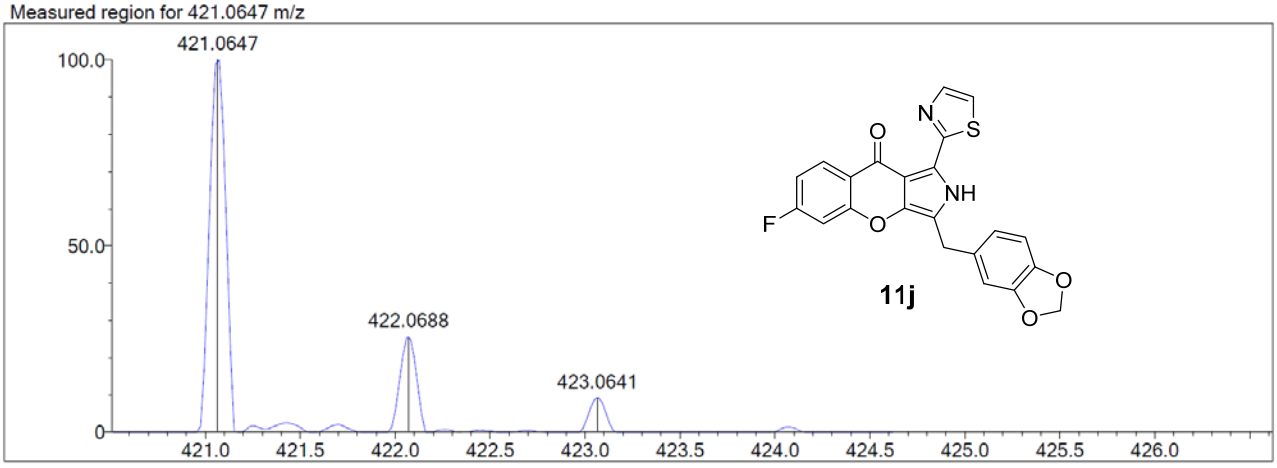
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.267 -> 1.267 Scan# : 254 -> 254

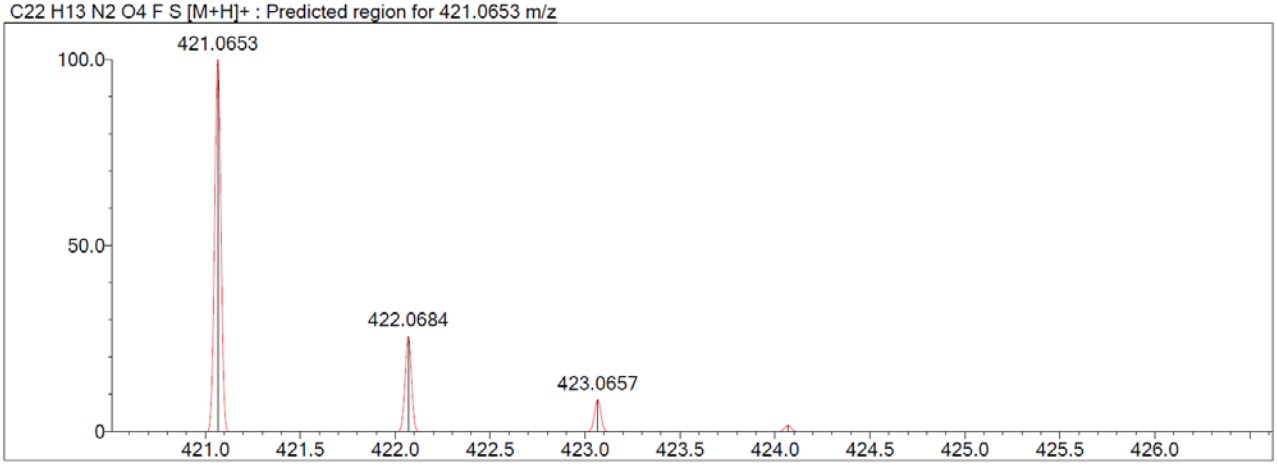


Measured region for 421.0647 m/z

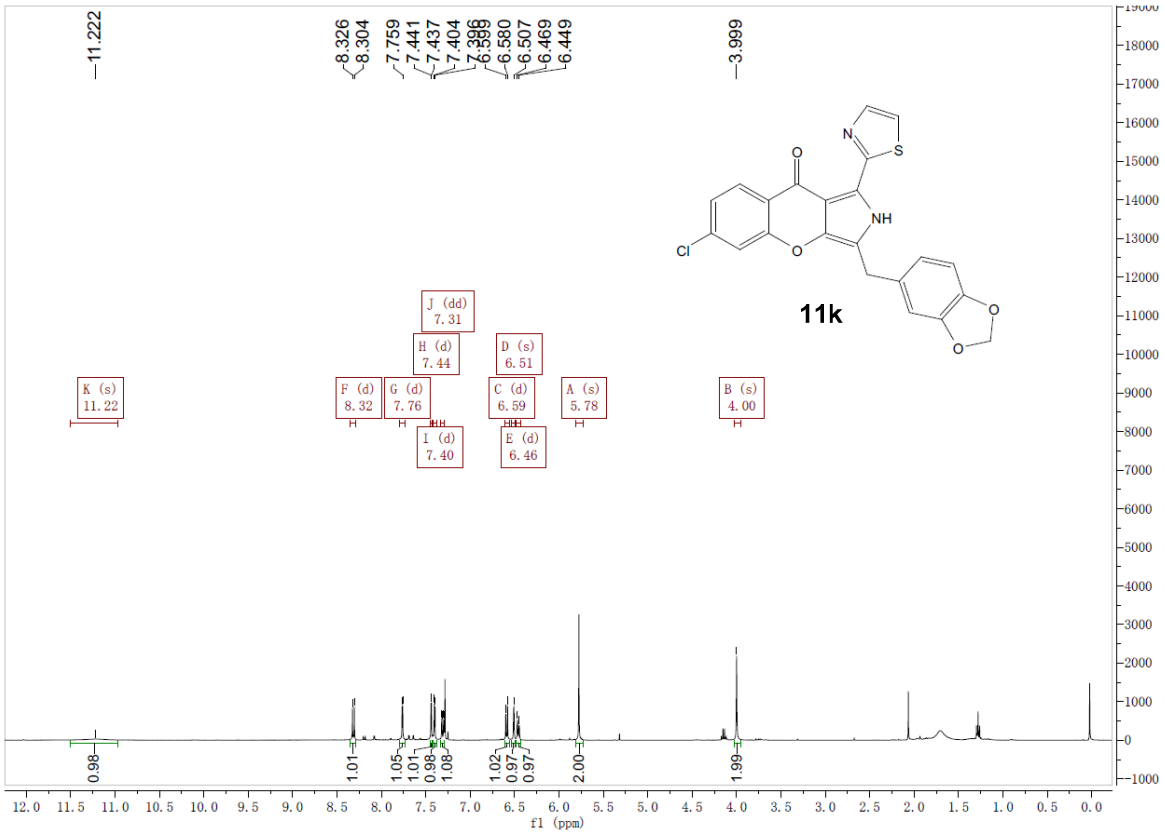


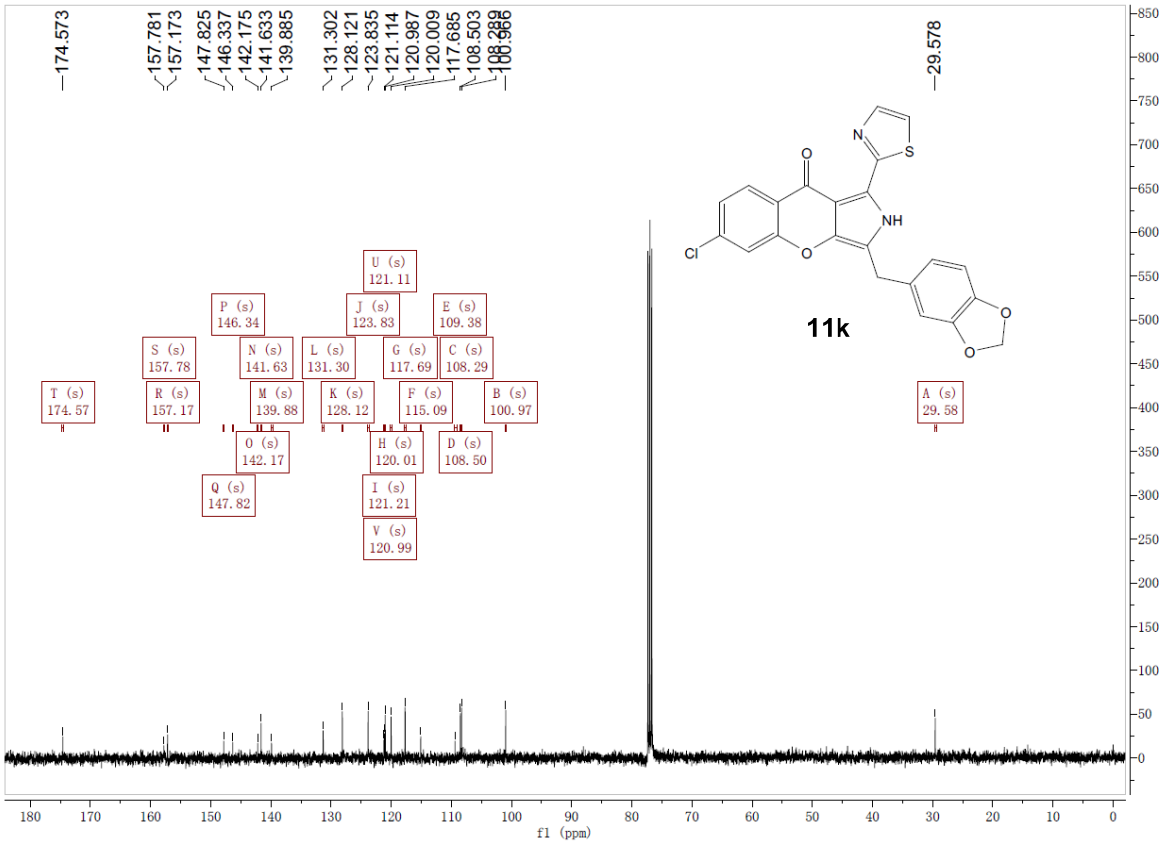
11j

C22 H13 N2 O4 F S [M+H]+ : Predicted region for 421.0653 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	93.37	C22 H13 N2 O4 F S	[M+H]+	421.0647	421.0653	-0.6	-1.42	94.37	17.0


11.222
8.326
8.304
7.759
7.441
7.437
7.404
6.580
6.507
6.469
6.449
3.999
11k
K (s) 11.22
F (d) 8.32
G (d) 7.76
J (dd) 7.31
H (d) 7.44
I (d) 7.40
C (d) 6.59
D (s) 6.51
E (d) 6.46
A (s) 5.78
B (s) 4.00
f1 (ppm)


174.573
157.781
157.173
147.825
146.337
142.175
141.633
139.885
131.302
128.121
123.835
121.114
120.987
120.009
117.685
108.503
29.578
11k
T (s) 174.57
S (s) 157.78
R (s) 157.17
P (s) 146.34
N (s) 141.63
M (s) 139.88
O (s) 142.17
Q (s) 147.82
L (s) 131.30
K (s) 128.12
U (s) 121.11
J (s) 123.83
G (s) 117.69
F (s) 115.09
H (s) 120.01
I (s) 121.21
V (s) 120.99
E (s) 109.38
C (s) 108.29
B (s) 100.97
D (s) 108.50
A (s) 29.58
f1 (ppm)

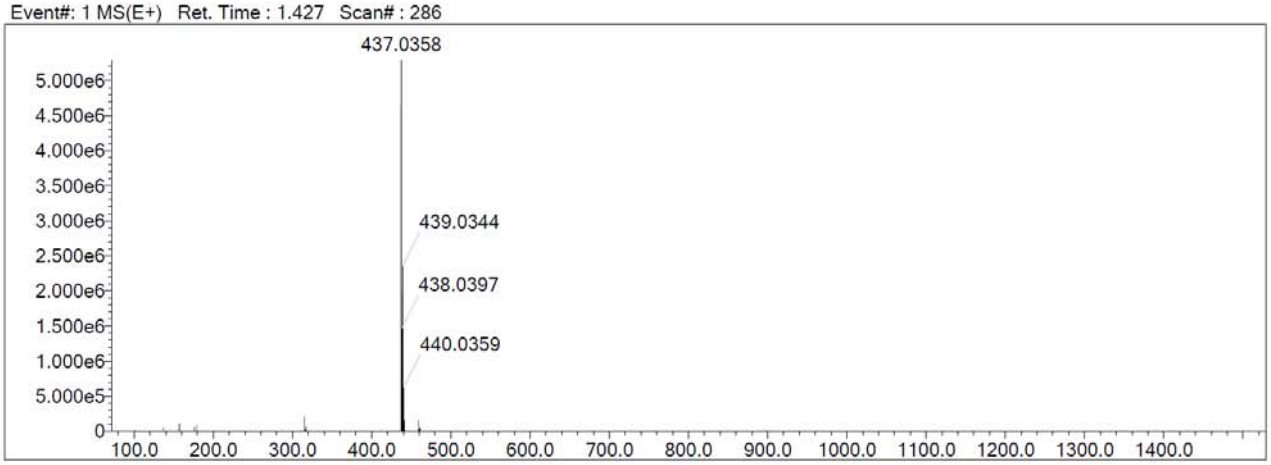
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

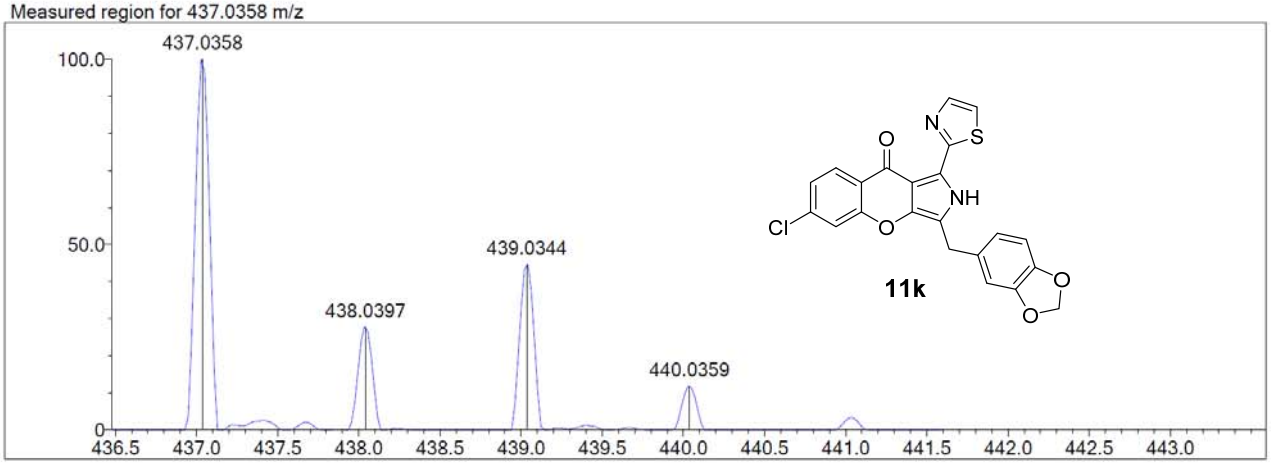
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

Event#: 1 MS(E+) Ret. Time : 1.427 Scan# : 286

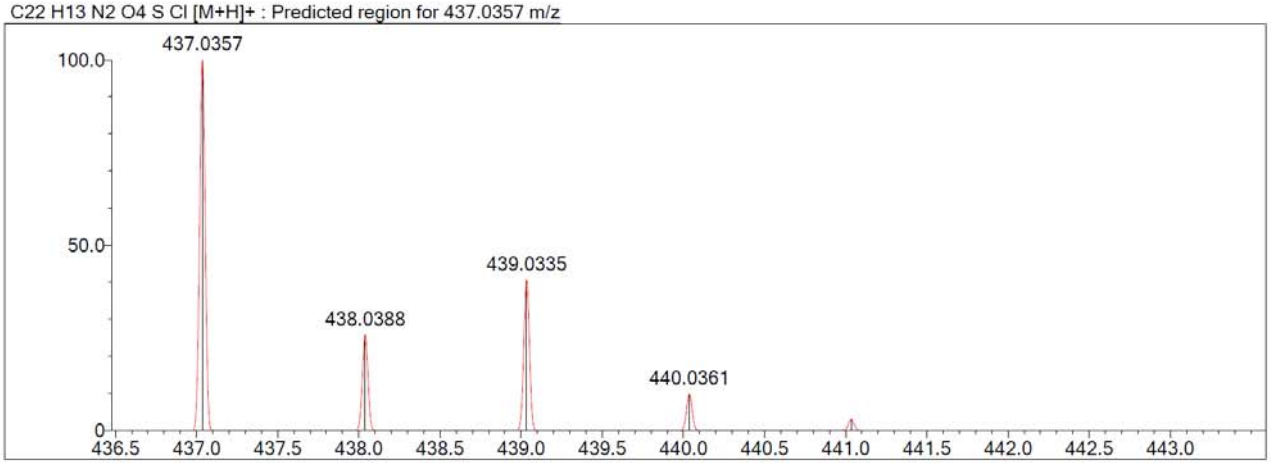


Measured region for 437.0358 m/z

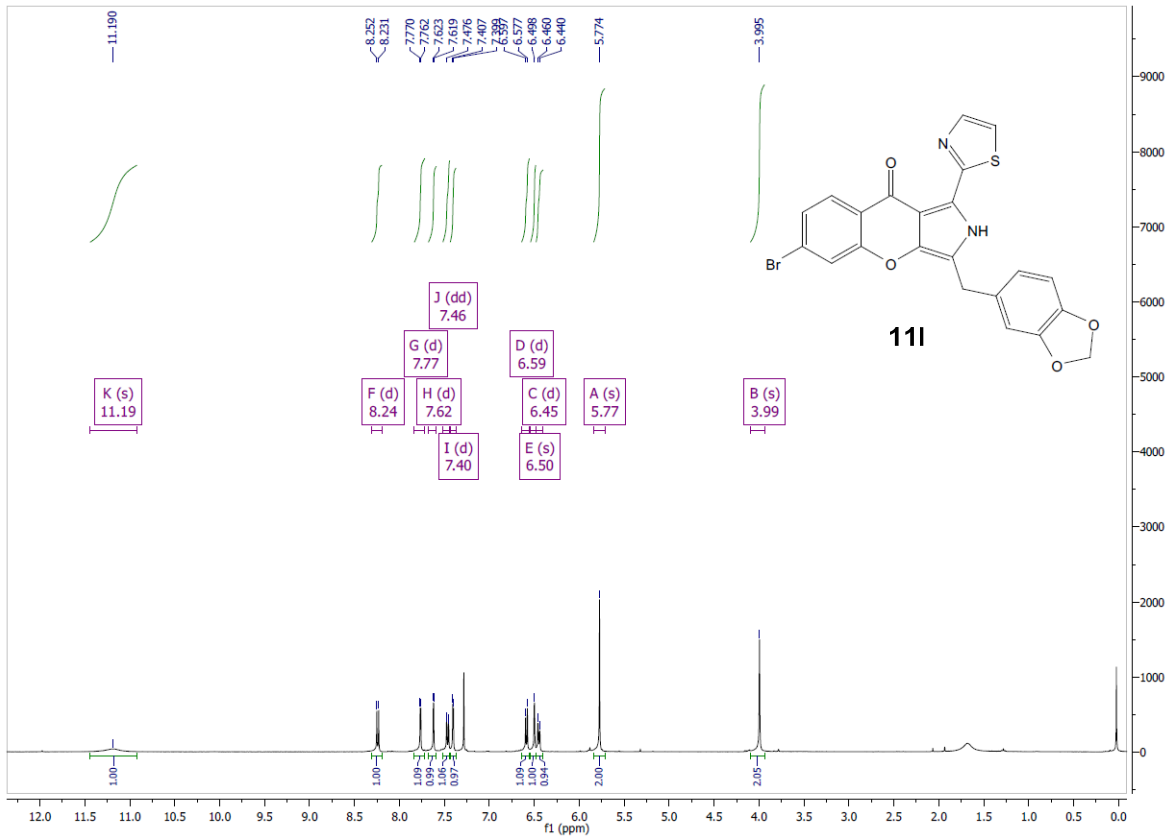


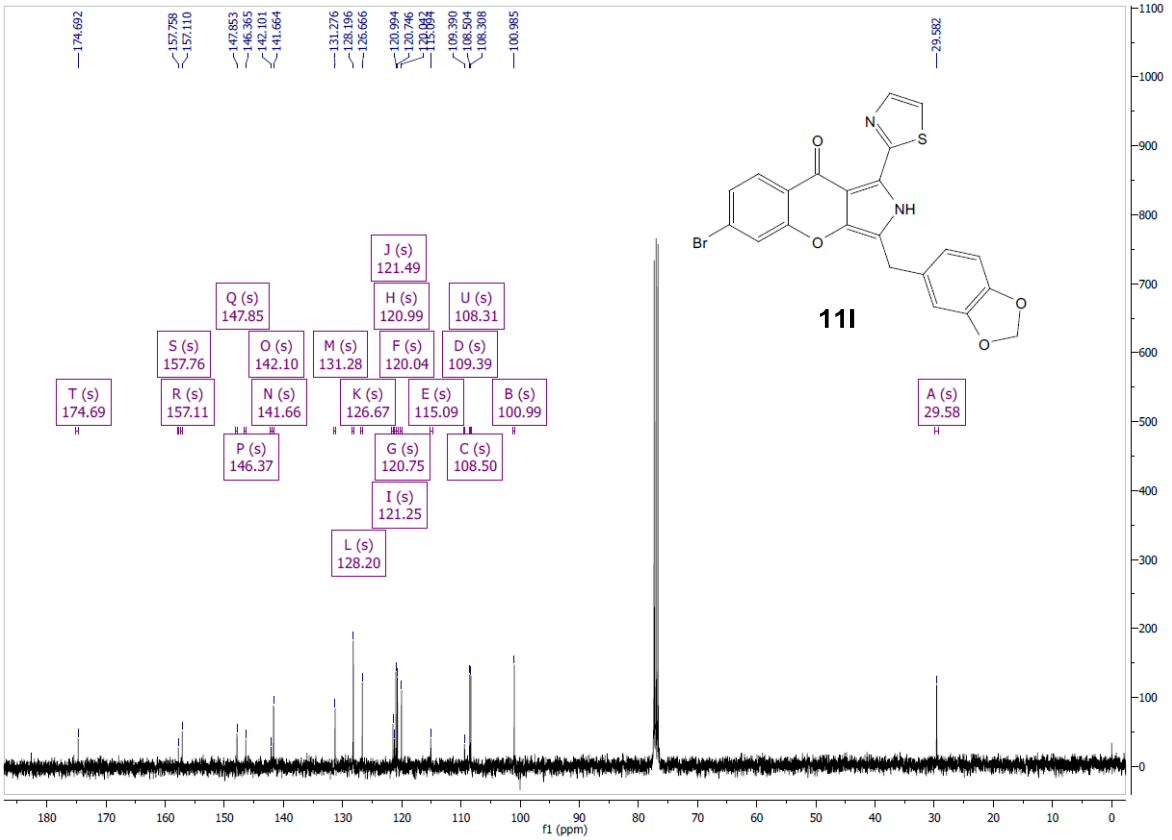
11k

C22 H13 N2 O4 S Cl [M+H]+ : Predicted region for 437.0357 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	87.02	C22 H13 N2 O4 S Cl	[M+H]+	437.0358	437.0357	0.1	0.23	87.02	17.0

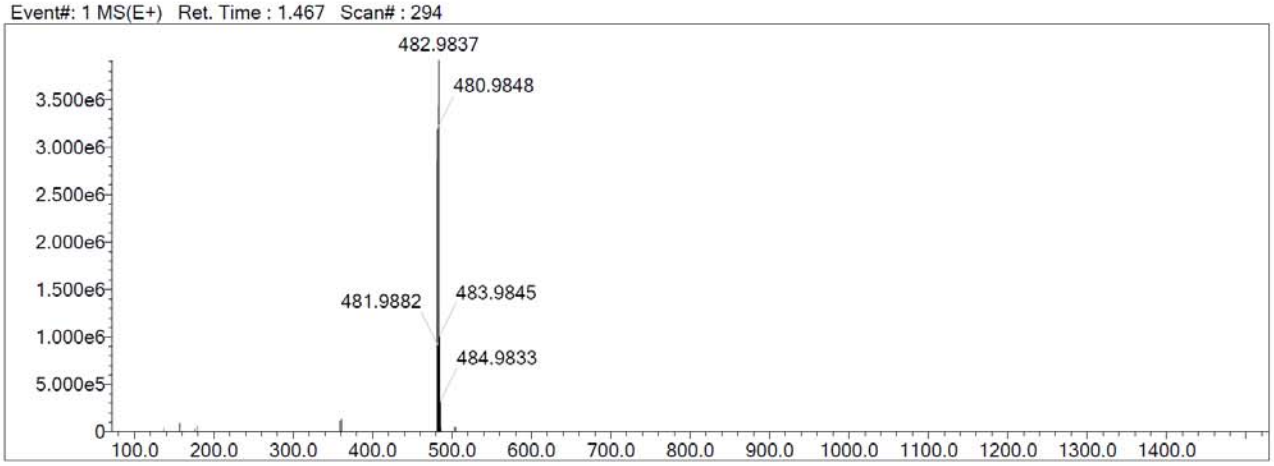

11.190
8.352
8.231
7.770
7.762
7.623
7.619
7.476
7.407
7.399
6.577
6.498
6.460
6.440
5.774
3.995
J (dd) 7.46
G (d) 7.77
D (d) 6.59
K (s) 11.19
F (d) 8.24
H (d) 7.62
C (d) 6.45
A (s) 5.77
B (s) 3.99
I (d) 7.40
E (s) 6.50
11l
f1 (ppm)


174.692
157.758
157.110
147.853
146.365
142.101
141.664
131.276
128.196
126.666
120.994
120.746
109.390
108.504
108.308
100.985
29.582
J (s) 121.49
Q (s) 147.85
H (s) 120.99
U (s) 108.31
S (s) 157.76
O (s) 142.10
M (s) 131.28
F (s) 120.04
D (s) 109.39
T (s) 174.69
R (s) 157.11
N (s) 141.66
K (s) 126.67
E (s) 115.09
B (s) 100.99
A (s) 29.58
P (s) 146.37
G (s) 120.75
C (s) 108.50
I (s) 121.25
L (s) 128.20
11l
f1 (ppm)

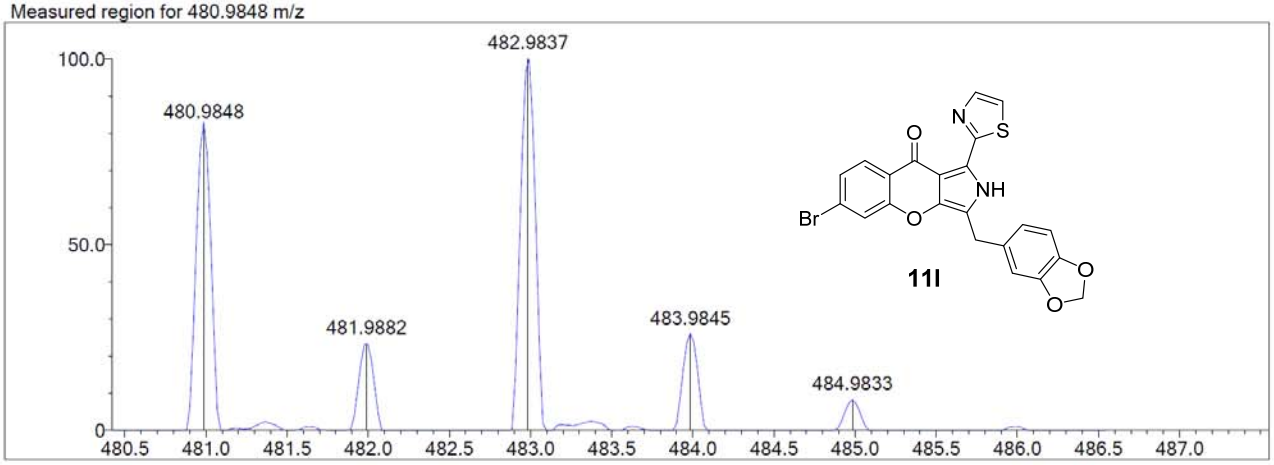
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	2	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm):	50	DBE Range:	-2.0 - 100.0	Electron Ions:	both
HC Ratio:	unlimited	Apply N Rule:	no	Use MSn Info:	no
Max Isotopes:	all	Isotope RI (%):	1.00	Isotope Res:	10000
MSn Iso RI (%):	75.00	MSn Logic Mode:	AND	Max Results:	15

Event#: 1 MS(E+) Ret. Time : 1.467 Scan# : 294

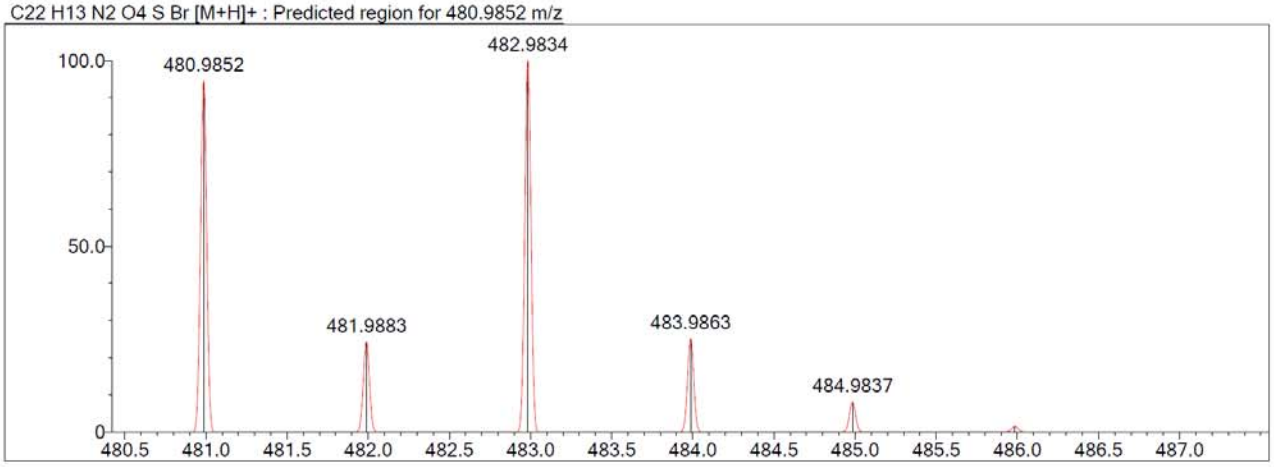


Measured region for 480.9848 m/z

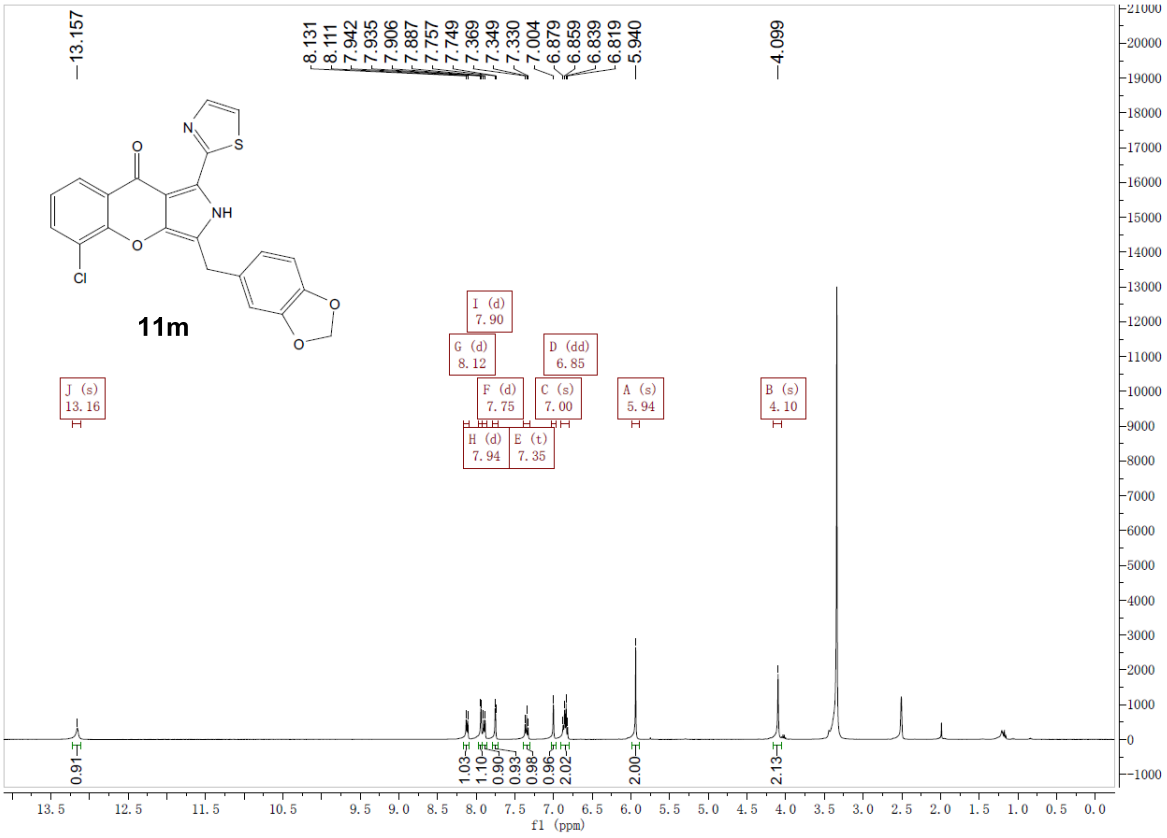


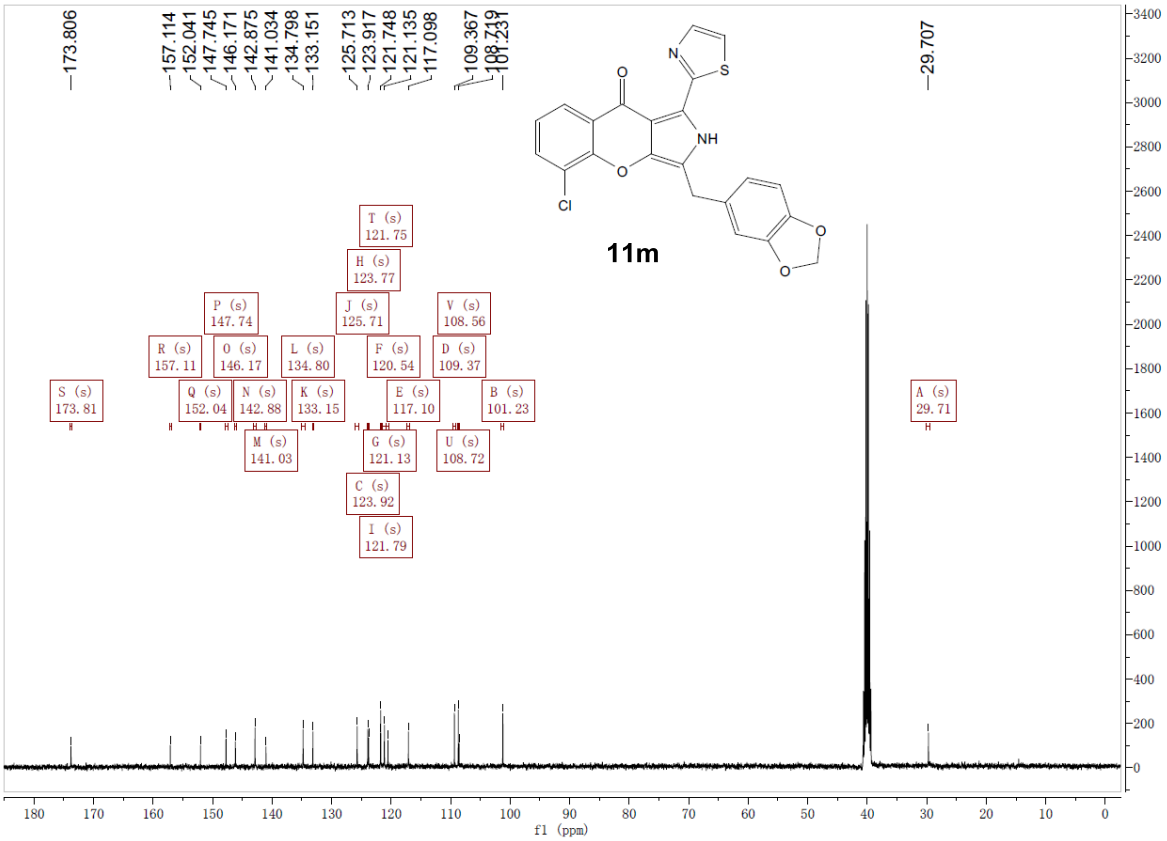
11l

C22 H13 N2 O4 S Br [M+H]+ : Predicted region for 480.9852 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
7	80.47	C22 H13 N2 O4 S Br	[M+H]+	480.9848	480.9852	-0.4	-0.83	80.47	17.0


13.157
8.131
8.111
7.942
7.935
7.906
7.887
7.757
7.749
7.369
7.349
7.330
7.004
6.879
6.859
6.839
6.819
5.940
4.099
11m
J (s) 13.16
I (d) 7.90
G (d) 8.12
D (dd) 6.85
F (d) 7.75
C (s) 7.00
A (s) 5.94
B (s) 4.10
H (d) 7.94
E (t) 7.35
0.91
1.03
1.10
0.90
0.93
0.98
0.96
2.02
2.00
2.13
f1 (ppm)


173.806
157.114
152.041
147.745
146.171
142.875
141.034
134.798
133.151
125.713
123.917
121.748
121.135
117.098
109.367
108.719
101.231
29.707
11m
T (s) 121.75
H (s) 123.77
P (s) 147.74
J (s) 125.71
V (s) 108.56
R (s) 157.11
O (s) 146.17
L (s) 134.80
F (s) 120.54
D (s) 109.37
S (s) 173.81
Q (s) 152.04
N (s) 142.88
K (s) 133.15
E (s) 117.10
B (s) 101.23
A (s) 29.71
M (s) 141.03
G (s) 121.13
U (s) 108.72
C (s) 123.92
I (s) 121.79
f1 (ppm)

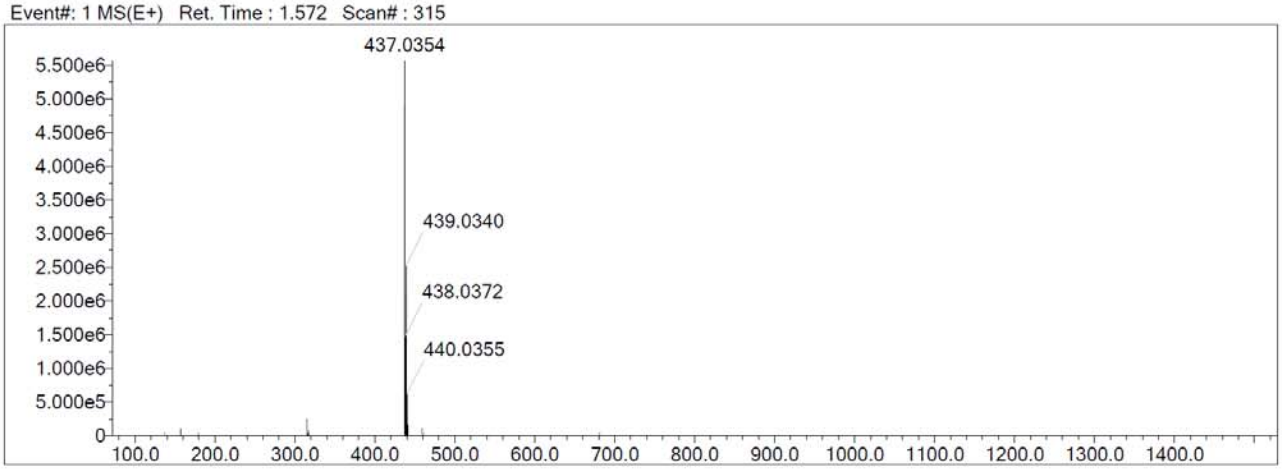
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	25	O	2	0	8	Cl	1	0	1	H
C	4	18	25	F	1	0	1	Br	1	0	1	Na
N	3	0	3	S	2	0	1	I	3	0	0	

Error Margin (ppm): 50
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

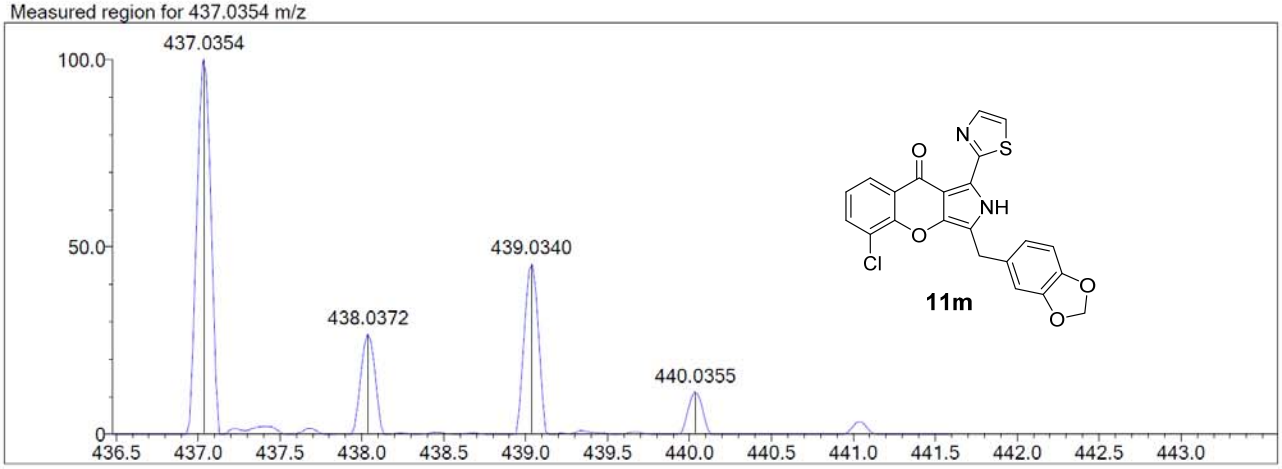
DBE Range: -2.0 - 100.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 15

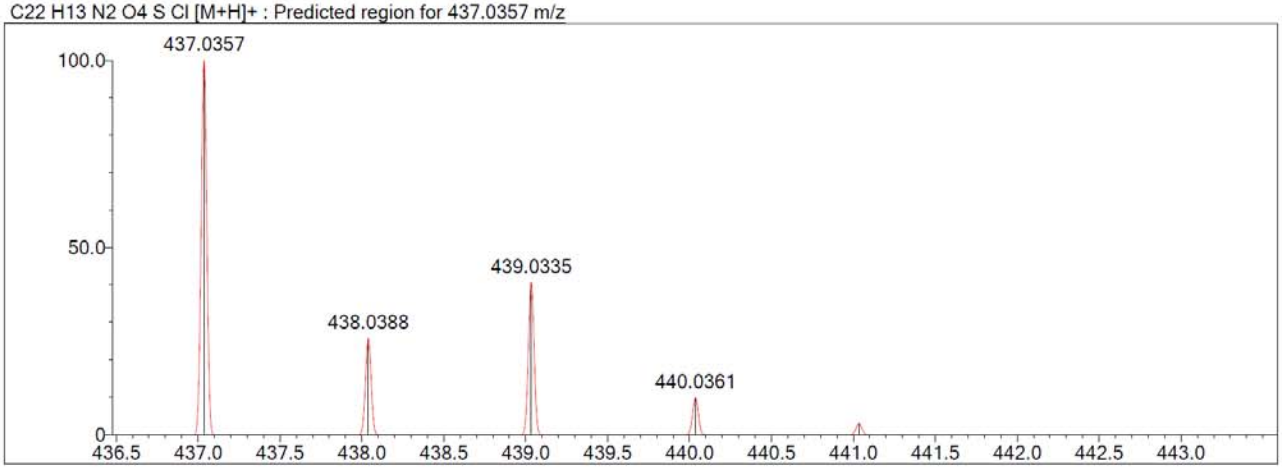
Event#: 1 MS(E+) Ret. Time : 1.572 Scan# : 315



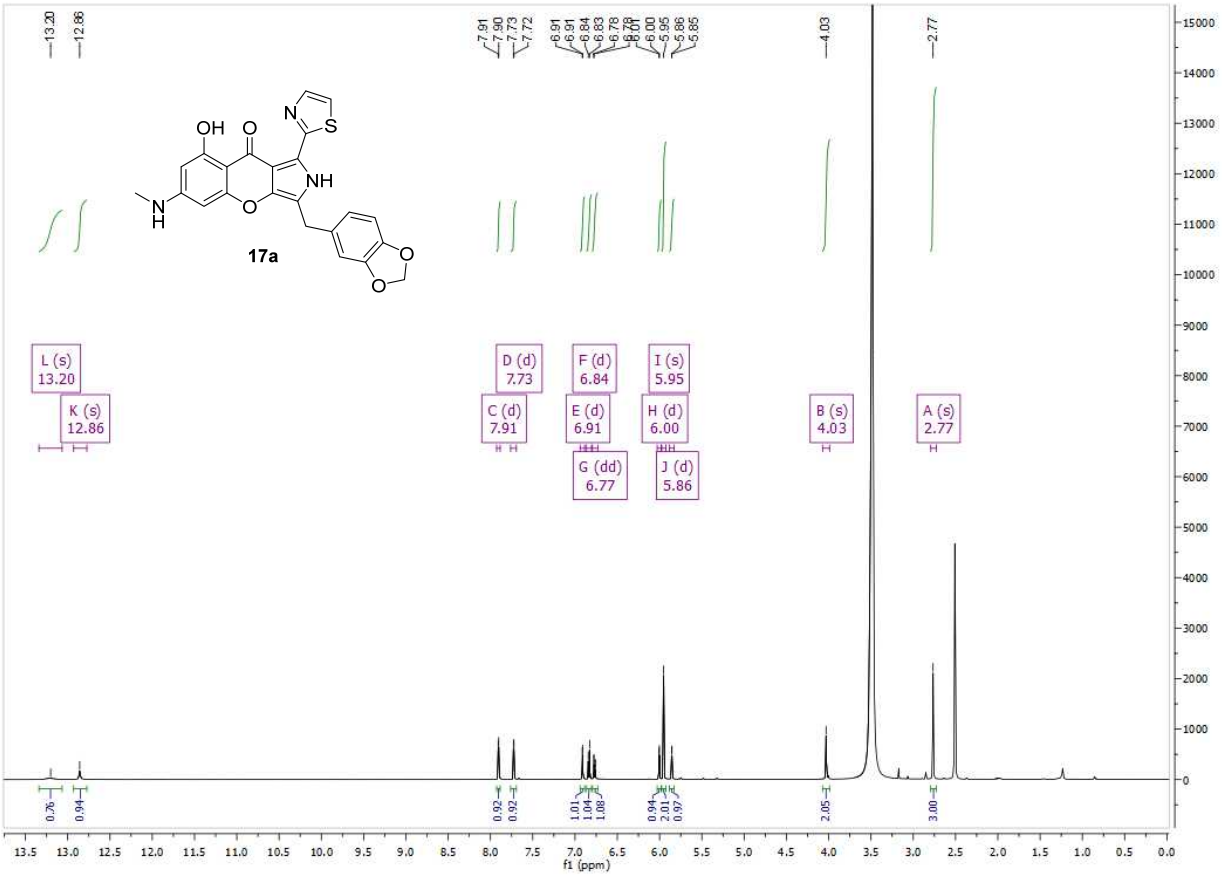
Measured region for 437.0354 m/z

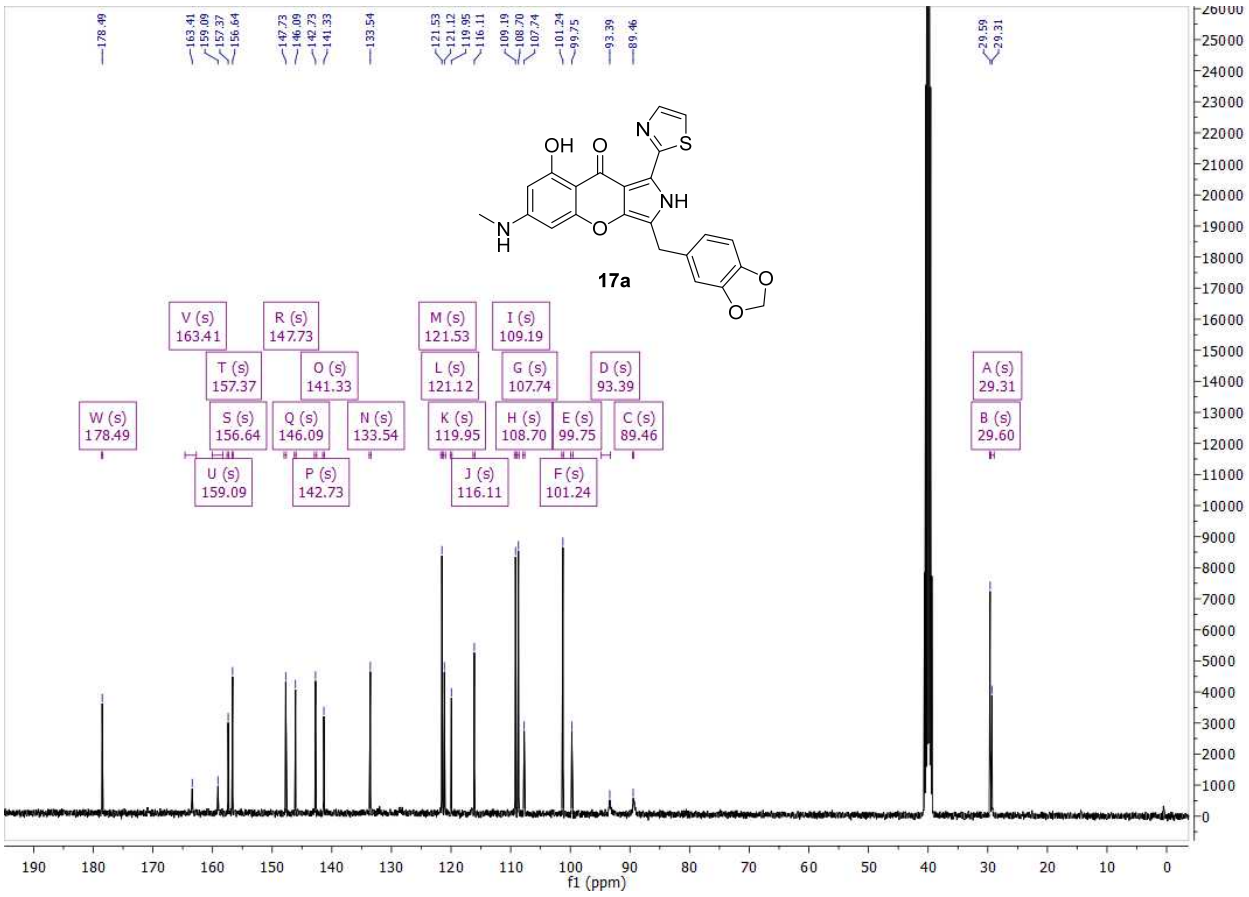


C22 H13 N2 O4 S Cl [M+H]+ : Predicted region for 437.0357 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.10	C22 H13 N2 O4 S Cl	[M+H]+	437.0354	437.0357	-0.3	-0.69	91.10	17.0


13.20
12.86
7.91
7.90
7.73
7.72
6.91
6.91
6.84
6.83
6.79
6.78
6.01
6.00
5.95
5.86
5.85
4.03
2.77
OH
O
N
S
NH
N
H
O
O
O
17a
L (s) 13.20
K (s) 12.86
D (d) 7.73
C (d) 7.91
F (d) 6.84
E (d) 6.91
G (dd) 6.77
I (s) 5.95
H (d) 6.00
J (d) 5.86
B (s) 4.03
A (s) 2.77
f1 (ppm)


178.49
163.41
159.09
157.37
156.64
147.73
146.09
142.73
141.33
133.54
121.53
121.12
119.95
116.11
109.19
108.70
107.74
101.24
99.75
93.39
89.46
29.59
29.31
OH
O
N
S
NH
N
H
O
O
O
17a
V (s) 163.41
R (s) 147.73
M (s) 121.53
I (s) 109.19
T (s) 157.37
O (s) 141.33
L (s) 121.12
G (s) 107.74
D (s) 93.39
A (s) 29.31
W (s) 178.49
S (s) 156.64
Q (s) 146.09
N (s) 133.54
K (s) 119.95
H (s) 108.70
E (s) 99.75
C (s) 89.46
B (s) 29.60
U (s) 159.09
P (s) 142.73
J (s) 116.11
F (s) 101.24
f1 (ppm)

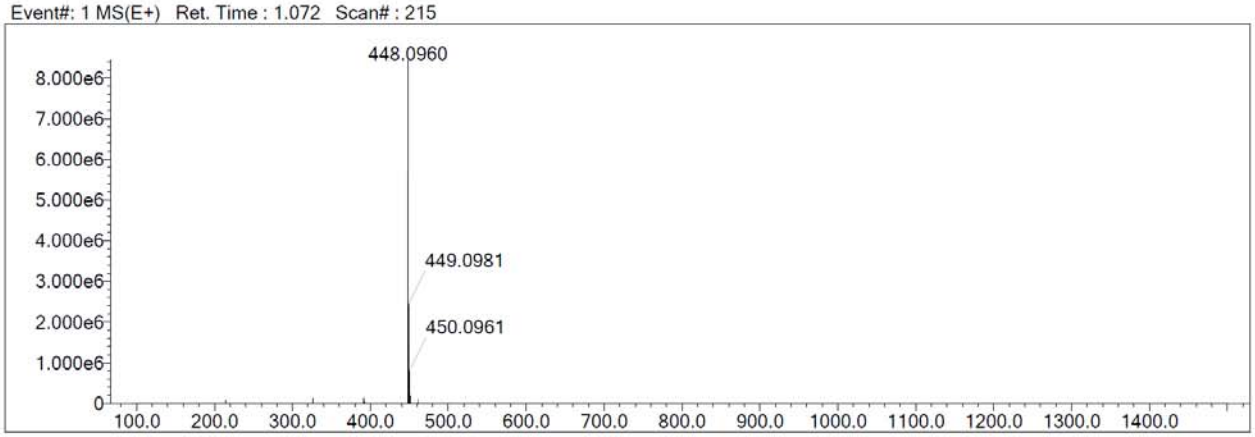
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	21	O	2	4	6	K	1	0	0	I	3	0	0	H
2H	1	0	0	F	1	0	1	Fe	2	0	0	Pt	2	0	0	Na
3H	1	0	0	Si	4	0	0	Cu	2	0	0	Au	1	0	0	
B	3	0	0	P	3	0	0	Se	2	0	0					
C	4	0	26	S	2	0	1	Br	1	0	0					
N	3	0	3	Cl	1	0	0	Pd	2	0	0					

Error Margin (ppm): 20
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

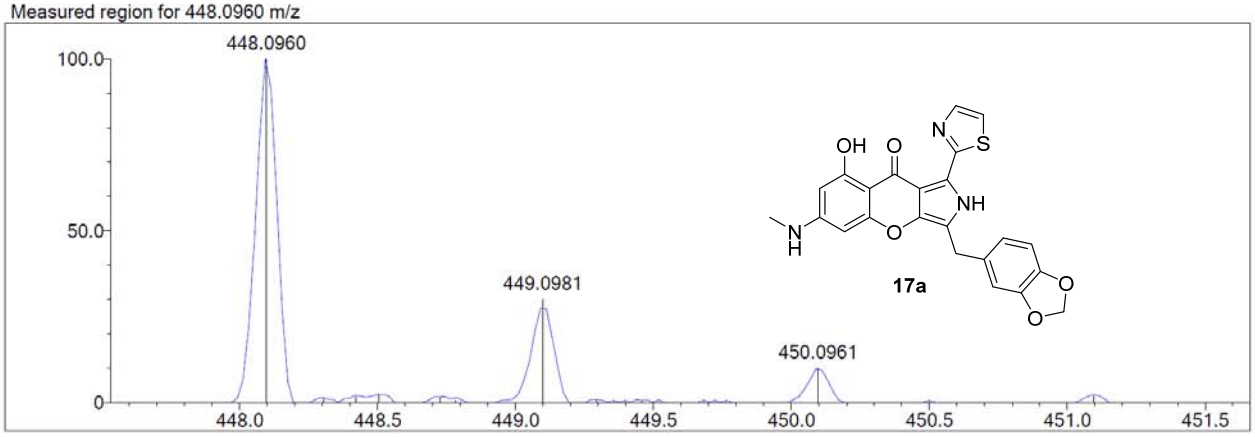
DBE Range: -50.0 - 50.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

Event#: 1 MS(E+) Ret. Time : 1.072 Scan# : 215

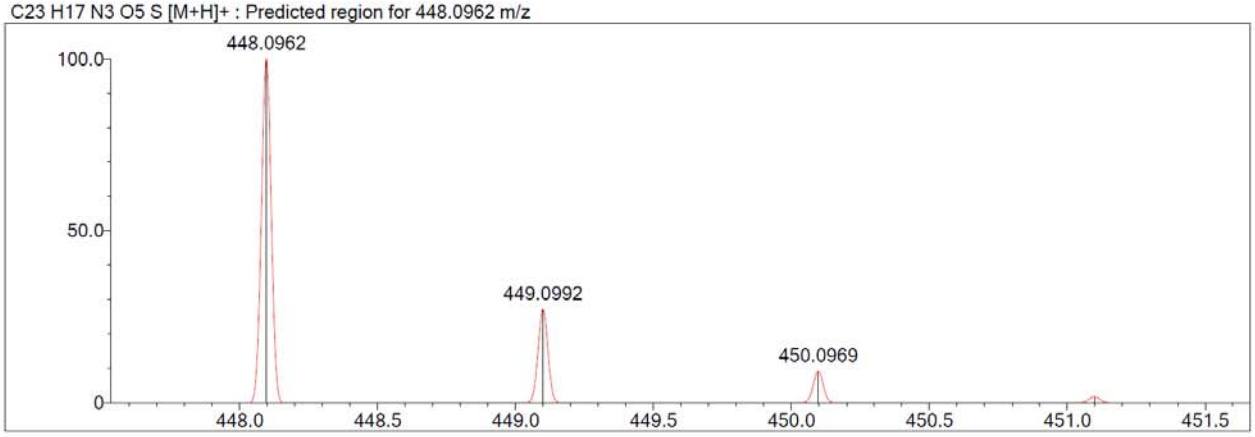


Measured region for 448.0960 m/z

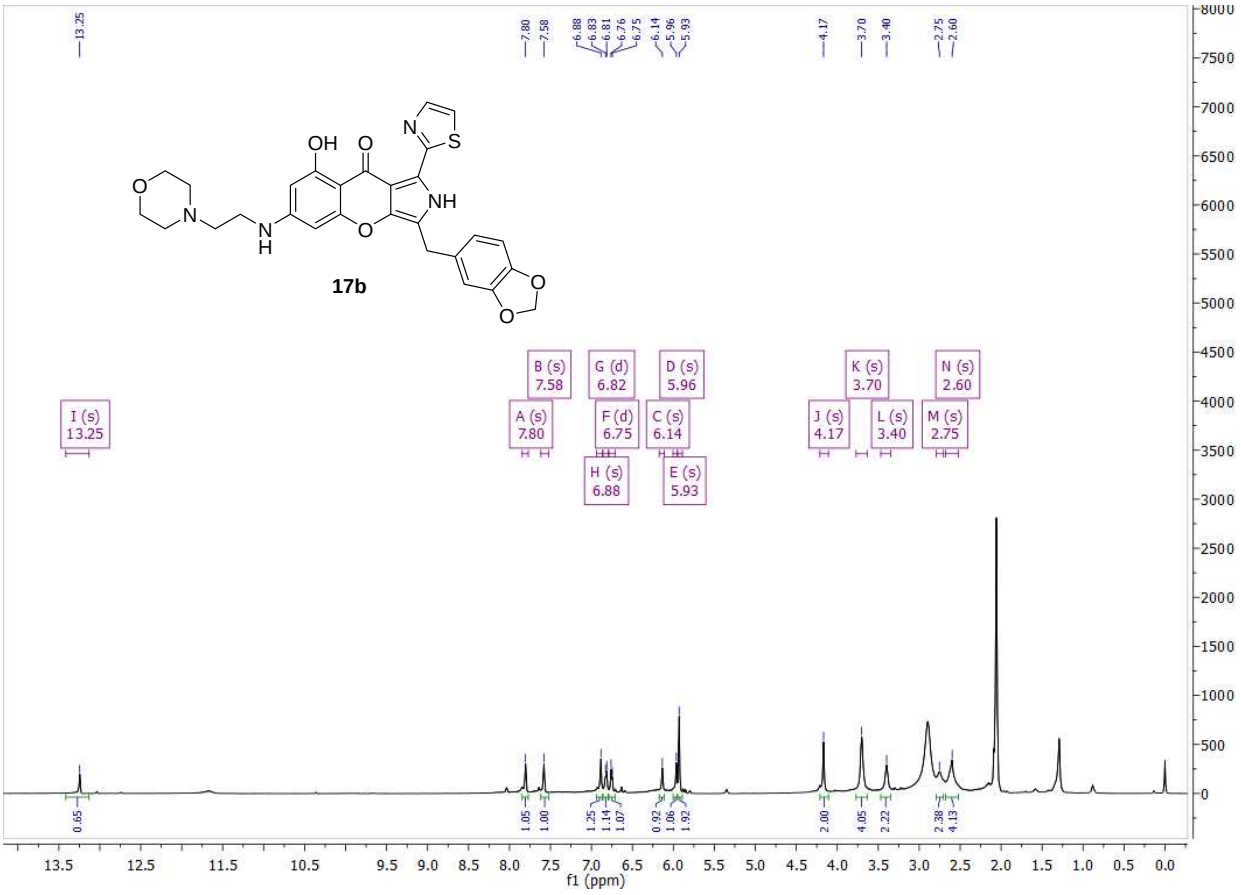


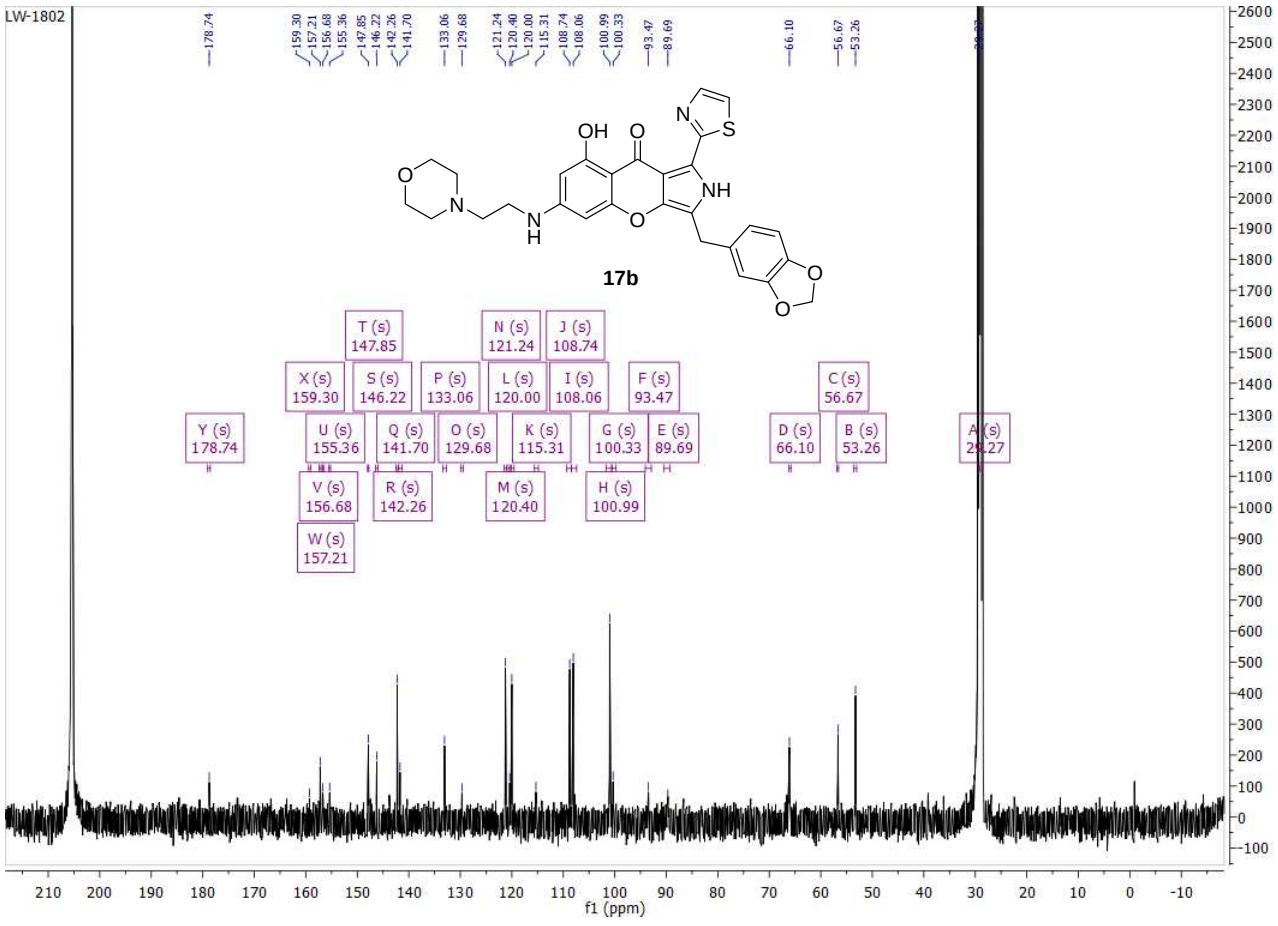
17a

C23 H17 N3 O5 S [M+H]+ : Predicted region for 448.0962 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	97.54	C23 H17 N3 O5 S	[M+H]+	448.0960	448.0962	-0.2	-0.45	97.54	17.0


17b
I (s) 13.25
A (s) 7.80
B (s) 7.58
F (d) 6.75
G (d) 6.82
H (s) 6.88
C (s) 6.14
D (s) 5.96
E (s) 5.93
J (s) 4.17
K (s) 3.70
L (s) 3.40
M (s) 2.75
N (s) 2.60
f1 (ppm)


LW-1802
17b
T (s) 147.85
X (s) 159.30
S (s) 146.22
P (s) 133.06
N (s) 121.24
L (s) 120.00
J (s) 108.74
I (s) 108.06
F (s) 93.47
C (s) 56.67
Y (s) 178.74
U (s) 155.36
Q (s) 141.70
O (s) 129.68
K (s) 115.31
G (s) 100.33
E (s) 89.69
D (s) 66.10
B (s) 53.26
A (s) 29.27
V (s) 156.68
R (s) 142.26
M (s) 120.40
H (s) 100.99
W (s) 157.21
f1 (ppm)

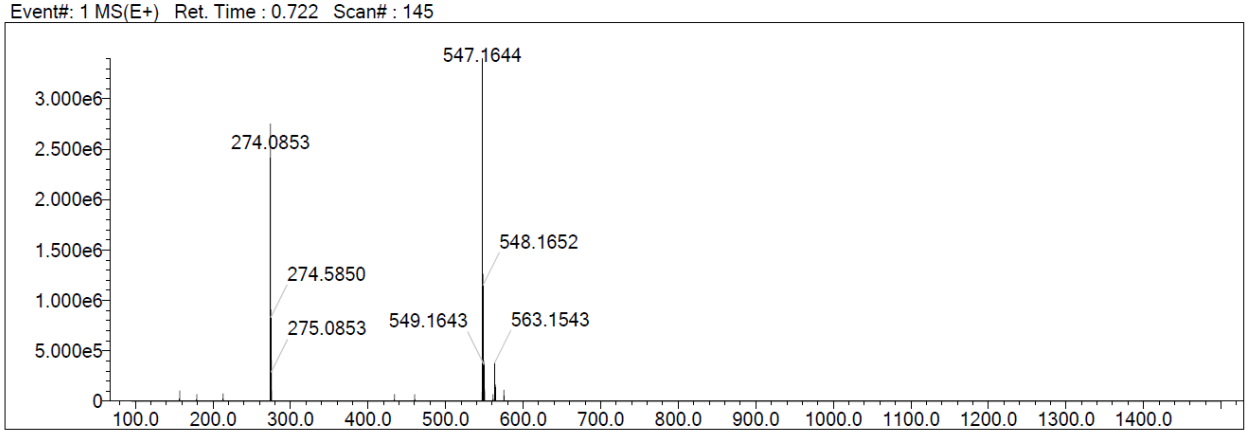
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	29	O	2	4	6	K	1	0	0	I	3	0	0	H
2H	1	0	0	F	1	0	1	Fe	2	0	0	Pt	2	0	0	Na
3H	1	0	0	Si	4	0	0	Cu	2	0	0	Au	1	0	0	
B	3	0	0	P	3	0	0	Se	2	0	0					
C	4	0	29	S	2	0	1	Br	1	0	0					
N	3	0	5	Cl	1	0	0	Pd	2	0	0					

Error Margin (ppm): 20
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

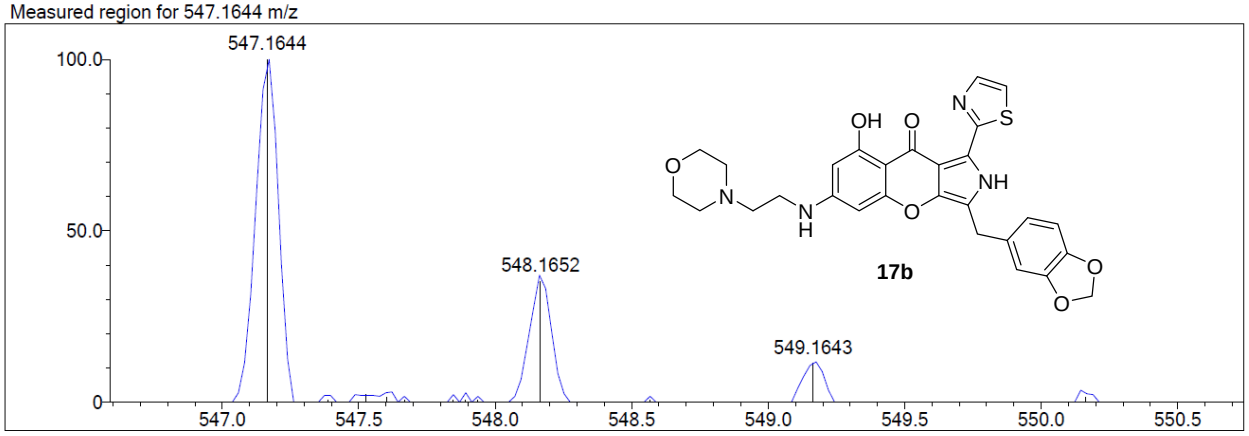
DBE Range: -50.0 - 50.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

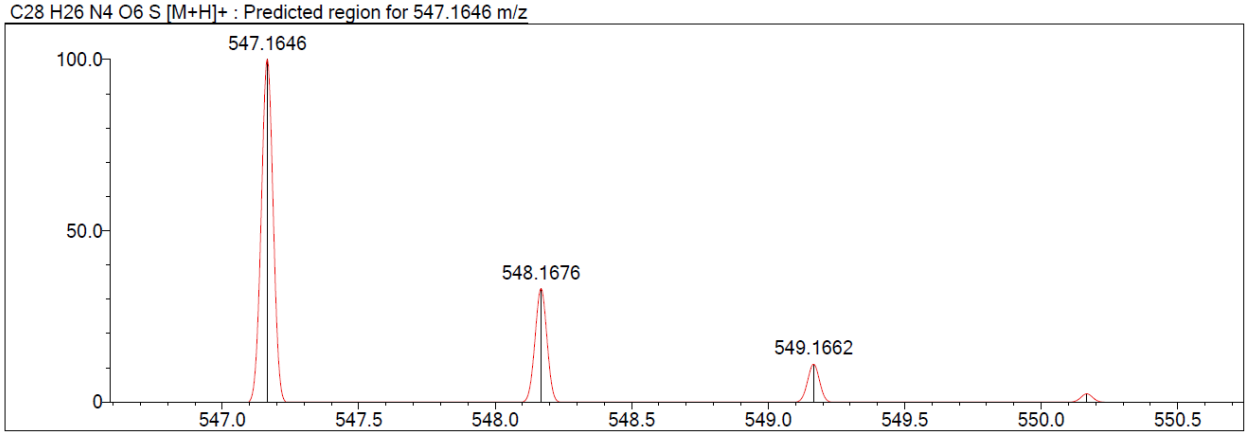
Event#: 1 MS(E+) Ret. Time : 0.722 Scan# : 145



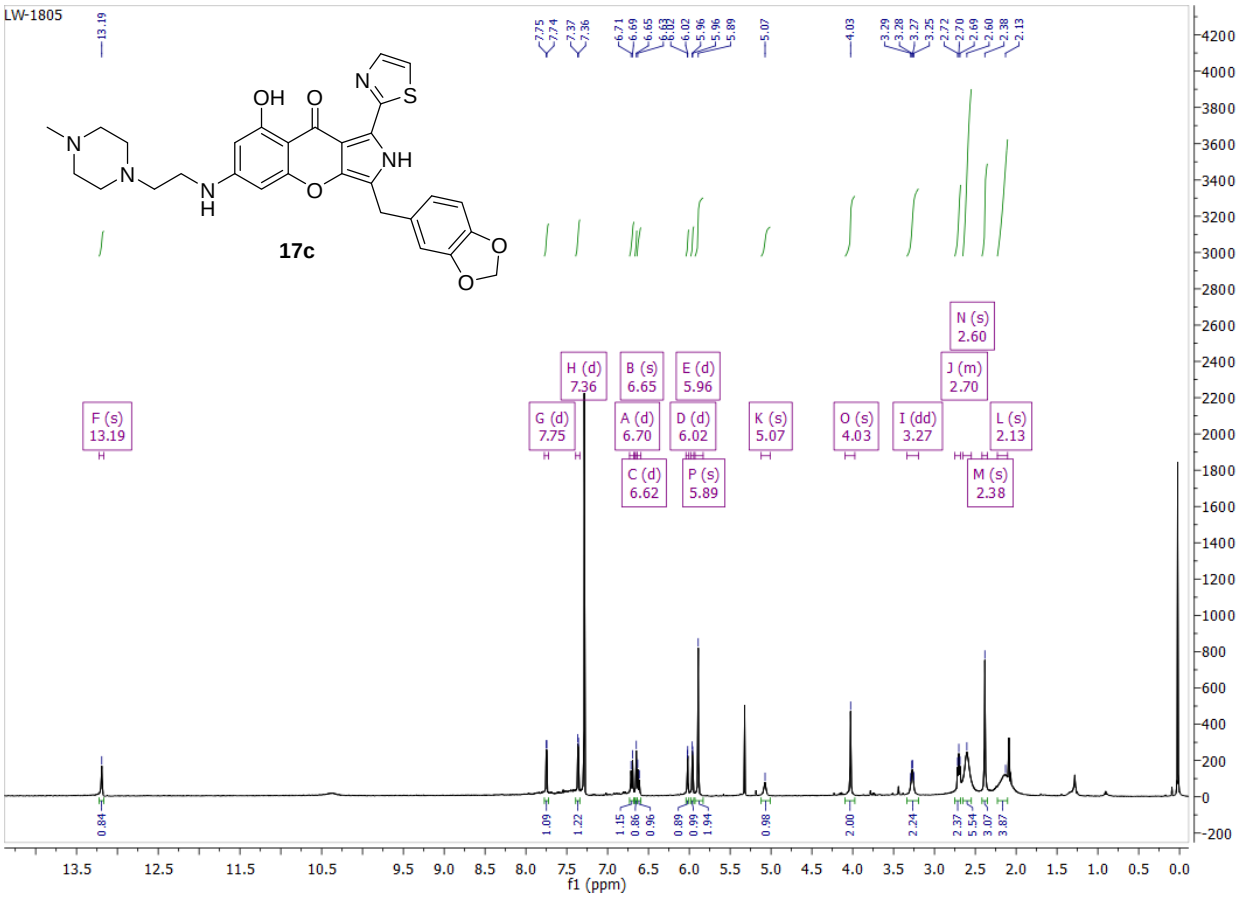
Measured region for 547.1644 m/z

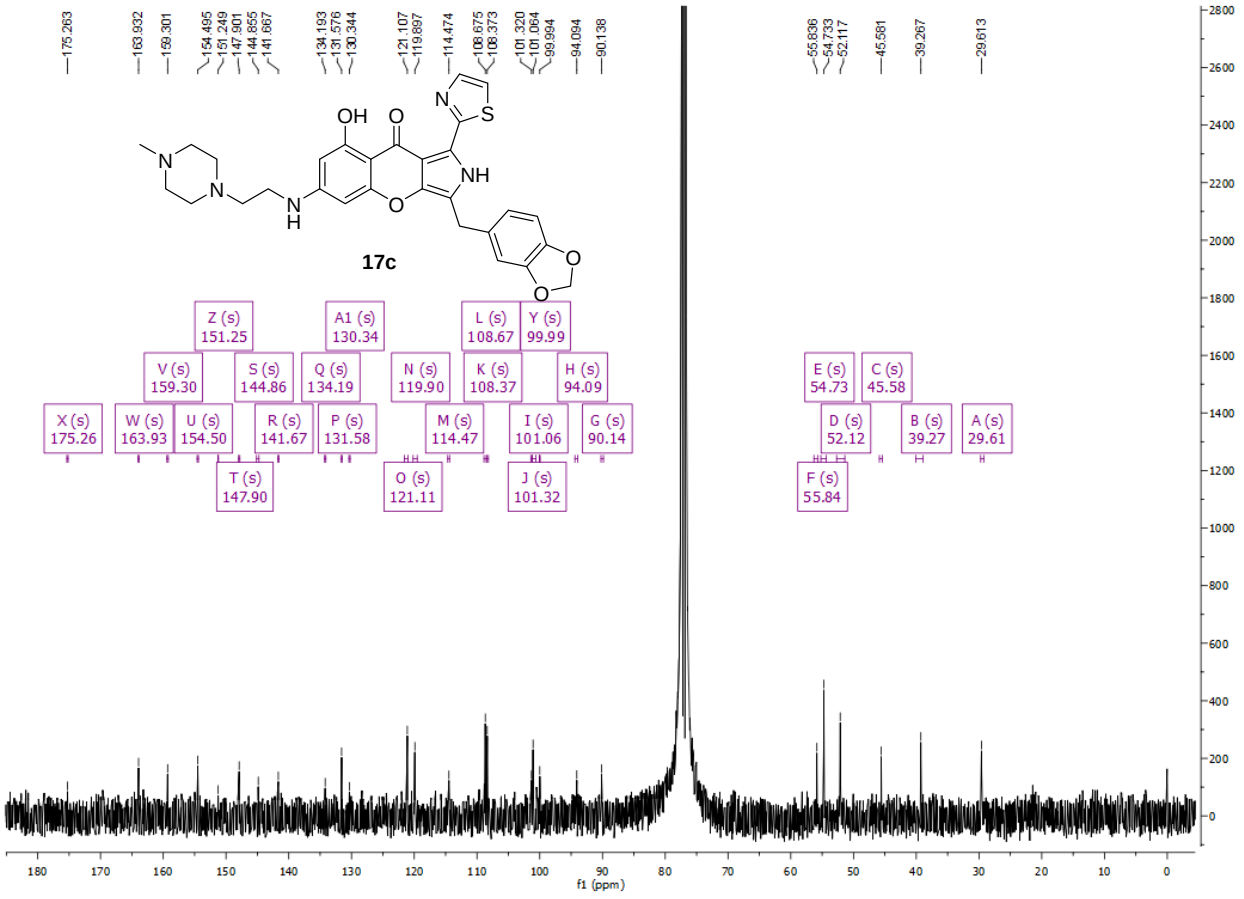


C28 H26 N4 O6 S [M+H]+ : Predicted region for 547.1646 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.14	C28 H26 N4 O6 S	[M+H]+	547.1644	547.1646	-0.2	-0.37	82.14	18.0


LW-1805
17c
F (s) 13.19
H (d) 7.36
G (d) 7.75
B (s) 6.65
A (d) 6.70
C (d) 6.62
E (d) 5.96
D (d) 6.02
P (s) 5.89
K (s) 5.07
O (s) 4.03
N (s) 2.60
J (m) 2.70
I (dd) 3.27
L (s) 2.13
M (s) 2.38
f1 (ppm)


17c
Z (s) 151.25
A1 (s) 130.34
L (s) 108.67
Y (s) 99.99
V (s) 159.30
S (s) 144.86
Q (s) 134.19
N (s) 119.90
K (s) 108.37
H (s) 94.09
X (s) 175.26
W (s) 163.93
U (s) 154.50
R (s) 141.67
P (s) 131.58
M (s) 114.47
I (s) 101.06
G (s) 90.14
T (s) 147.90
O (s) 121.11
J (s) 101.32
E (s) 54.73
C (s) 45.58
D (s) 52.12
B (s) 39.27
A (s) 29.61
F (s) 55.84
f1 (ppm)

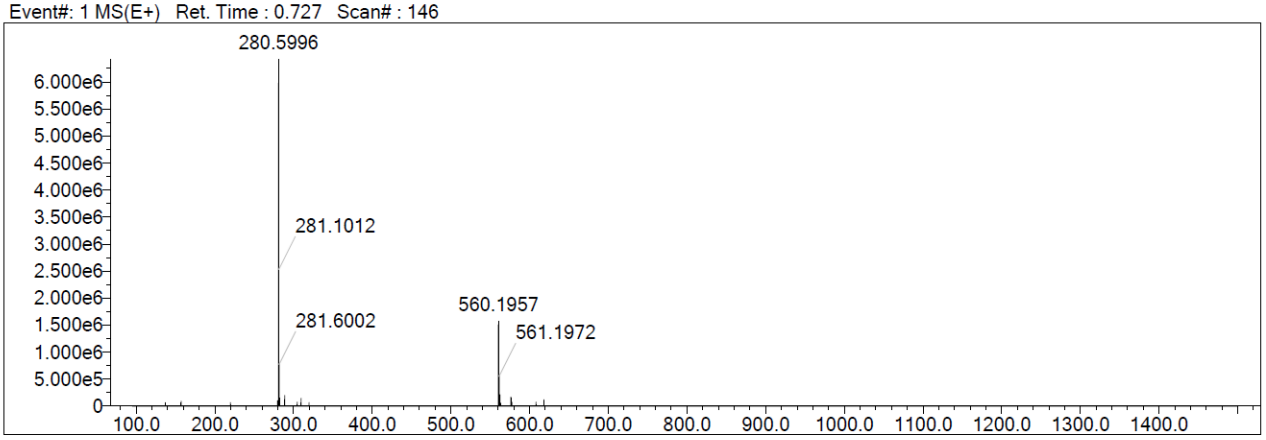
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	29	O	2	4	6	K	1	0	0	I	3	0	0	H
2H	1	0	0	F	1	0	1	Fe	2	0	0	Pt	2	0	0	Na
3H	1	0	0	Si	4	0	0	Cu	2	0	0	Au	1	0	0	
B	3	0	0	P	3	0	0	Se	2	0	0					
C	4	0	29	S	2	0	1	Br	1	0	0					
N	3	0	5	Cl	1	0	0	Pd	2	0	0					

Error Margin (ppm): 20
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

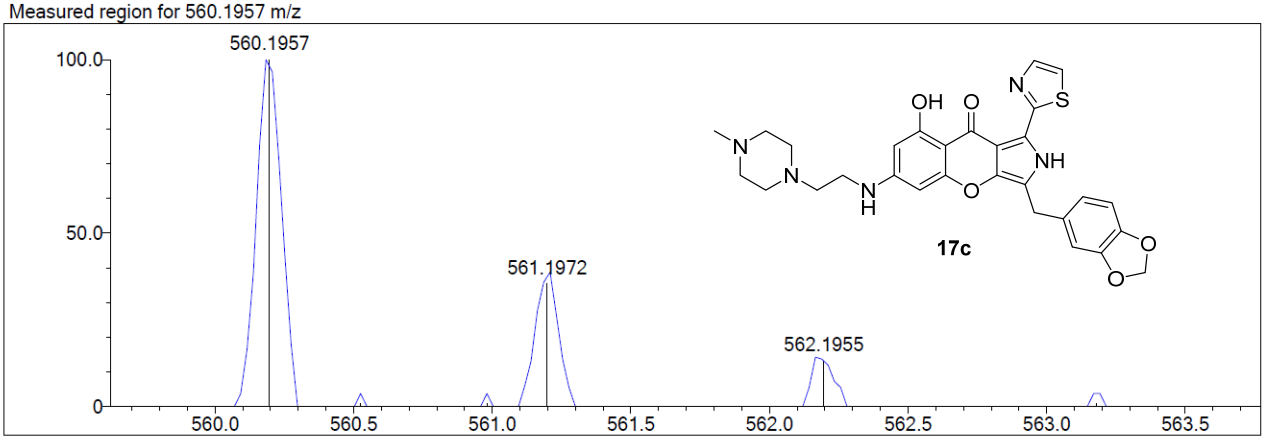
DBE Range: -50.0 - 50.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 500

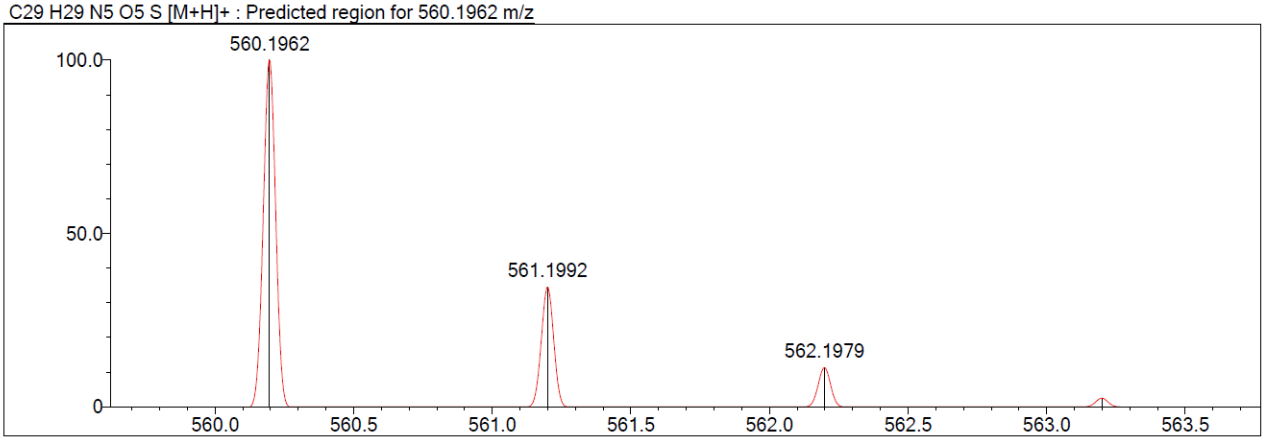
Event#: 1 MS(E+) Ret. Time : 0.727 Scan# : 146



Measured region for 560.1957 m/z



C29 H29 N5 O5 S [M+H]+ : Predicted region for 560.1962 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	71.17	C29 H29 N5 O5 S	[M+H]+	560.1957	560.1962	-0.5	-0.89	71.17	18.0

References.

1. Huang, Y.; Liu, X.; Wu, D.; Tang, G.; Lai, Z.; Zheng, X. ; Yin. S.; Luo, H.-B. The discovery, complex crystal structure, and recognition mechanism of a novel natural PDE4 inhibitor from Selaginella pulvinata. *Biochem. Pharmacol.* **2017**,130, 51-59.

2. Li, Z.; Cai, Y. H.; Cheng, Y. K.; Lu, X.; Shao, Y. X.; Li, X.; Liu, M.; Liu, P.; Luo, H.-B. Identification of Novel Phosphodiesterase-4D Inhibitors Prescreened by Molecular Dynamics-augmented Modeling and Validated by Bioassay. *J. Chem. Inf. Model.* **2013,** *53*, 972−981.

3. Lin, T. T.; Huang, Y. Y.; Tang, G. H.; Cheng, Z. B.; Liu, X.; Luo, H.-B.; Yin, S. Prenylated Coumarins: Natural Phosphodiesterase-4 Inhibitors from Toddalia Asiatica. *J. Nat. Prod.* **2014,** *77*, 955−962.

4. Wang, H.; Yan, Z.; Yang, S.; Cai, J.; Robinson, H.; Ke, H. Kinetic and Structural Studies of Phosphodiesterase-8A and Implication on the Inhibitor Selectivity. *Biochemistry* **2008,** *47*, 12760−12768.

5. Shang, N. N.; Shao, Y. X.; Cai, Y. H.; Guan, M.; Huang, M.; Cui, W.; He, L.; Yu, Y. J.; Huang, L.; Li, Z.; Bu, X. Z.; Ke, H.; Luo, H.-B. Discovery of 3-(4-Hydroxybenzyl)-1-(thiophen-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one as a Phosphodiesterase-5 Inhibitor and Its Complex Crystal Structure. *Biochem. Pharmacol.* **2014,** *89*, 86−98.

6. Shao, Y. X.; Huang, M.; Cui, W.; Feng, L. J.; Wu, Y.; Cai, Y.; Li, Z.; Zhu, X.; Liu, P.; Wan, Y.; Ke, H.; Luo, H.-B. Discovery of a Phosphodiesterase 9A Inhibitor as a Potential Hypoglycemic Agent. *J. Med. Chem.* **2014,** *57*, 10304−10313.