Supplementary Information

Targeting host integrated stress response: lead discovery of flavonoid compounds active against coronaviruses PEDV and PDCoV

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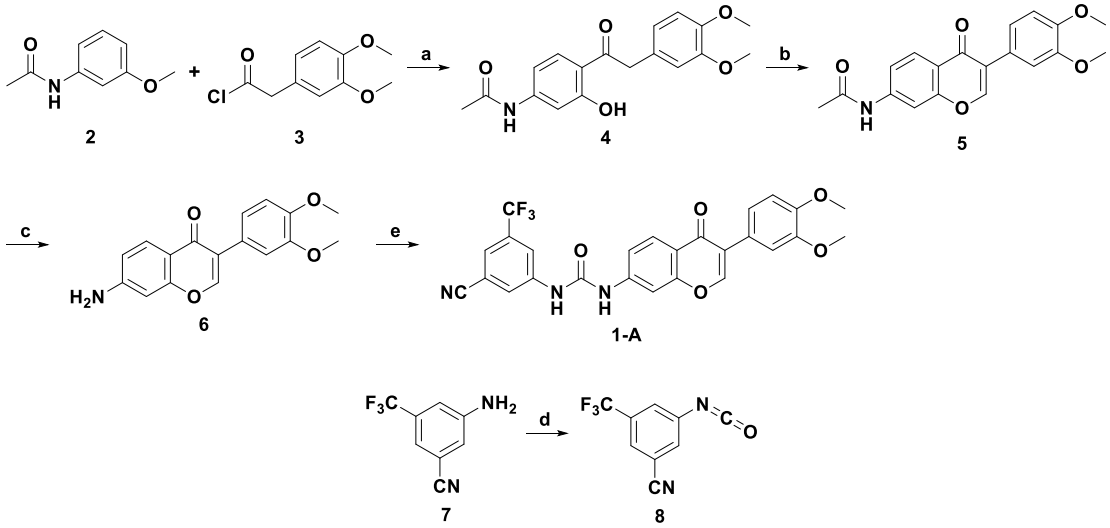
Part 1. Synthetic procedures and characterization data of title compounds **1-A** and **1-D**.

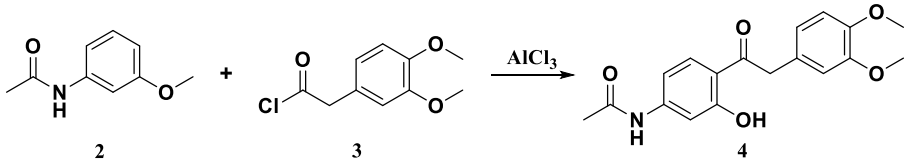
General. All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. Silica gel H was employed for column chromatography purification. ^{1}H NMR, ^{13}C and ^{19}F NMR data were recorded on either a Bruker Avance III 400 MHz or a Bruker Avance Neo 600 MHz spectrometer as specified. Chemical shifts were reported in parts per million (ppm). ^{1}H and ^{13}C spectra were referenced to residual protons (DMSO-d_6, δ 2.50 ppm) and carbons (DMSO-d_6, δ 39.5 ppm) in the deuterated solvents, respectively. Multiplicities are designated as s (singlet), br s (broad singlet), d (doublet), dd (doublet doublet), t (triplet), and m (multiplet). High-resolution mass (HRMS) analyses were run on a ThermoScientific Q Exactive Plus spectrometer operating with electrospray ionization (ESI) in positive ion mode, and a suitable external calibrant was used. Melting points were recorded using a TIANDA TIANFA YRT-3 melting point apparatus and were uncorrected.

Preparation of 1-A



Scheme S1 Synthesis of **1-A**. a. $AlCl_3$, ethylene chloride, ice cooling, 45 min, then r.t., 4 h, 49%; b. $BF_3{\cdot}Et_2O$, PCl_5, DMF, ice cooling, 45 min, r.t., 5 h, then 85–95 °C, 1 h, 45%; c. concentrated aqueous HCl-H_2O (1:1), reflux, 3 h, then NH_4OH, 61%; d. triphosgene, CH_2Cl_2, reflux, 1.5 h, 100%; e. **8**, Et_3N, CH_2Cl_2, reflux, 4 h, 29%.

***N*-(4-(2-(3,4-Dimethoxyphenyl)acetyl)-3-hydroxyphenyl)acetamide (4)**

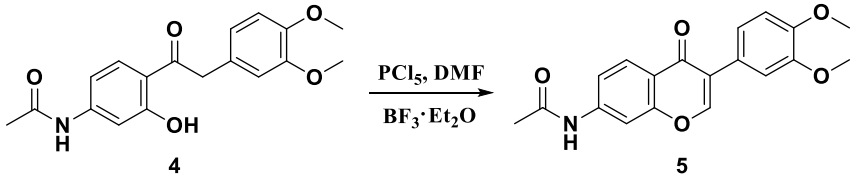


To a solution of **2** (10 g, 60.54 mmol) and **3** (15.6 g, 72.68 mmol) in dry ethylene chloride (50 mL) under icebath cooling was added anhydrous aluminum chloride (20.2 g, 151.5 mmol) in three portions. The reaction was allowed to stir under icebath cooling for 45 min, then at room temperature for 4 h, and quenched with water (20 mL). To the resulting black viscous mixture was added 4 mol/L aqueous HCl (100 mL), and a gray suspension was observed after 4 h, which was

filtered. The filter was washed with methylene chloride (20 mL), and the combined red filtration was extracted with methylene chloride (3 × 40 mL). The combined organic extract was washed with saturated brine (40 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a black oil. This residue was chromatographed on silica gel with gradient eluting of a mixture of petroleum ether (60–90 °C) and ethyl acetate (5:1–2:1) to furnish **4** (9.8 g, yield 49%) as a gray oil. **4** could be obtained as an off-white solid after further chromatography followed by recrystallization from ethanol. mp: 138.2−138.7 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (s, 1H), 10.27 (s, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 2.1 Hz, 1H), 7.06 (dd, J = 8.8, 2.1 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.79 (dd, J = 8.2, 2.1 Hz, 1H), 4.24 (s, 2H), 3.72 (s, 3H), 3.71 (s, 3H), 2.08 (s, 3H).

***N*-(3-(3,4-Dimethoxyphenyl)-4-oxo-4*H*-chromen-7-yl)acetamide (5)**

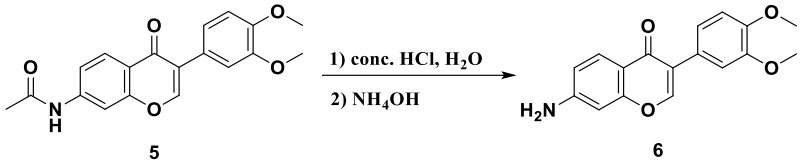


4 5

To a solution of **4** (9.3 g, 28.2 mmol) in dry DMF (20 mL) under icebath cooling was added boron trifluoride diethyl etherate (23.0 g, 162.1 mmol), followed by phosphorus pentachloride (5.1 g, 24.5 mmol) in portions. The mixture was allowed to stir under icebath cooling for 45 min, then at room temperature for 5 h. 90 °C Hot water (40 mL) was added, and the mixture was allowed to stir at 85−95 °C for 1 h to obtain a crimson solution. Water (200 mL) was added, and the mixture was allowed to stir at room temperature to crystallize a yellow solid, which was filtered and washed with water. The filter was subjected to recrystallization from ethanol (10 mL), followed by drying *in vacuo* (80 °C, 5 h) to afford **5** (4.3 g, yield 45%) as a yellow solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.44 (s, 1H), 8.08 (d, J = 1.9 Hz, 1H), 8.06 (d, J = 8.7 Hz, 1H), 7.49 (dd, J = 8.7, 2.0 Hz, 1H), 7.20 (d, J = 2.0 Hz, 1H), 7.14 (dd, J = 8.2, 2.0 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 3.78 (s, 3H), 3.78 (s, 3H), 2.13 (s, 3H).

7-Amino-3-(3,4-dimethoxyphenyl)-4*H*-chromen-4-one (6)

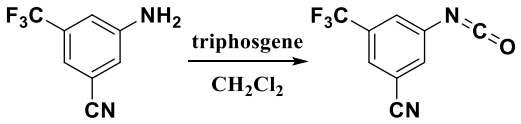


5 6

5 (4.3 g, 12.7 mmol) was added to a mixture of concentrated aqueous HCl (6 mL) and water (6 mL). The reaction was stirred under reflux for 3 h, and concentrated *in vacuo*. The residue was dissolved in water (15 mL), and the pH was adjusted from 1 to 10 with concentrated NH_4OH. The resulting slurry was filtered, and the filter was washed with water, recrystallized from ethanol, and dried *in vacuo* (80 °C, 3 h) to deliver **6** (2.3 g, yield 61%) as an off-white powder. mp: 183.8−184.5 °C.

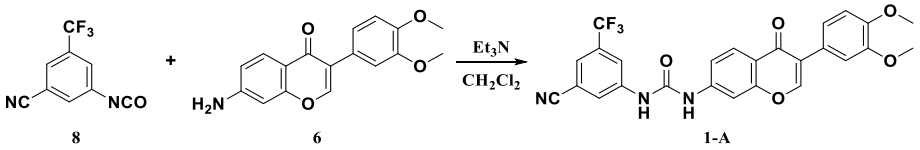
^{1}H NMR (600 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.3, 2.0 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.69 (dd, J = 8.7, 2.0 Hz, 1H), 6.51 (d, J = 2.0 Hz, 1H), 6.31 (s, 2H), 3.77 (s, 3H), 3.77 (s, 3H).

1-(3-Cyano-5-(trifluoromethyl)phenyl)-3-(3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-7-yl)urea (1-A)



7 8

7 (0.2 g, 1.1 mmol) was added under icebath cooling to a solution of triphosgene (0.2 g, 0.68 mmol) in dry THF (10 mL), and the mixture was allowed to stir at room temperature for 0.5 h, and then under reflux for 1.5 h under nitrogen. The reaction was evaporated *in vacuo* (55 °C) to deliver **8** in quantitative yield as a brown oil.



8 6 1-A

To a solution of **6** (54 mg, 0.18 mmol) and triethylamine (73 mg, 0.72 mmol) in dry methylene chloride (3.5 mL) under nitrogen and reflux was added dropwise a solution of **8** (154 mg, 0.72 mmol) in dry methylene chloride (4 mL). The reaction was then allowed to stir under reflux for 4 h, and quenched with water. The aqueous phase was separated and extracted with methylene chloride (3 × 10 mL). The combined organic phase was washed with saturated brine (20 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a yellow solid. The residue was subjected to silica gel chromatography eluting with a mixture of petroleum ether (60–90 °C) and ethyl acetate (1:1, v/v) to afford a yellow solid, which was treated with activated charcoal in hot ethyl acetate. The obtained solid was slurried in ethyl ether to deliver **1-A** (27 mg, yield 29%) as a yellow solid.

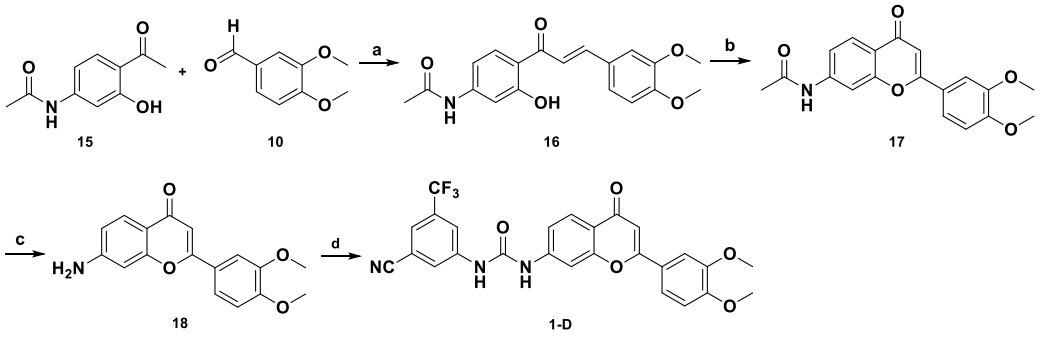
^{1}H NMR (600 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.57 (s, 1H), 8.46 (s, 1H), 8.25 – 8.23 (m, 1H), 8.14 – 8.13 (m, 1H), 8.07 (d, J = 8.7 Hz, 1H), 7.96 – 7.94 (m, 2H), 7.42 (dd, J = 8.8, 2.1 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.16 (dd, J = 8.3, 2.1 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 3.79 (s, 6H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 175.05, 156.85, 154.17, 152.48, 148.93, 148.58, 144.53, 141.32, 131.19 (q, J = 33.2 Hz), 126.77, 125.55, 124.61, 123.34 (q, J = 271.7 Hz), 123.84, 121.59, 118.85, 117.79, 116.97, 113.37, 112.92, 111.85, 105.59, 55.84.

^{19}F NMR (376 MHz, DMSO-d_6) δ -61.79.

ESI-HRMS: calcd for $[M + H]^+$, m/z: 510.12713; found, m/z: 510.12611; calcd for $[M + Na]^+$, m/z: 532.10908; found, m/z: 532.10799.

Preparation of 1-D

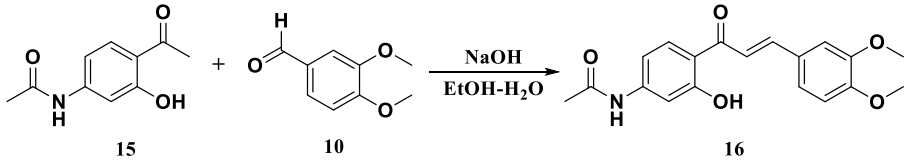


15 10 16 17 18 1-D

Scheme 2S Synthesis of **1-D**. a. NaOH, EtOH-H_2O (4:3), r.t., 24 h, 58%; b. I_2, DMSO, 180 °C, 12

h, 41%; c. conc. aqueous HCl-H_2O (1:1), reflux, 3 h, 67%; d. **8**, Et_3N, CH_2Cl_2, reflux, 1 h, 50%.

(*E*)-*N*-(4-(3-(3,4-Dimethoxyphenyl)acryloyl)-3-hydroxyphenyl)acetamide (16)

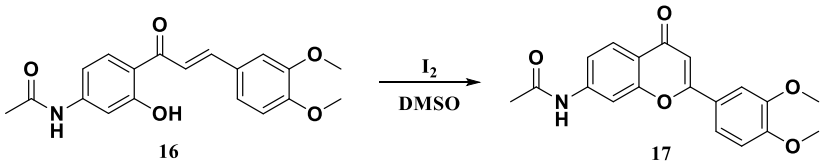


15 10 16

To a solution of **15** (7 g, 36.23 mmol) in ethanol (20 mL) was added a solution of sodium hydroxide (7.25 g, 181.25 mmol) in water (15 mL), and the mixture was allowed to stir at room temperature for 30 min. Upon addition of **10** (7.22 g, 43.45 mmol), the reaction was allowed to stir at room temperature for 24 h. After adding concentrated aqueous HCl (2 mL), the resulting mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic extract was washed with saturated brine (20 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was chromatographed on silica gel eluting with a mixture of petroleum ether (60–90 °C) and ethyl acetate (1:1) to deliver **16** (7.21 g, yield 58%) as a yellow solid. mp: 126.3−128.2 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.30 (s, 1H), 10.50 (s, 1H), 8.28 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 15.3 Hz, 1H), 7.79 (d, J = 15.3 Hz, 1H), 7.57 (s, 1H), 7.42 – 7.38 (m, 2H), 7.15 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 2.10 (s, 3H).

***N*-(2-(3,4-Dimethoxyphenyl)-4-oxo-4H-chromen-7-yl)acetamide (17)**

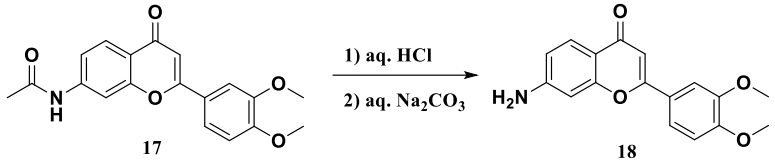


16 17

To a solution of **16** (3 g, 8.8 mmol) in DMSO (15 mL) was added iodine (2.23 g, 8.8 mmol), and the mixture was allowed to stir at 180 °C for 12 h. The resulting reaction was washed with saturated aqueous sodium thiosulfate (20 mL). The aqueous phase was back-extracted with methylene chloride (3 × 15 mL). The combined organic extract was washed with saturated brine (20 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was chromatographed on silica gel eluting with a mixture of methylene chloride and methanol (40:1) to furnish **17** (1.24 g, yield 41%) as an off-white solid. mp: 267.8−268.5 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.20 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.67 (dd, J = 8.6, 2.2 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 6.95 (s, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 2.13 (s, 3H).

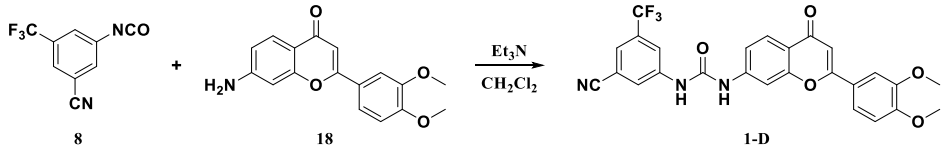
7-Amino-2-(3,4-dimethoxyphenyl)-4*H*-chromen-4-one (18)



A mixture of **17** (1.4 g, 4.13 mmol), concentrated aqueous HCl (7 mL) and water (7 mL) was allowed to reflux for 3 h. The resulting reaction was cooled to room temperature, and evaporated *in vacuo*. The residue was recrystallized from ethanol to give red crude hydrochloride product, which was dissolved in methylene chloride (40 mL). Saturated aqueous sodium carbonate (30 mL) was added to the solution, and the mixture was allowed to stir for 30 min. The organic phase was separated and washed with saturated brine (25 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The red solid residue was chromatographed on silica gel with gradient eluting of a mixture of methylene chloride and methanol (50:0−50:1) to furnish **18** (0.825 g, yield 67%) as an orange solid. mp: 217.4−218.5 °C.

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.68 (d, J = 8.5 Hz, 1H), 7.60 (dd, J = 8.5, 2.2 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 6.76 (s, 1H), 6.66 (dd, J = 8.6, 2.0 Hz, 1H), 6.64 (d, J = 2.0 Hz, 1H), 6.27 (br s, 2H), 3.87 (s, 3H), 3.84 (s, 3H).

1-(3-Cyano-5-(trifluoromethyl)phenyl)-3-(2-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-7-yl)urea (1-D)



To a solution of **18** (0.2 g, 0.67 mmol) and triethylamine (0.2 g, 2 mmol) in dry methylene chloride (10 mL) under nitrogen and reflux was added dropwise a solution of **8** (0.21 g, 1 mmol) in dry methylene chloride (5 mL). The reaction was then allowed to stir under reflux for 1 h, quenched with water (10 mL), and extracted with methylene chloride (3 × 15 mL). The combined organic extract was washed with saturated brine (20 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The solid residue was subjected to silica gel chromatography with gradient eluting of a mixture of methylene chloride and methanol (30:0−30:1). After drying *in vacuo* (100 °C, 10 h), **1-D** (0.17 g, yield 50%) was obtained as a pale yellow solid. mp: 214.6−215.9 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 9.56 (s, 1H), 8.26 (t, J = 1.9 Hz, 1H), 8.13 (t, J = 1.8 Hz, 1H), 8.11 (d, J = 2.0 Hz, 1H), 7.96 (d, J = 6.2 Hz, 1H), 7.95 (s, 1H), 7.71 (dd, J = 8.5, 2.1 Hz, 1H), 7.58 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.7, 2.1 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H), 6.97 (s, 1H), 3.90 (s, 3H), 3.86 (s, 3H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 176.38, 162.56, 156.70, 152.33, 151.88, 149.09, 144.34, 141.62, 125.74, 124.76, 122.65 (d, J = 270.0 Hz), 120.80, 120.65, 120.38, 119.88, 119.18, 118.12, 117.53, 116.23, 113.20, 111.76, 109.31, 108.08, 105.41, 55.92, 55.76.

ESI-HRMS: calcd for $[M + H]^+$, m/z: 510.12713; found, m/z: 510.12701; calcd for $[M + Na]^+$, m/z: 532.10908; found, m/z: 532.10786.

Part 2. NMR and HRMS spectra.

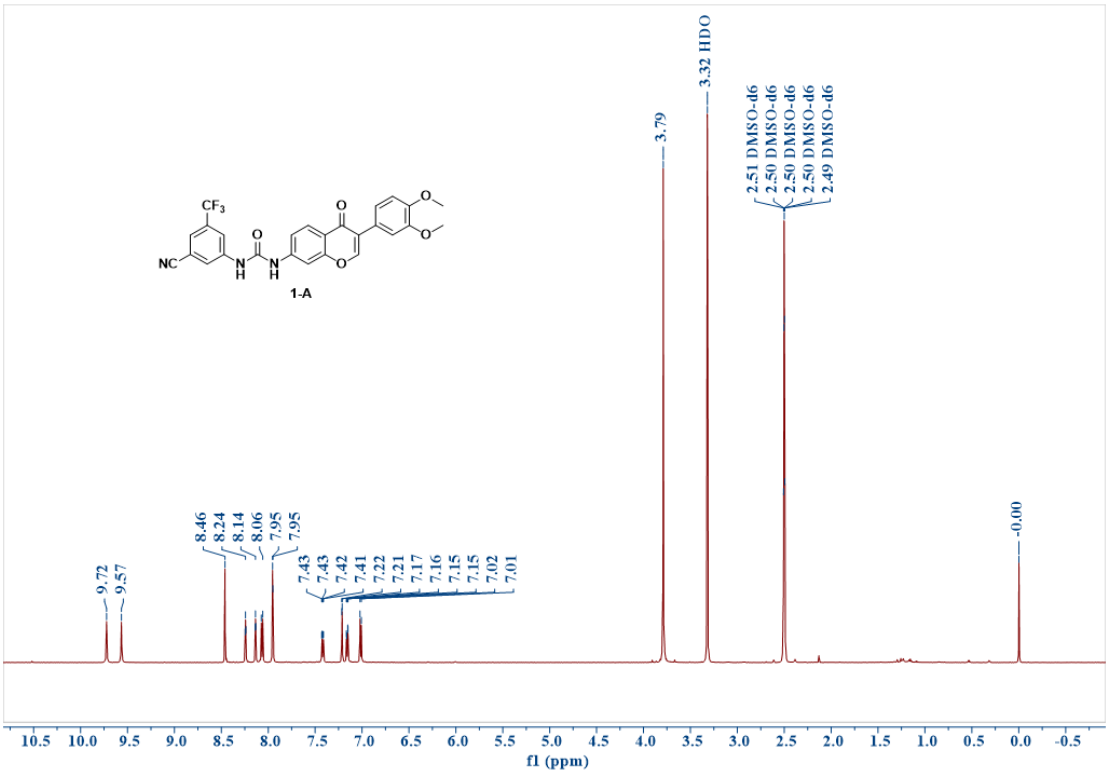


Figure S1 ^{1}H NMR of title compound **1-A**.

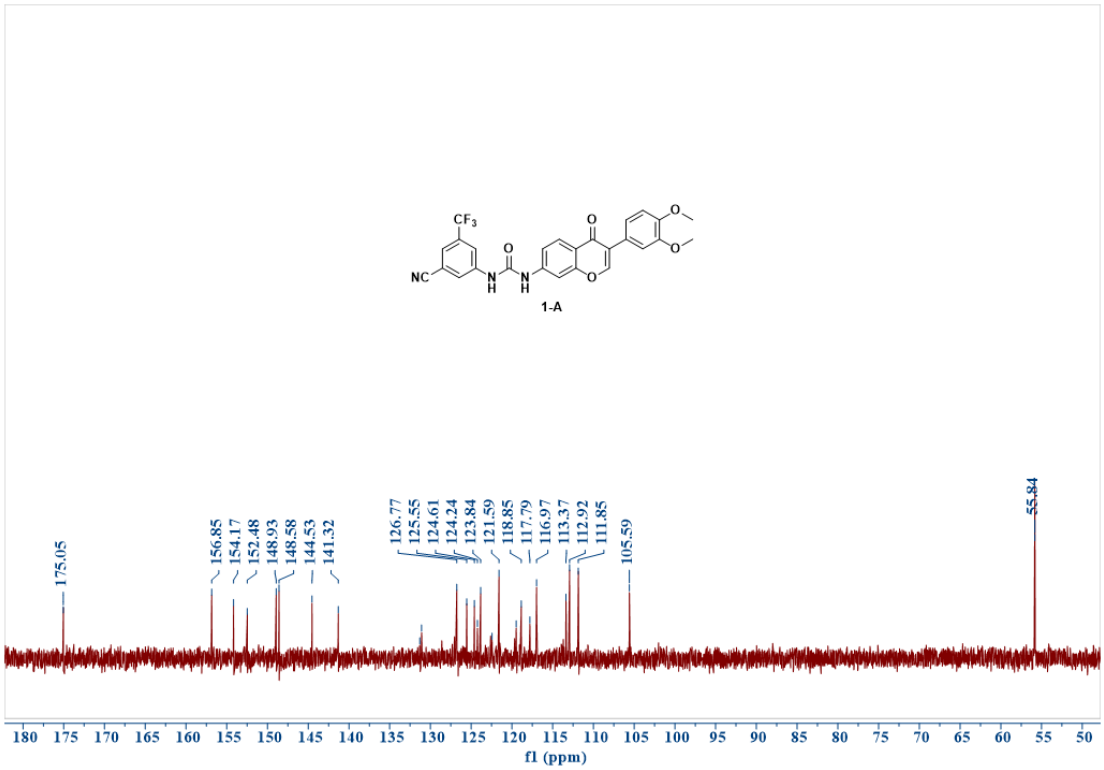


Figure S2 ^{13}C NMR of title compound **1-A**.

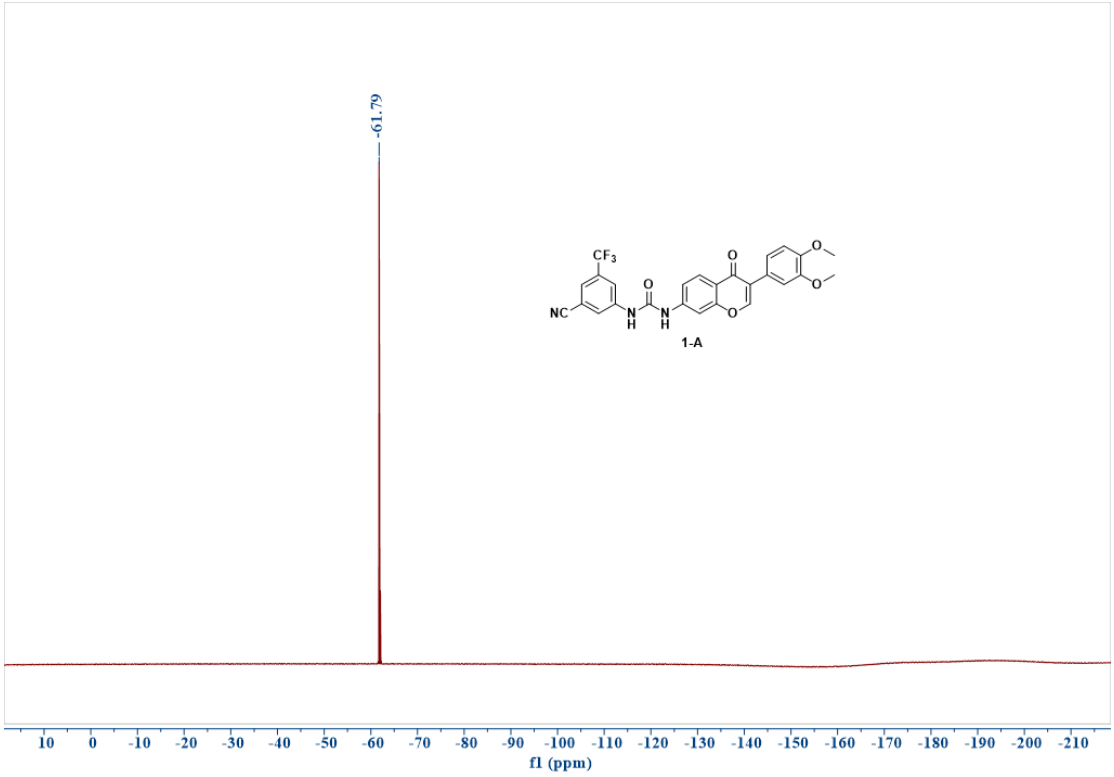
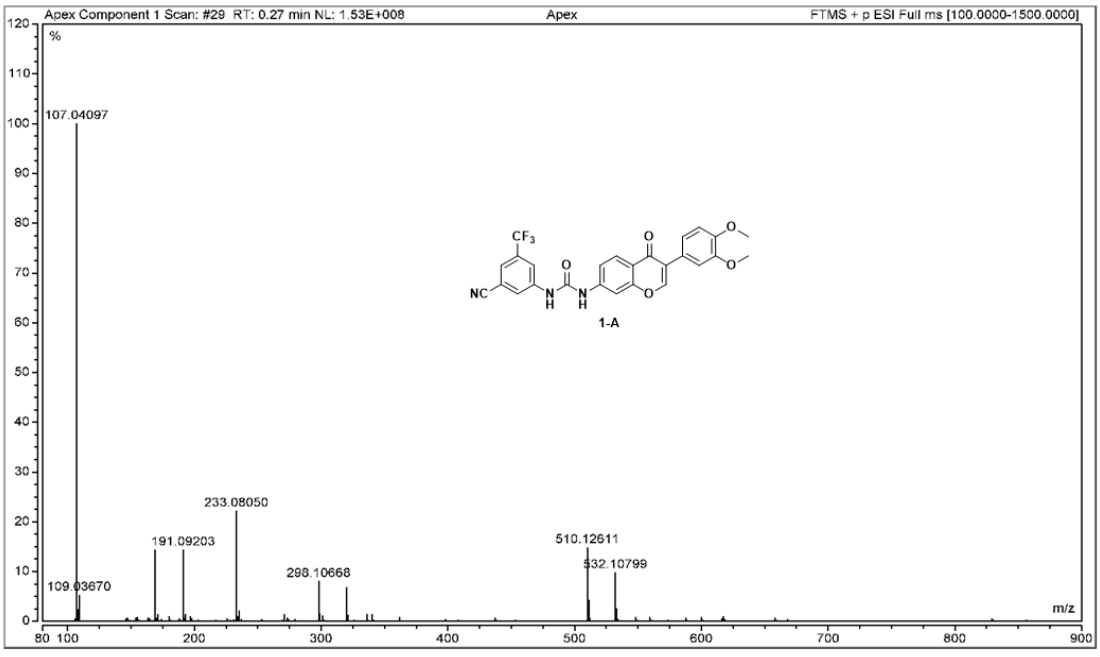


Figure S3 ^{19}F NMR of title compound **1-A**.



Mass spectrum Peak List						
RT (min)	m/z	Theo. Mass	Charge	Delta (ppm)	Ion	Formula
0.27	510.12611	510.12713	1	-2.00	[M+H]+	C26H18F3N3O5
0.27	532.10799	532.10908	1	-2.04	[M+Na]+	C26H18F3N3O5

Figure S4 ESI-HRMS of title compound **1-A**.

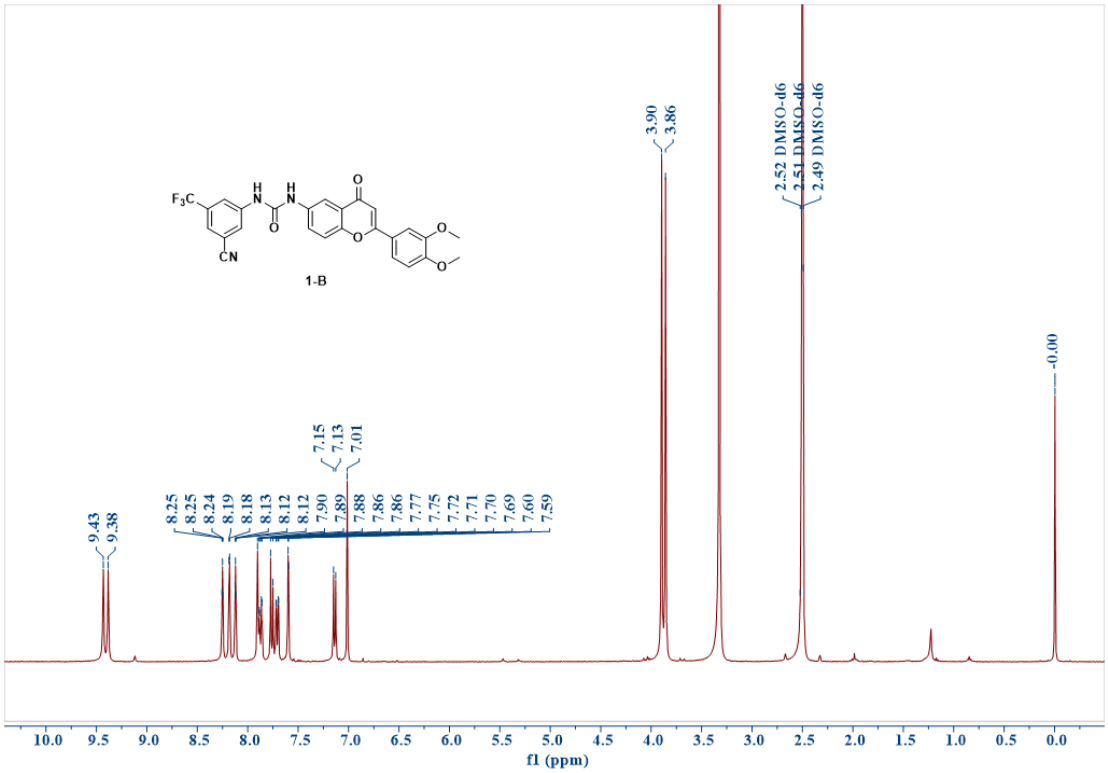


Figure S5 ^{1}H NMR of title compound **1-B**.

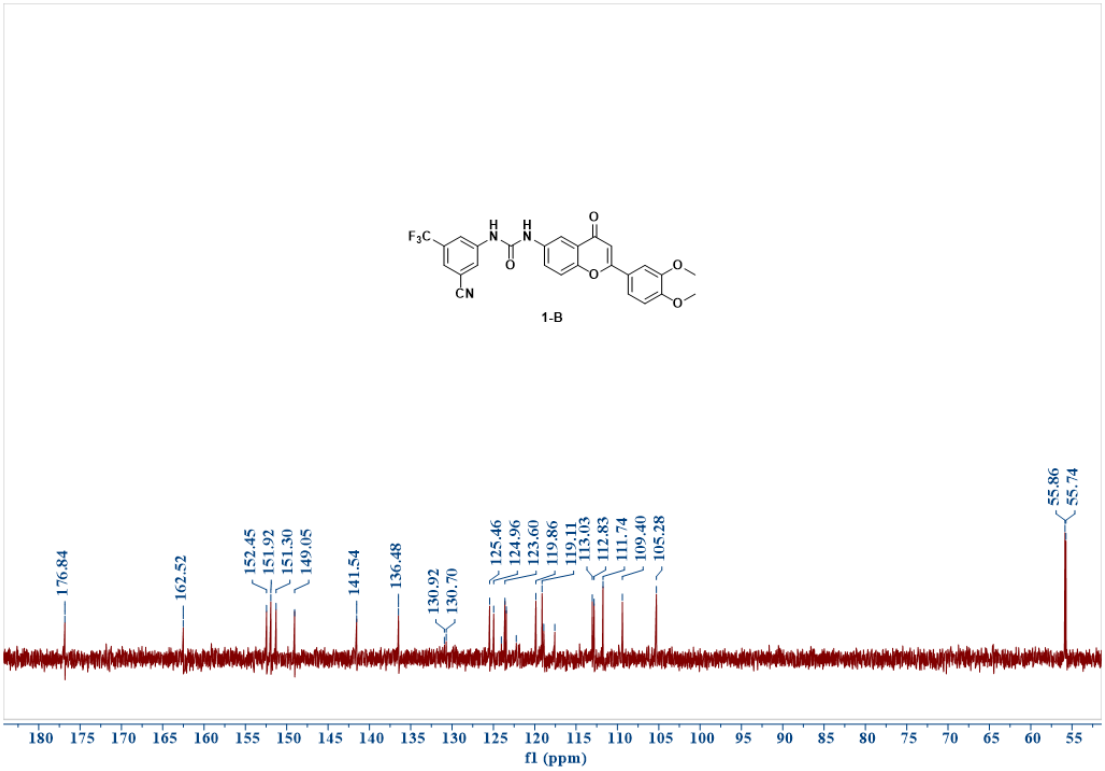


Figure S6 ^{13}C NMR of title compound **1-B**.

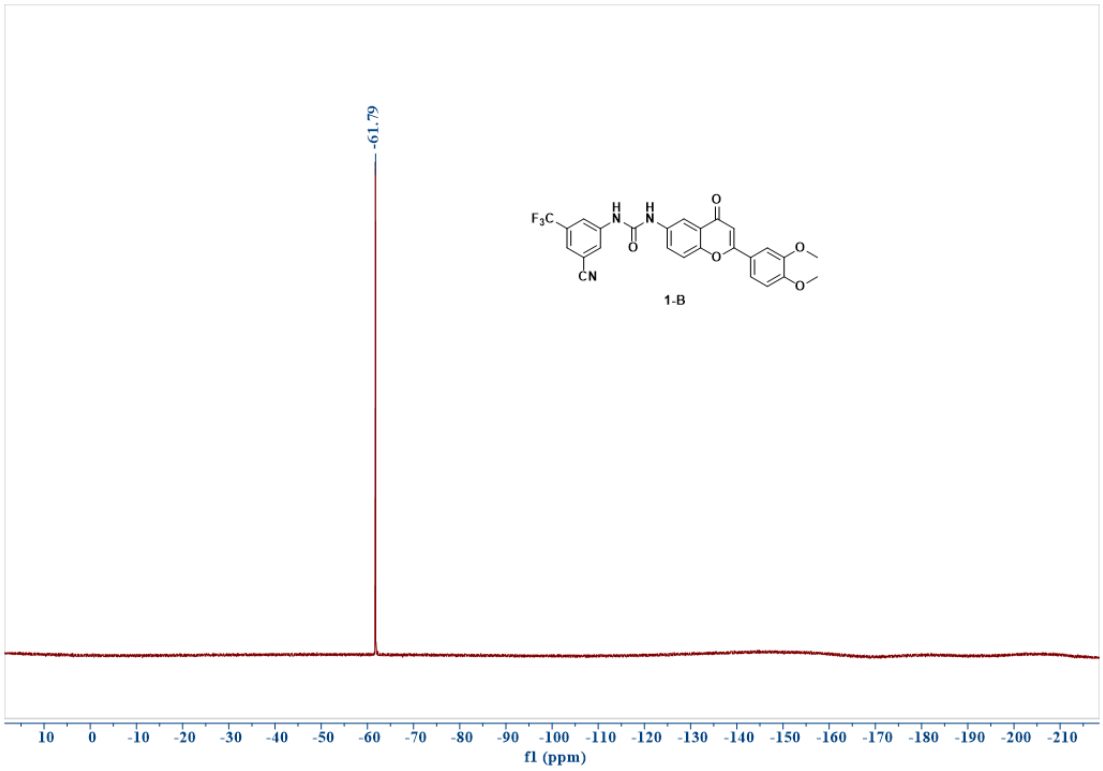
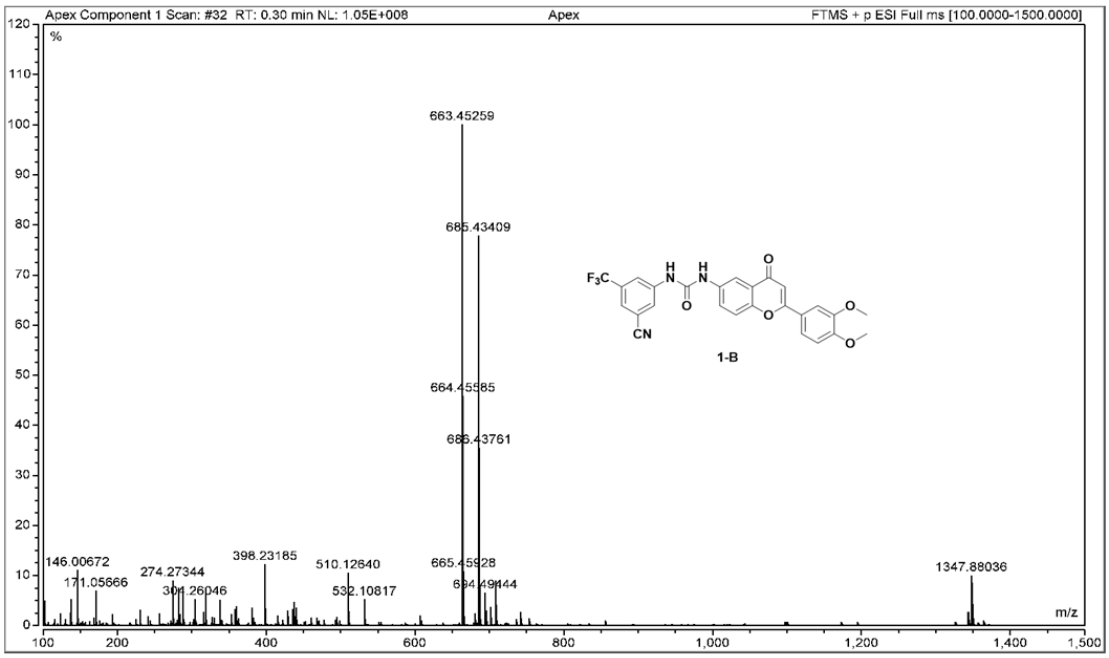


Figure S7 ^{19}F NMR of title compound **1-B**.



Mass spectrum Peak List						
RT (min)	m/z	Theo. Mass	Charge	Delta (ppm)	Ion	Formula
0.30	510.12640	510.12713	1	-1.43	[M+H]+	C26H18F3N3O5
0.30	532.10817	532.10908	1	-1.70	[M+Na]+	C26H18F3N3O5

Figure S8 ESI-HRMS of title compound **1-B**.

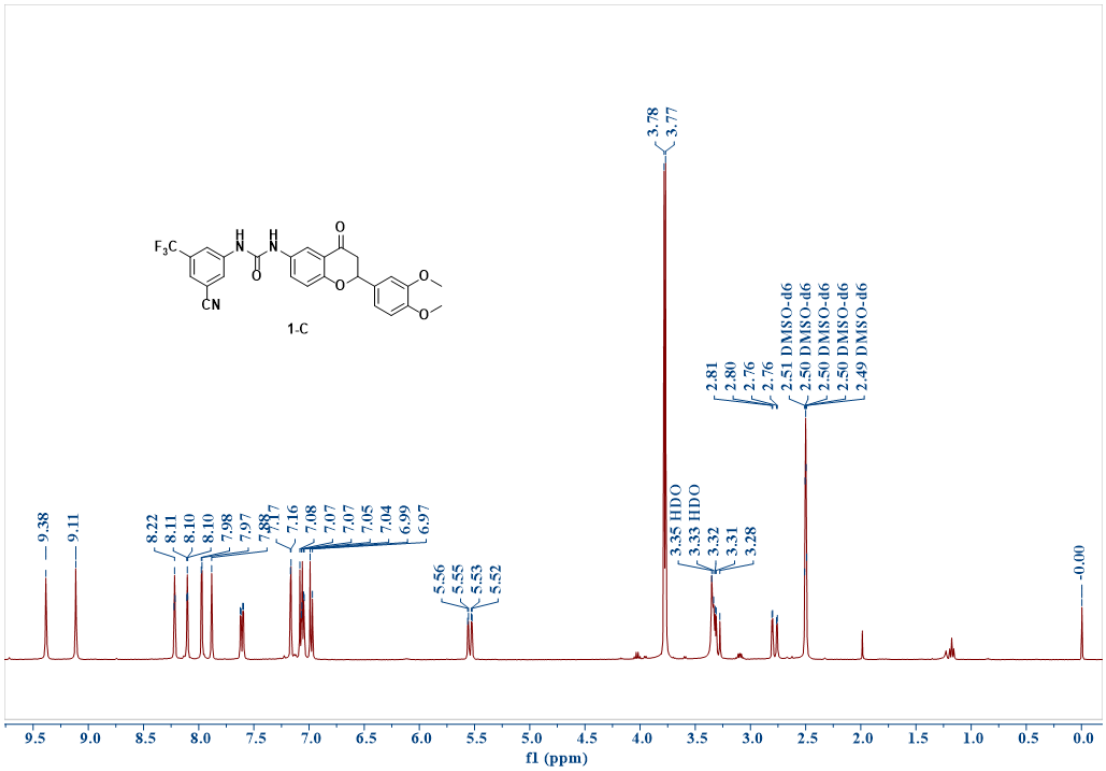


Figure S9 ^{1}H NMR of title compound **1-C**.

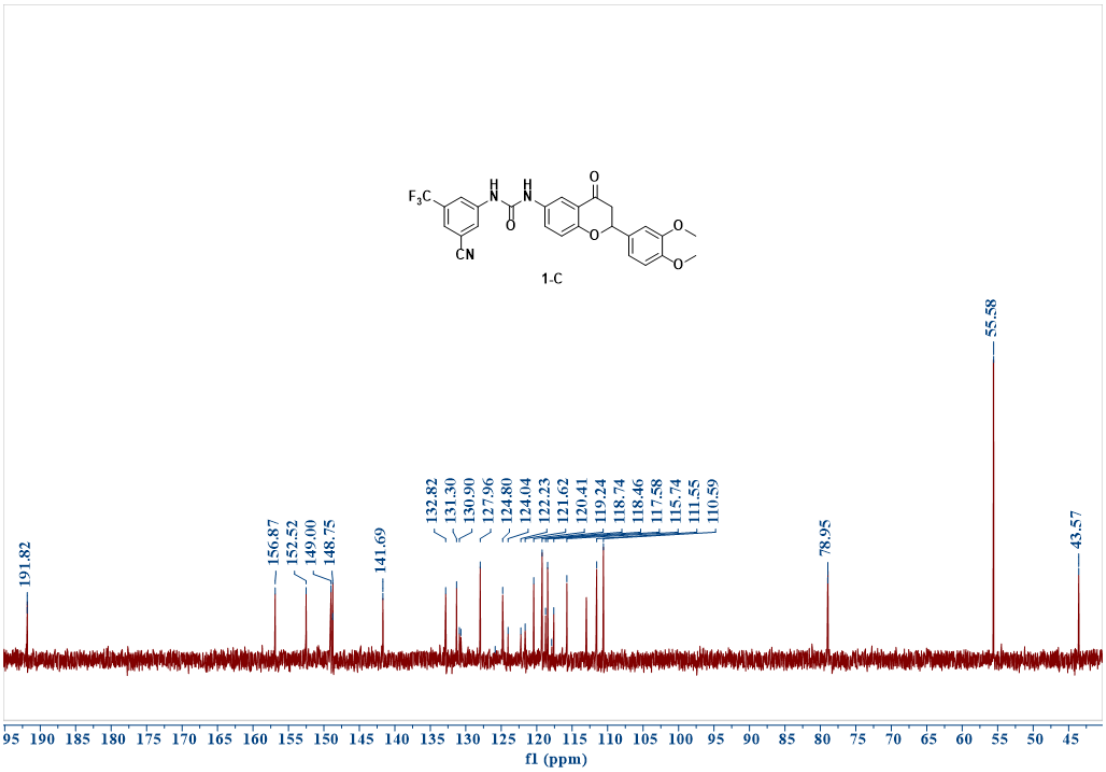


Figure S10 ^{13}C NMR of title compound **1-C**.

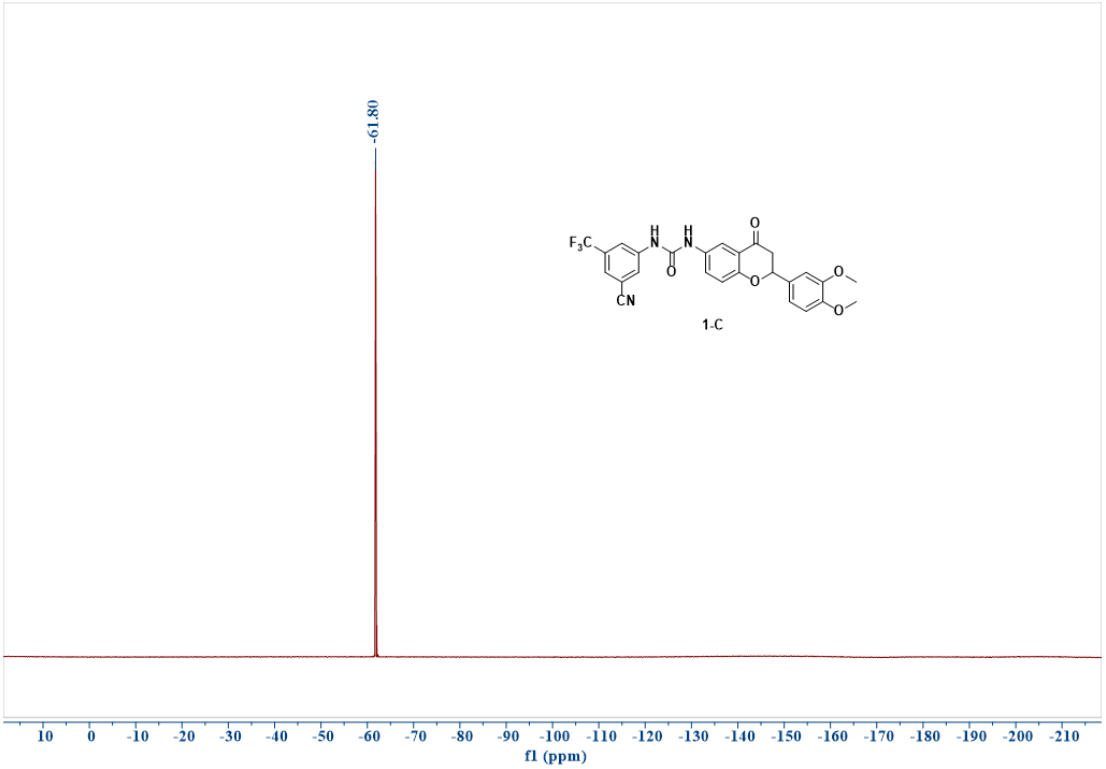
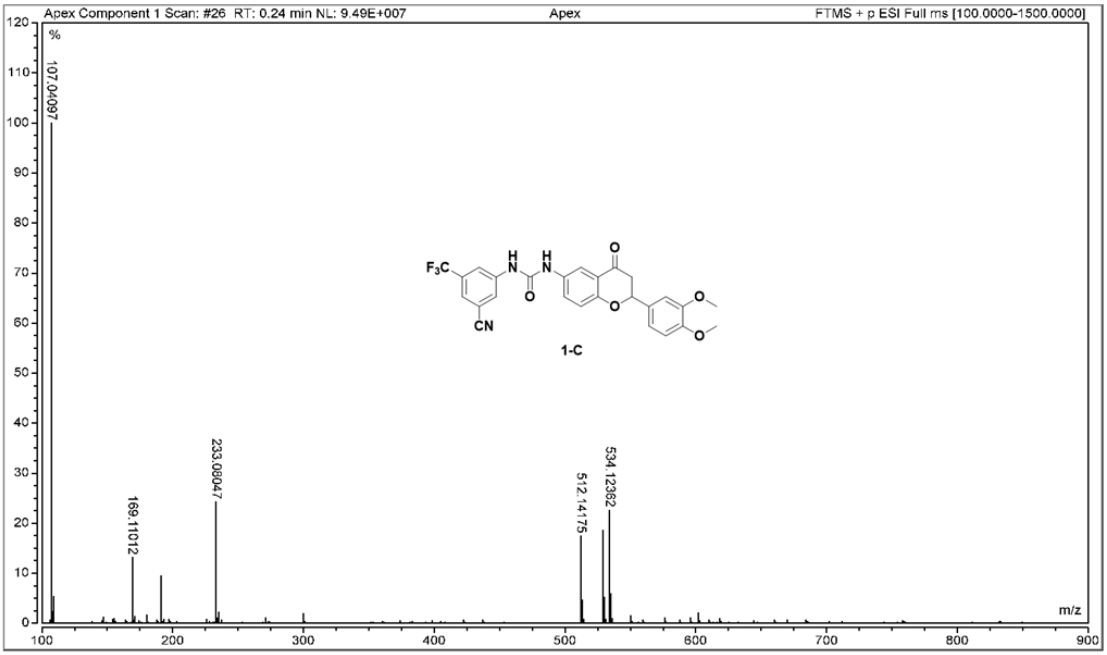


Figure S11 ^{19}F NMR of title compound **1-C**.



Mass spectrum Peak List						
RT (min)	m/z	Theo. Mass	Charge	Delta (ppm)	Ion	Formula
0.24	512.14175	512.14278	1	-2.01	[M+H]+	C26H20F3N3O5
0.24	529.16837	529.16933	1	-1.82	[M+NH4]+	C26H20F3N3O5
0.24	534.12362	534.12473	1	-2.07	[M+Na]+	C26H20F3N3O5

Figure S12 ESI-HRMS of title compound **1-C**.

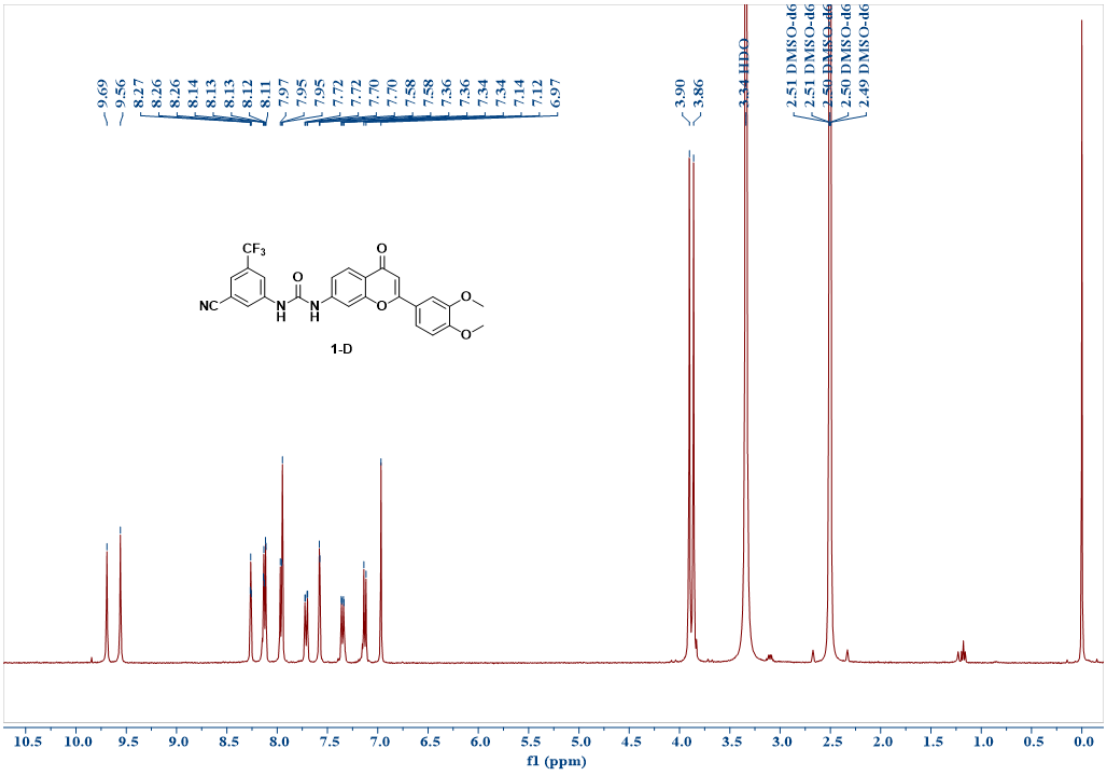


Figure S13 ^{1}H NMR of title compound **1-D**.

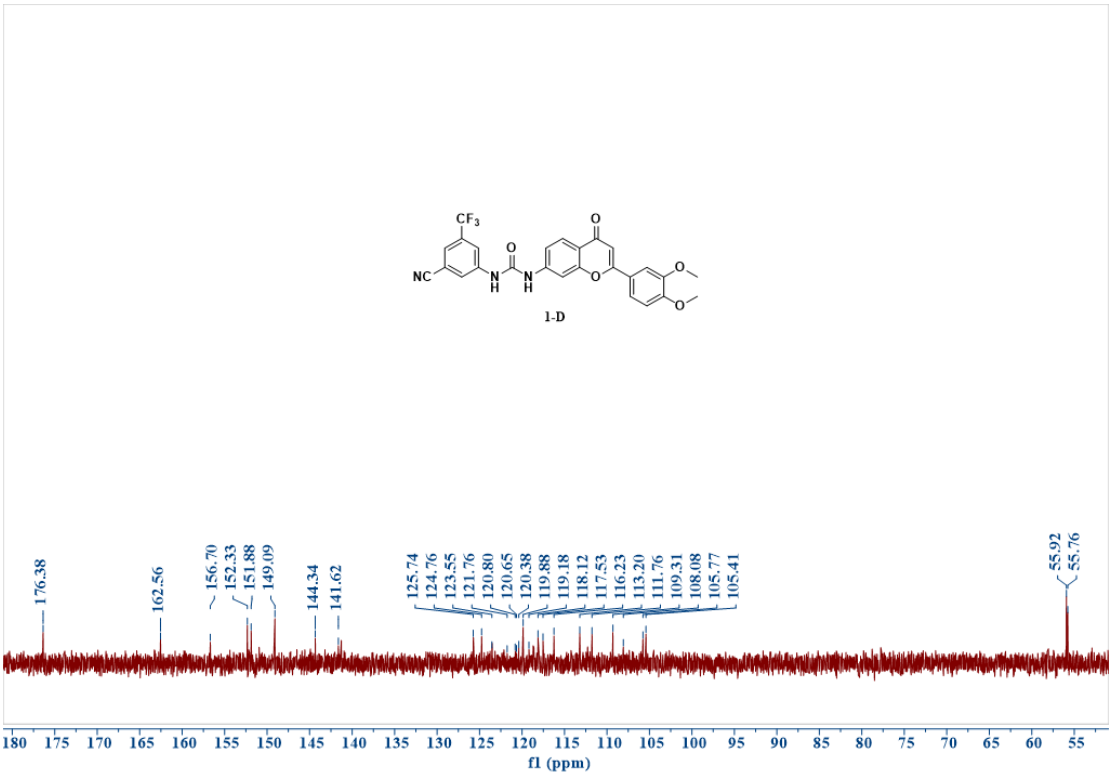
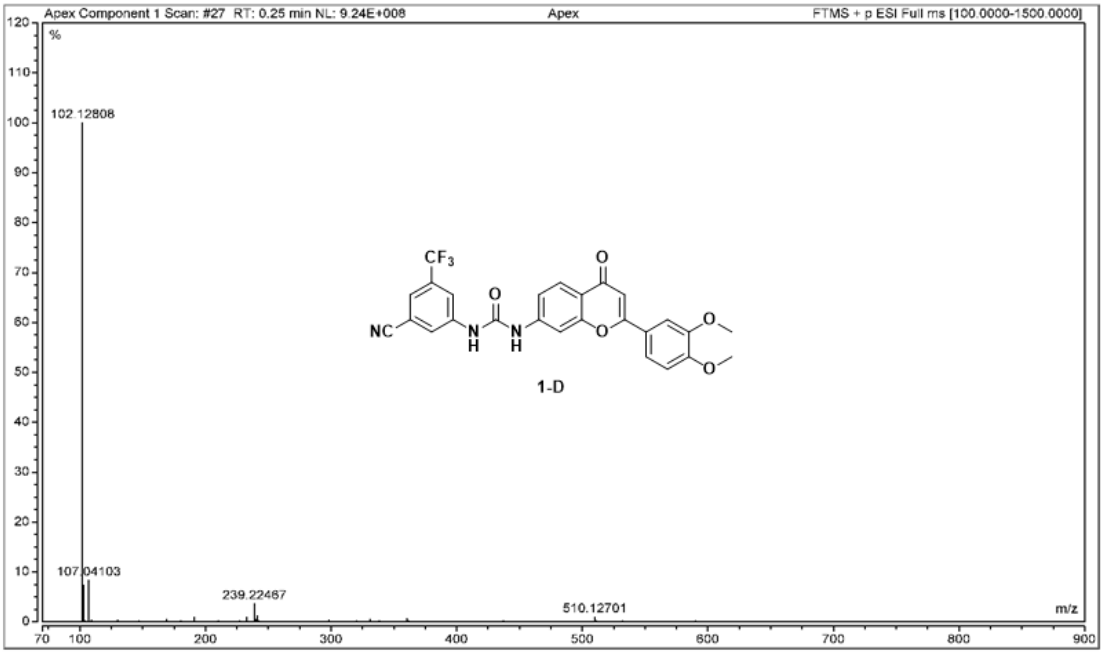


Figure S14 ^{13}C NMR of title compound **1-D**.



Mass spectrum Peak List						
RT (min)	m/z	Theo. Mass	Charge	Delta (ppm)	Ion	Formula
0.25	510.12701	510.12713	1	-0.24	[M+H]+	C26H18F3N3O5
0.25	532.10786	532.10908	1	-2.29	[M+Na]+	C26H18F3N3O5

Figure S15 ESI-HRMS of title compound **1-D**.

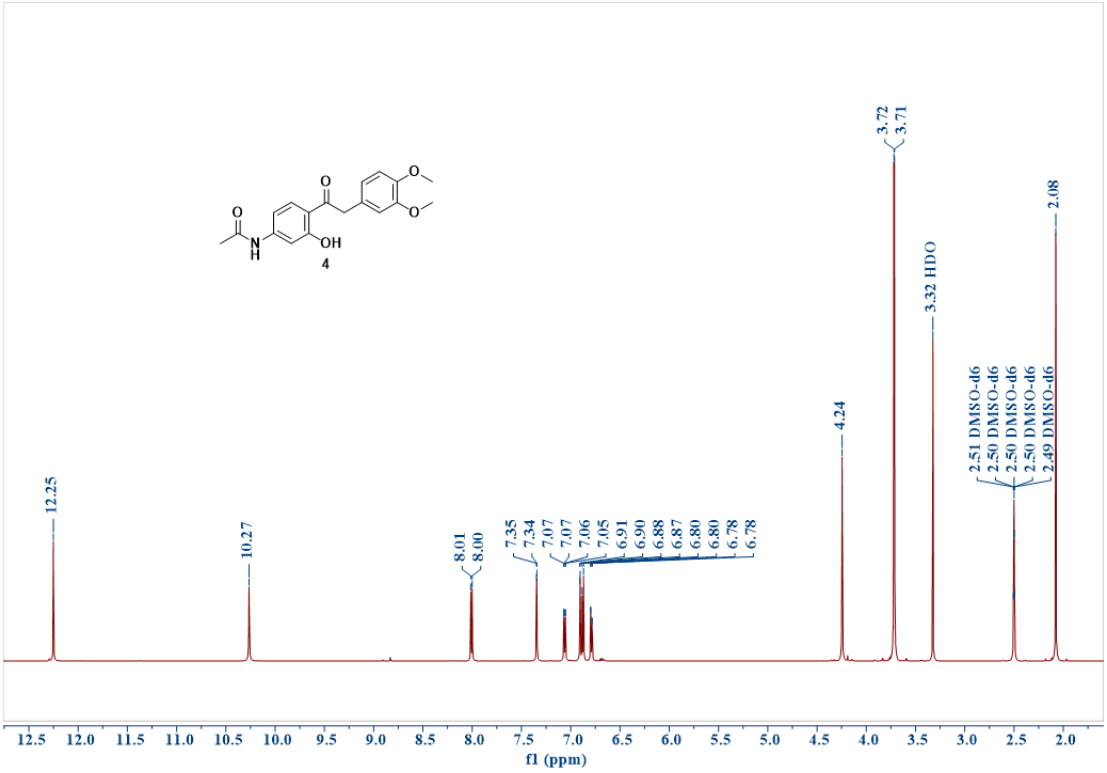


Figure S16 ^{1}H NMR of intermediate **4**.

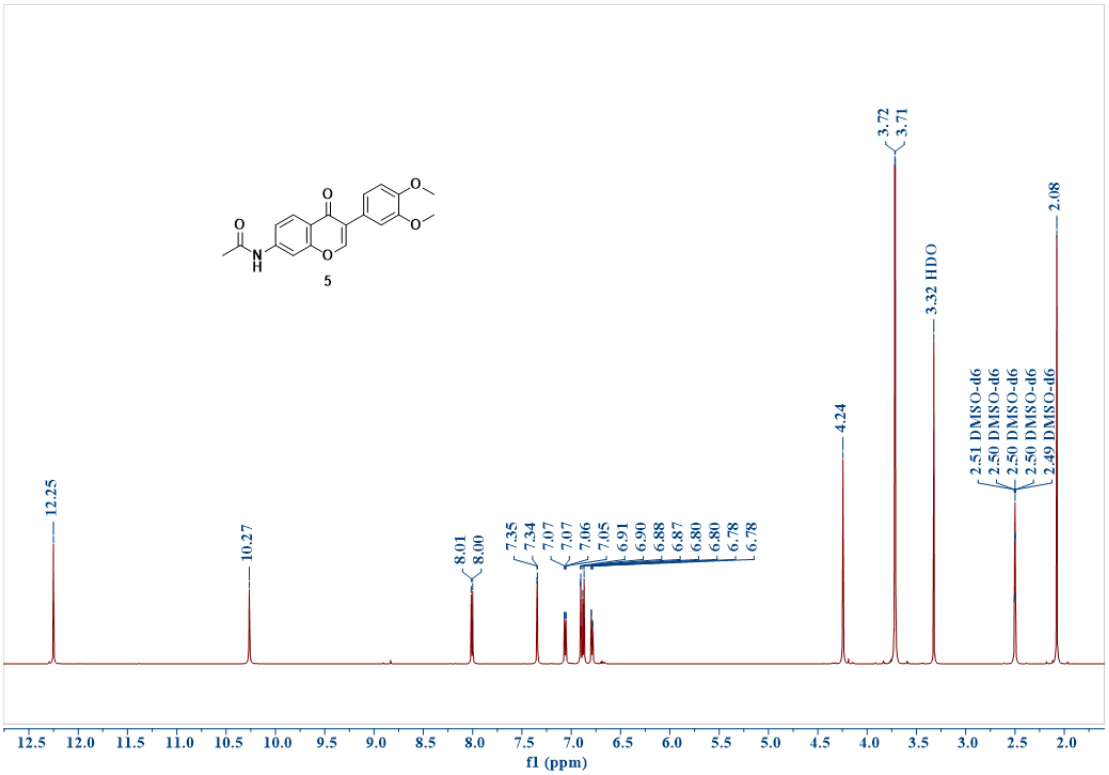


Figure S17 ^{1}H NMR of intermediate **5**.

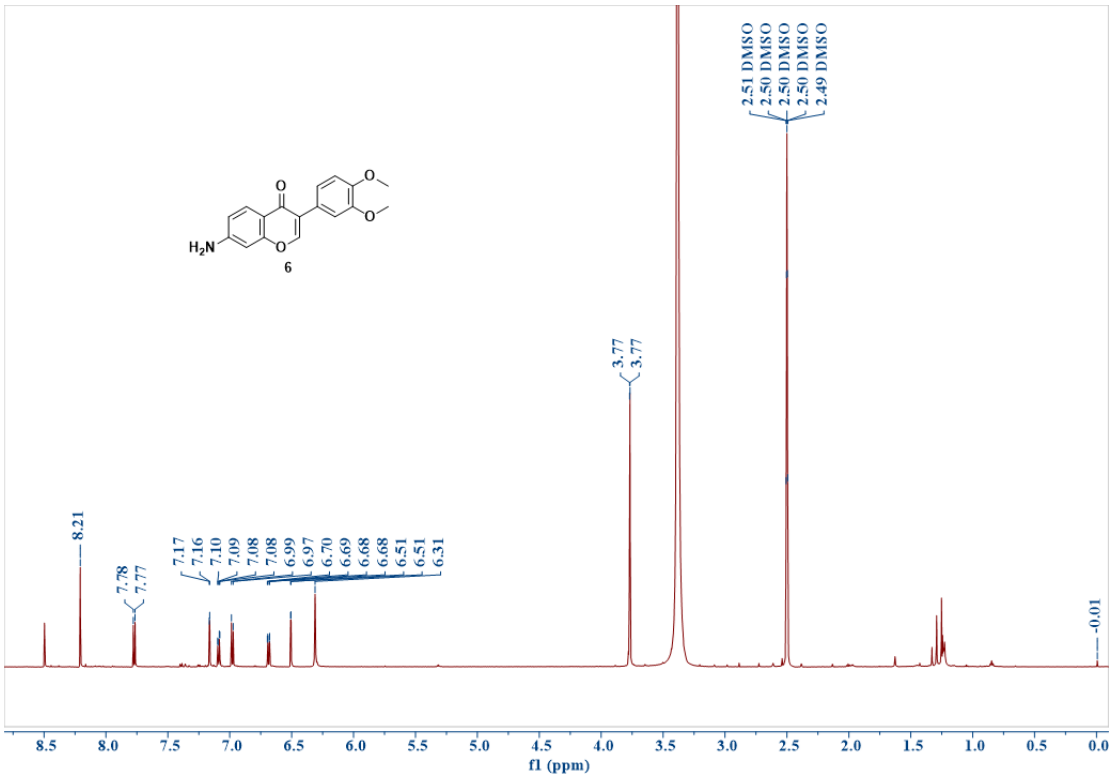


Figure S18 ^{1}H NMR of intermediate **6**.

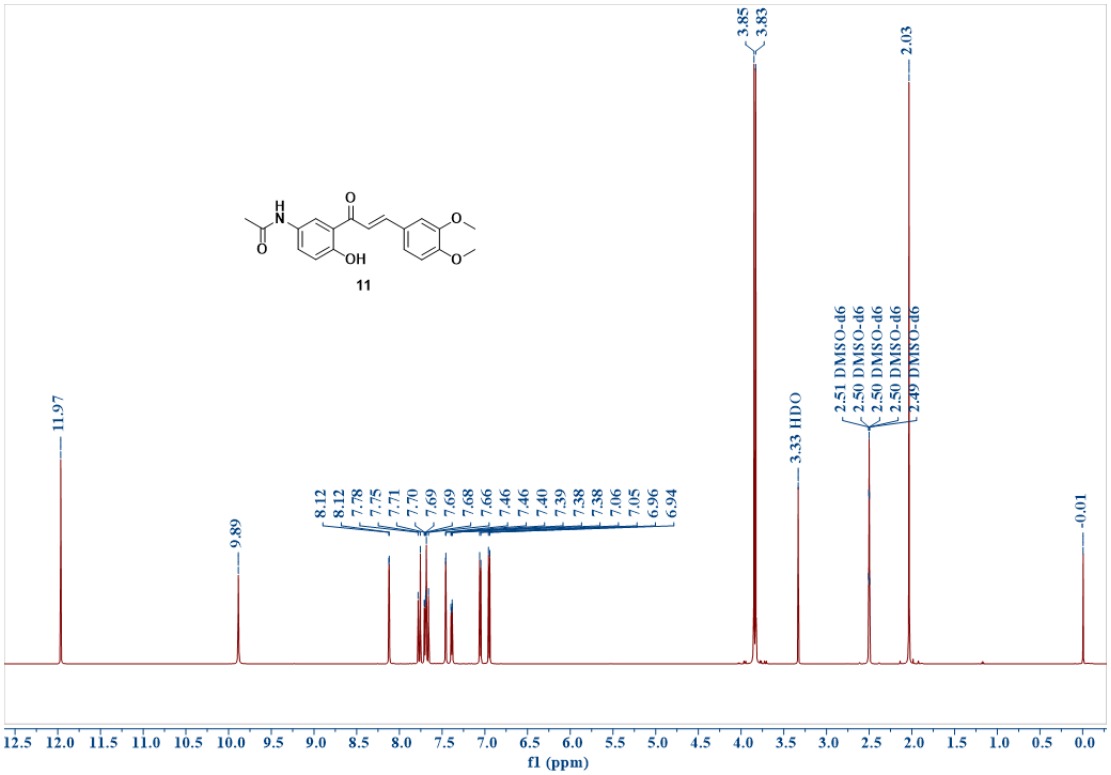


Figure S19 ^{1}H NMR of intermediate **11**.

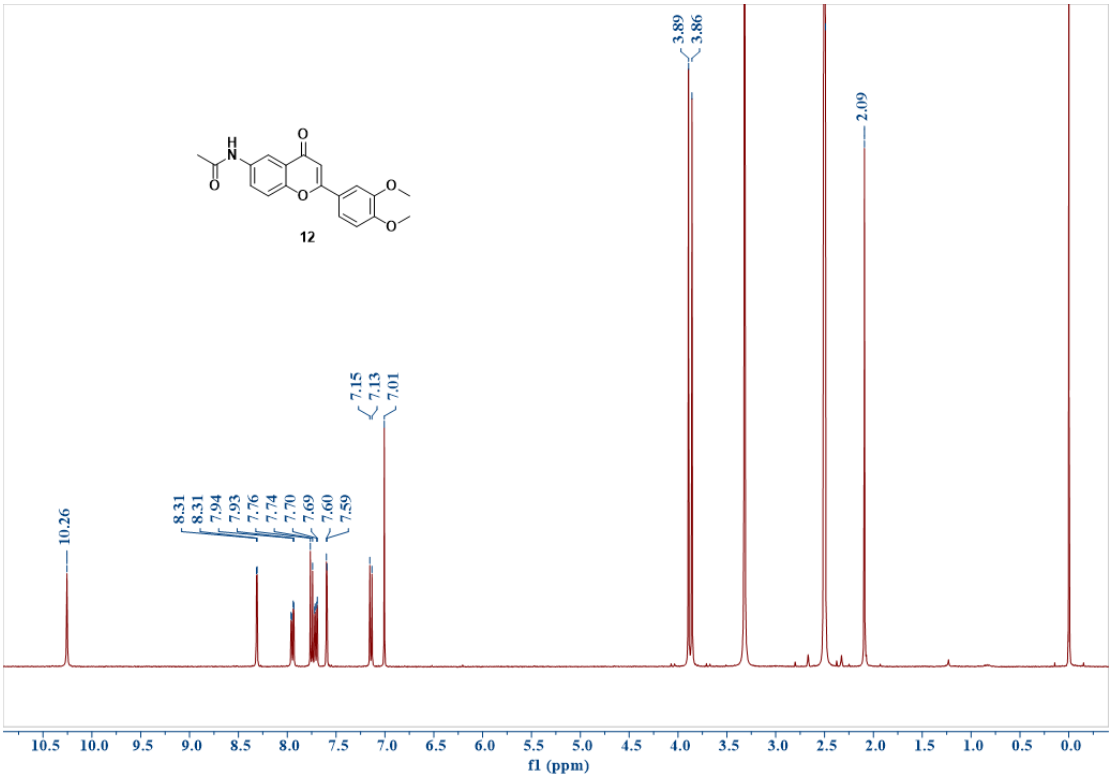


Figure S20 ^{1}H NMR of intermediate **12**.

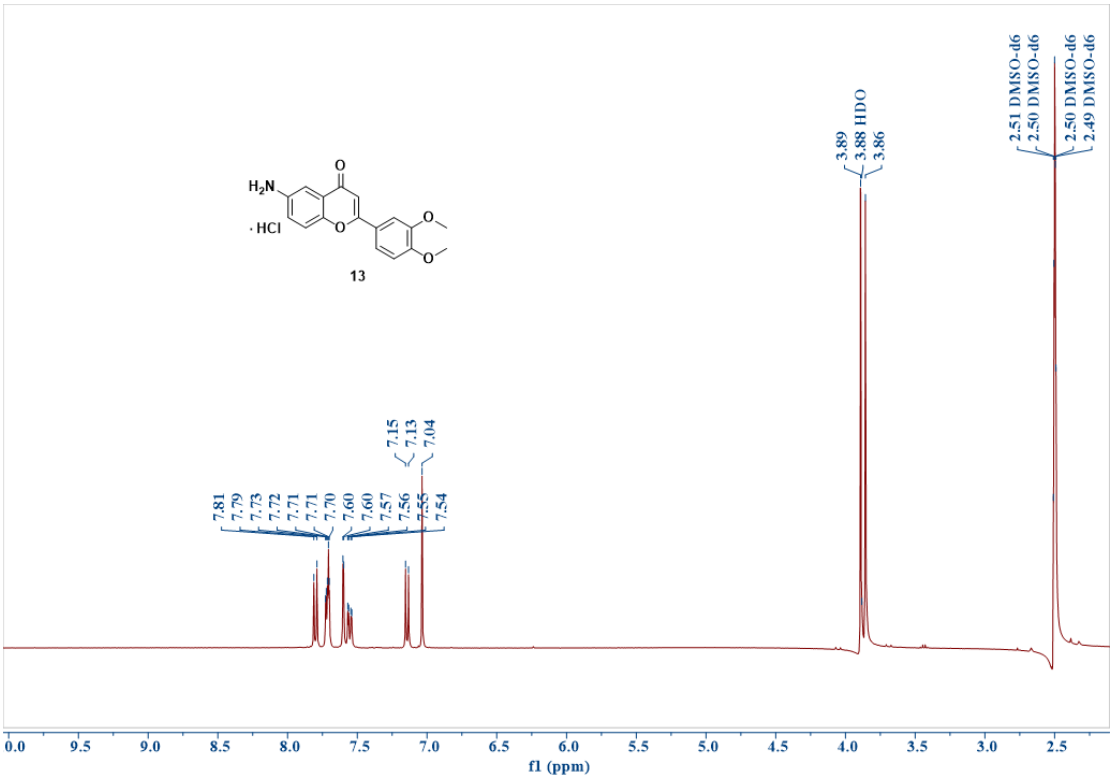


Figure S21 ^{1}H NMR of intermediate **13**.

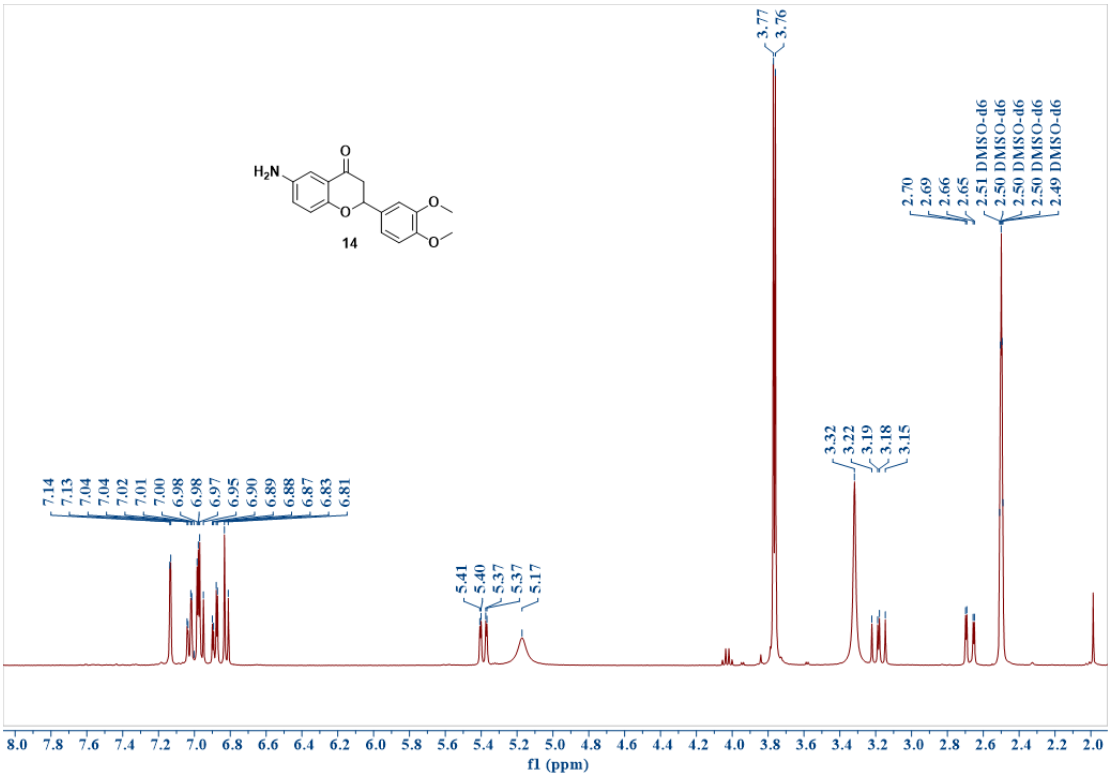


Figure S22 ^{1}H NMR of intermediate **14**.

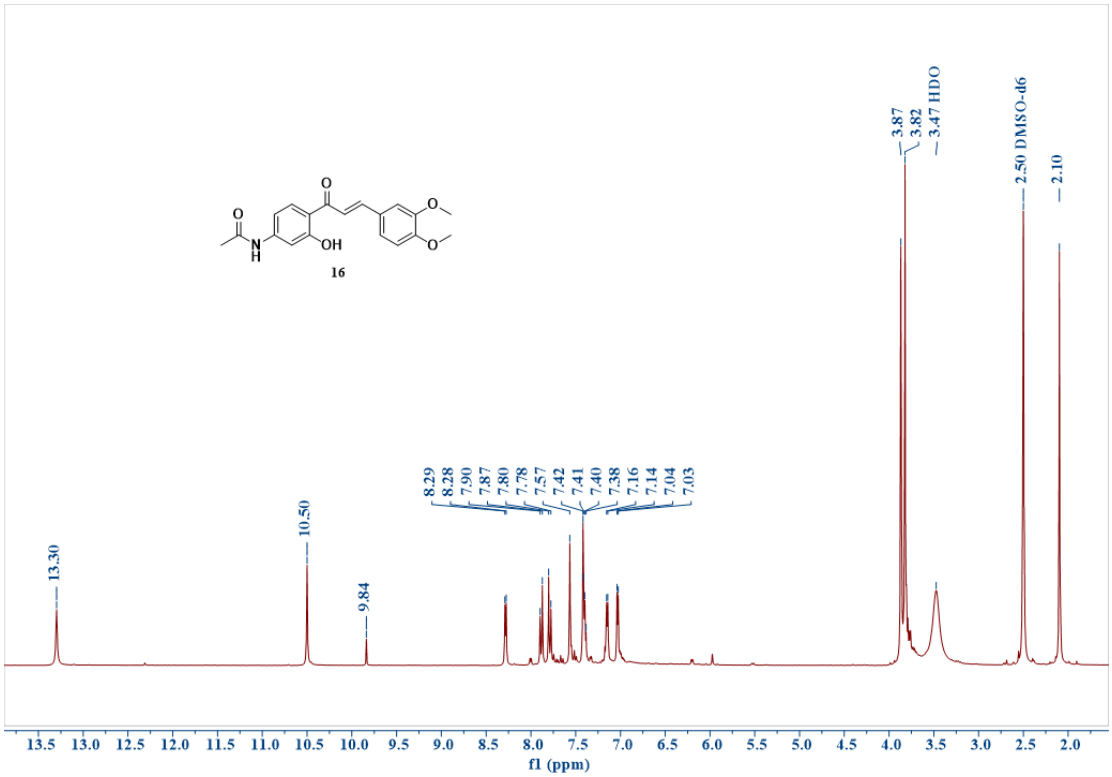


Figure S23 ^{1}H NMR of intermediate **16**.

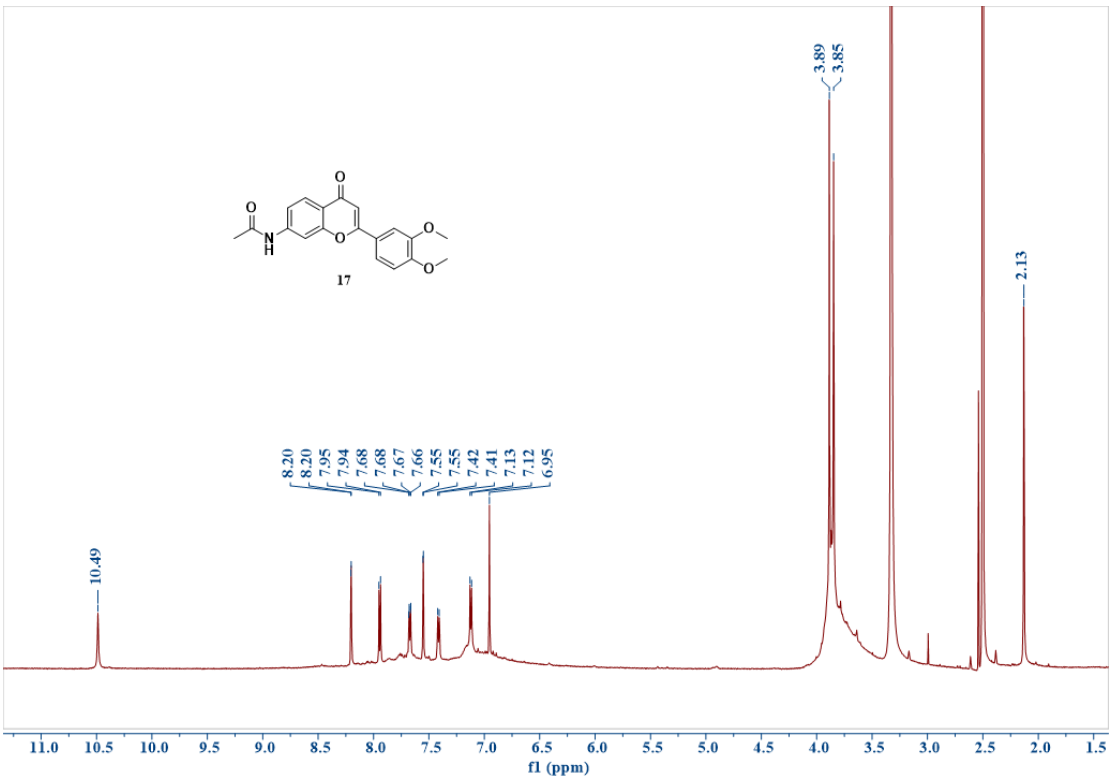


Figure S24 ^{1}H NMR of intermediate **17**.

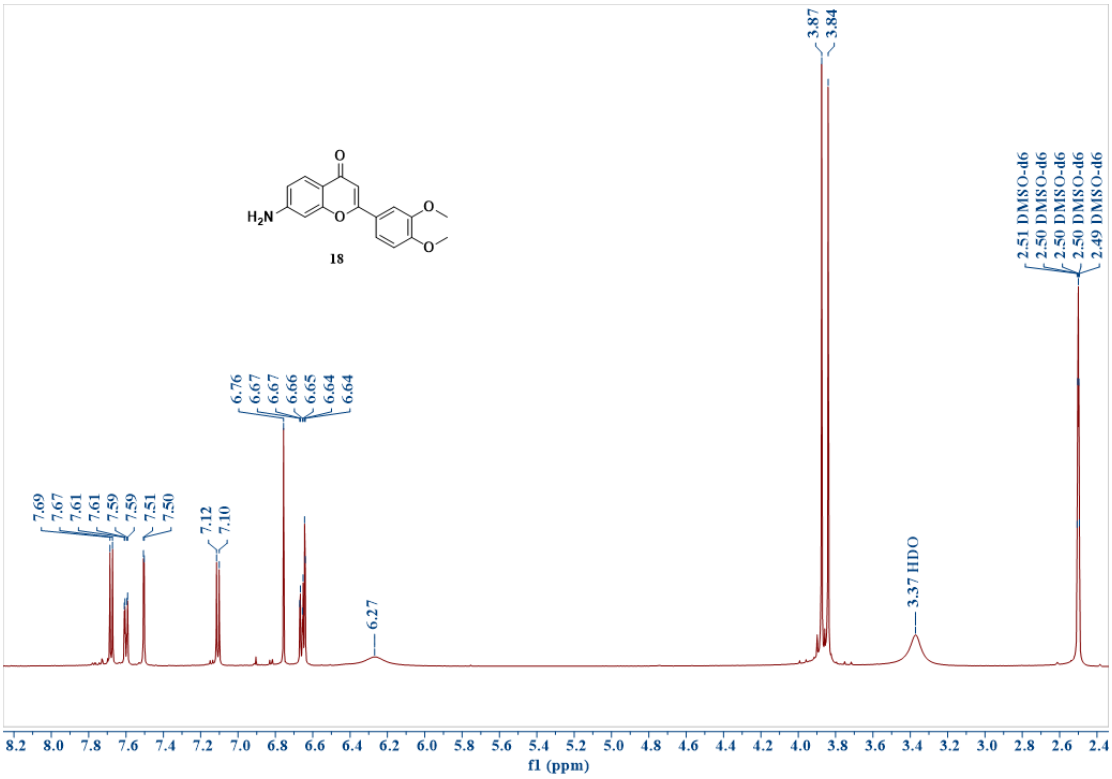


Figure S25 ^{1}H NMR of intermediate **18**.

Part 3. Biological experiments.

Cell lines and virus strains

African green monkey kidney Vero cells and pig kidney epithelial LLC-PK1 cells were provided by Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS). Vero cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The mouse epithelial-like cell line NCTC clone 1469 was purchased from Procell and cultured in DMEM supplemented with 10% horse serum. LLC-PK1 cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% FBS. All cell lines were routinely incubated at 37 °C with 5% CO_2. The maintenance media used for dilution are as follows: Vero, DMEM supplemented with 2% FBS; NCTC clone 1469, DMEM supplemented with 2% horse serum; LLC-PK1, MEM supplemented with 2% FBS.

The PEDV HLJBY strain was obtained from Shanghai Veterinary Research Institute, CAAS and propagated in Vero cells. MHV A59 strain was sourced from Shanghai Veterinary Research Institute, CAAS and propagated in NCTC clone 1469 cells. IBV Beaudette strain (ATCC VR-22) was a generous gift from Professor Dingxiang Liu at South China Agricultural University and propagated in Vero cells. PDCoV CHN-GD-2016 strain was acquired from Shanghai Veterinary Research Institute, CAAS and cultured in LLC-PK1 cells.

Antiviral activity assay

Determination of CC_{50}: Stock solutions (20 mM) of title compounds in DMSO were diluted into eight 2-fold serial concentrations with 1000 μM as the highest. Cells were seeded at a density of 5×10^4 cells per well and cultured to confluence. Title compounds were added in triplicate. Mock-infected cells treated with 0.1% DMSO served as the negative control, while maintenance medium treated with 0.1% DMSO served as the blank control. After incubated for 72 h, CCK-8 reagent diluted to 0.1 mL/mL was added to each well and incubated for 1 h. Absorbance at 450 nm was measured with a Multiskan SkyHigh Microplate Reader. CC_{50} was calculated by the formula: $CC_{50} = (A_1–A_2) / (A_3−A_2) \times 100\%$, where A_1 is the average absorbance of the compound wells, A_2 is the average absorbance of the wells without cells and compound (blank control), and A_3 is the average absorbance of the wells with cells and vehicle (negative control).

Determination of EC_{50}: Stock solutions (20 mM) of title compounds in DMSO were diluted into six 2-fold serial concentrations with CC_{50} as the highest. Cells were seeded at a density of 5×10^4 cells per well and cultured. Upon cells formed a confluent monolayer, they were infected with respective viral inoculum (MOI = 1), and then incubated for 1 h. Title compounds were added in triplicate. Cells mock-infected and treated with 0.1% DMSO served as the negative control, while cells infected with virus and treated with 0.1% DMSO served as the blank control. The cultures were incubated for 48 h. CCK-8 reagent diluted to 0.1 mL/mL was added to each well and incubated for 1 h. Absorbance at 450 nm was measured. EC_{50} was calculated by the formula: $EC_{50} = (A_4–A_5) / (A_6−A_5) \times 100\%$, where A_4 is the average absorbance of the compound wells, A_5 is the average absorbance of the wells with virus-infected cells and vehicle (blank control), and A_6 is the average absorbance of the wells with mock-infected cells and vehicle (negative control). Each experiment was independently repeated three times. Data analysis was performed with GraphPad Prism 8 software. Data are presented as mean ± standard deviation.