Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Substituted 4-Methylcoumarin Inhibitors of SLC26A3 (DRA) for Treatment of Constipation and Hyperoxaluria

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Biology: General

The DRA functional plate-reader inhibition assay was done as previously described.[1,2] Briefly, each well of 96-well plate (Corning-Costar Corp., New York NY) containing FRT cells expressing DRA and iodide-sensitive YFP was washed with phosphate buffered saline (PBS; 2x100 μL). Test compounds (0.5 μL in DMSO) were diluted into PBS (60 μL) and added to each well at specified final concentration. After 10 min each well was assayed individually for SLC26A3-mediated I^- influx by recording fluorescence continuously (400 ms/point) for 2 s (baseline). Then 60 μL of 140 mM I^- solution was added at 2 s, and fluorescence was further read for 12 s. The initial rate of I^- influx following each of the solution addition was computed from fluorescence data by nonlinear regression, and % inhibition was determined by interpolation of data obtained from test compounds with positive and negative controls. Initial concentration-dependence studies were done in triplicate at eight-point serial dilutions starting at 5 μM. For compounds with the greatest potency, the measurements were repeated at a lower starting concentration of 1 μM. A curve was fitted through the dose-response data, which allowed for determination of IC50 values using GraphPad Prism v6.0 (San Diego, CA) to determine IC_{50} values. Efficacy of the compounds were tested in loperamide-induced constipation model in mice as previously described[1]. **4b** (10 mg/kg), **4k** (10 mg/kg) or vehicle control (5% DMSO, 10% Kolliphor HS in saline) were administered by oral gavage one hour before loperamide treatment (0.3 mg/kg, intraperitoneal). For pharmacokinetics, mice were treated with single-dose of **4k** (10 mg / kg, oral gavage) and blood samples were obtained at specified time points and processed as previously described.[1] Compound concentrations were measured in serum using HPLC by UV-absorbance at λ_{max} determined to be 320 nm, which gave sensitive detection down to 1 μM.

Animals

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health and approved by the UCSF Institutional Animal Care and Use Committee.

Chemistry: General

Unless otherwise indicated, all reaction solvents were anhydrous and obtained as such from commercial sources. Chemical and solvents were commercially available and no further purification was required. RP-HPLC analysis was performed using a Dionex Ultimate 3000 system, using a C18 column [3x150 mm]. Low resolution ESI-LCMS was carried out with an Agilent 1100 HPLC coupled to an Agilent 1956B MSD. RP-HPLC runs typically employed gradients of two solvents: [A]= H_2O (0.05% TFA) and [B] CH_3CN (0.05% TFA); RP-LCMS used the same solvent system using the modifier formic acid (88% aq). The standard HPLC and LCMS gradients proceeded with [A:B]= 95:5 to [A:B]= 5:95 over 15 minutes. HRMS was performed using an Agilent 1200 HPLC coupled to an Agilent 6530 QTOF instrument with an electrospray ionization source. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz instrument. ^{1}H NMR chemical shifts are relative to TMS (δ = 0.00 ppm), $CDCl_3$ (δ 7.26), acetone-*d6* (δ 2.05), or DMSO-*d6* (δ 2.5). ^{13}C NMR chemical shifts are relative to DMSO-*d6* (δ 39.5) or $CDCl_3$ (δ 77.2). Purity of assayed compounds was >95% based on HPLC-LCMS analysis at 254 nm, and absence of impurities was confirmed by examination at least one other wavelength (320 nm) as well as careful inspection of ^{1}H and ^{13}C-NMR spectra.

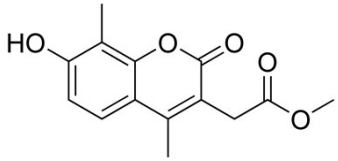
General procedure 1: synthesis of methyl substituted substituted coumarin esters (7a-7e) prepared from resorcinol (5a-5e) and dimethyl acetylsuccinate. Substituted resorcinol (1.0 eq) (**5a-5e**) and dimethyl acetylsuccinate (1.0 eq) were mixed and treated with

dropwise H_2SO_4 (2.8 eq). The reaction suspensions were gently stirred at room temperature for 24 hr and an LCMS was taken to confirm consumption of starting material and formation of product. For workup, di-alkylsubstituted coumarins (**7a-7b**) were precipitated by pouring the reaction mixtures into ice water and the resulting solid materials were filtered and pumped down via high vacuum (500 mTorr) for 1 hr to yield the title products typically as off white solids. The halogen-substituted coumarins (**7c-7e**) were worked up by aqueous extraction: the crude products were neutralized with $NaHCO_3$, transferred to a separatory funnel and extracted with ethyl acetate. The organic phase was washed with water, NaCl (saturated aqueous), and was then dried over Na_2SO_4 and concentrated *in vacuo* to yield the title products typically as off-white to pink solids (16-63% yield).

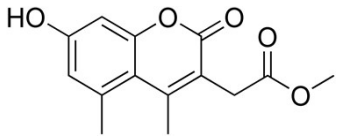
General procedure 2: synthesis of benzyl ether (9a-9t) from substituted 7-hydroxycoumarin (7a-7e) and substituted benzyl bromides (8a-8d). A mixture of the substituted hydroxycoumarin (**7a-7e**) was mixed with substituted benzyl bromide (1.4 eq) (**8a-8d**), potassium carbonate (2.0 eq), and anhydrous acetone was heated at reflux (56°C) for 24 hr. The reaction mixture was mixed at room temperature for 24 hr and an LCMS was taken to confirm consumption of starting material and formation of product. The mixture was poured over water and the resulting precipiate was placed in a high vacuum (500mTorr) for 1 hr to yield the title products (**9a-9t**) typically as an off white solid (43-100%).

General procedure 3: synthesis of coumarin 3-acetic acids (4a-4t) via the hydrolysis of the coumarin methyl esters (9a-9t). 1 N NaOH (4.0 eq) was added to a solution of the benzyl ether methyl ester (**9a-9t**) in methanol. The reaction mixture was mixed at reflux (65°C) for 24 hr and an LCMS was taken to confirm consumption of starting material and formation of product. To avoid acid catalyzed re-esterification during the workup, we recommend evaporating

reaction mixtures prior to acidification. After concentration, the mixtures were acidified using 1M HCl to a pH ~3. For subsequent workup, products **4a-4h** were precipitated by pouring the reaction mixtures on ice, and the resulting precipitates were placed in a high vacuum (500 mTorr) for 1 hr to yield the title products typically as off white solids. The halogen-substituted coumarin acid products **4i-4t** were worked up by aqueous extraction: the crude products were transferred to a separatory funnel and extracted with methylene chloride. The organic phase was washed with water, NaCl (saturated aqueous), and was then dried over Na_2SO_4 and concentrated *in vacuo* to yield the title products typically as off-white to pink solids (20%-to-quantitative). In cases where crude yields were greater than quantitative initially, additional trituration was performed to remove contamination, typically by solvent.

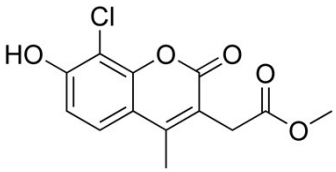


(7-Hydroxy-4,8-dimethyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (7a). Using general procedure 1, 2-methylresorcinol (**5a**) (500 mg, 4.028mmol) was converted to the dimethyl coumarin (**7a**) as an off-white solid (662mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.29 (s, 3H), 2.35 (s, 3H), 3.74 (s, 2H), 3.77 (s, 3H), 6.03 (bs, 1H), 6.70 (d, *J*=9 Hz, 1H), 7.24 (d, *J*=9 Hz, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{14}H_{14}O_5$ [M+H] 263.1, found [M+H] 263.2, matching the previously characterized material.[1]

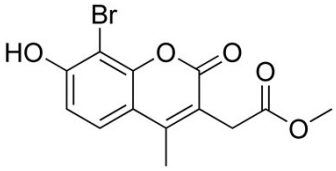


(7-Hydroxy-4,5-dimethyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (7b). Using general procedure 1, 5-methylresorcinol (**5b**) (300mg, 2.417 mmol) was converted to the substituted coumarin (**7b**) as an off-white solid (267mg; 42%). ^{1}H NMR (500 MHz, $CDCl_3$): δ

2.28 (s, 3H), 2.59 (s, 3H), 3.79 (s, 2H), 3.85 (s, 3H), 6.22 (s, 1H), 6.37 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.8, 21.2, 32.4, 52.9, 107.0, 109.3, 111.8, 115.3, 142.7, 152.4, 153.1, 154.8, 161.7, 173.7. ESI-LCMS (low resolution) m/z calculated for $C_{14}H_{14}O_5$ [M+H] 263.1, found [M+H] 263.0. The crude material was used without further purification or characterization.

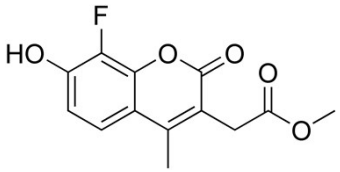


(8-Chloro-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (7c). Using general procedure 1, 2-chlororesorcinol (**5c**) (400mg, 2.767 mmol) was converted to the substituted coumarin (**7c**) as a pink solid (130.1 mg; 16%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.75 (s, 5H), 3.77 (s, 3H), 6.38 (bs, 1H), 6.98 (d, *J*=9 Hz, 1H), 7.47 (d, *J*=9 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.7, 33.4, 70.6, 99.2, 110.5, 115. 3 (t, *J* = 235 Hz), 115.6, 118.3, 124.9 (t, *J* = 8 Hz), 125.9, 126.3, 129.7, 130.3, 134.9 (t, *J* = 23 Hz), 137.6, 149.3, 150.2, 157.4, 160.8, 171.8. ESI-LCMS (high resolution) m/z calculated for $C_{13}H_{11}ClO_5$ assuming ^{35}Cl stable isotope [M+Na] 305.0193, found [M+Na] 305.0192. ESI-LCMS (high resolution) m/z calculated for $C_{13}H_{11}ClO_5$ assuming ^{37}Cl stable isotope [M+Na] 307.0164, found [M+Na] 307.0166.

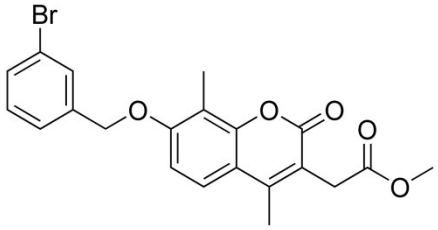


(8-Bromo-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (7d). Using general procedure 1, 2-bromoresorcinol (**5d**) (300 mg, 1.058 mmol) was converted to the substituted coumarin (**7d**) as an off-white solid (224 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.74 (s, 3H), 3.75 (s, 2H), 7.00 (d, *J*=9 Hz, 1H), 7.52 (d, *J*=9 Hz, 1H). ESI-LCMS

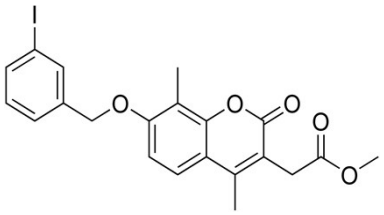
(low resolution) m/z calculated for $C_{13}H_{11}BrO_5$ assuming ^{79}Br stable isotope [M+H] 327.0, found [M+H] 326.8.



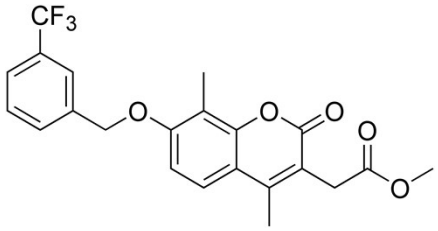
(8-Fluoro-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (7e). Using general procedure 1, 2-fluororesorcinol (**5e**) (300 mg, 2.342 mmol) was converted to the substituted coumarin (**7e**) as an off-white solid (61 mg, 29%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.36 (s, 3H), 3.62 (s, 3H), 3.67 (s, 2H), 6.96 (t, *J*=9 Hz, 1H), 7.48 (dd, *J*=2,9 Hz, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{13}H_{11}FO_5$ [M+H] 267.0, found [M+H] 267.1.



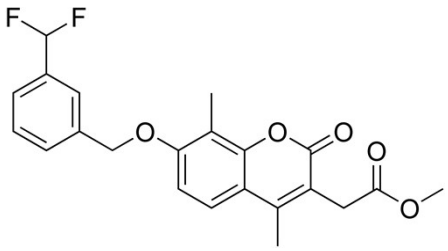
[7-(3-bromo-benzyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9a). Using general procedure 2, the 7-hydroxycoumarin (**7a**) (150 mg, 0.572 mmol) was converted to the benzyl ether (**9a**) as an off-white solid. (202 mg, 81.3%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.38 (s, 3H), 3.71 (s, 3H), 3.73 (s, 1H), 5.15 (s, 2H), 6.85 (d, *J*=9 Hz, 1H), 7.26-7.29 (m, 2H), 7.37 (d, *J*=8 Hz, 1H), 7.43 (d, *J=9* Hz, 1H), 7.48 (d, *J*=8 Hz, 1H), 7.59 (s, 1H). The material matched the compound as reported previously.[1]



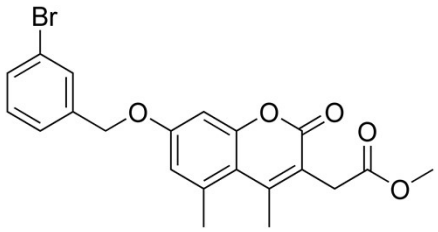
[7-(3-Iodo-benzyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9b). Using general procedure 2, the 7-hydroxycoumarin (**7a**) (130 mg, 0.496 mmol) was converted to the benzyl ether (**9b**) as an off-white solid. (239.1 mg, quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.38 (s, 6H), 3.72 (s, 3H), 3.74 (s, 2H), 5.13 (s, 2H), 6.86 (d, *J*=9 Hz, 1H), 7.147 (t, *J*=8 Hz, 1H), 7.41 (d, *J*=8 Hz, 1H), 7.44 (d, *J*=9 Hz, 1H), 7.69 (d, *J*=8 Hz, 1H), 7.80 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{21}H_{19}IO_5$ [M+H] 478.0, found [M+H] 478.8.



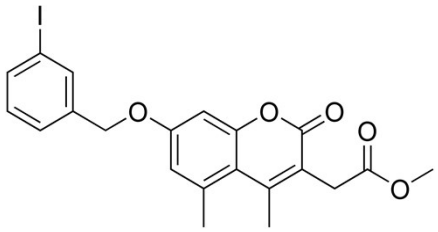
[4,8-Dimethyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl]-acetic acid methyl ester (9c). Using general procedure 2, the 7-hydroxycoumarin (**7a**) (29 mg, 0.111 mmol) was converted to the benzyl ether (**9c**) as an off-white solid. (37.2 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 6H), 3.72 (s, 3H), 3.75 (s, 2H), 5.24 (s, 2H), 6.89 (d, *J*=9 Hz, 1H), 7.55 (t, *J*=8 Hz, 1H), 7.63 (d, *J*=8 Hz, 1H), 7.66 (d, *J*=8 Hz, 1H), 7.72 (s, 1H). The material matched the compound as reported previously.[1]



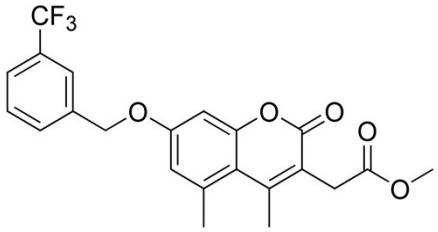
[7-(3-Difluoromethyl-benzyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9d). Using general procedure 2, the 7-hydroxycoumarin (**7a**) (29 mg, 0.111) was converted to the benzyl ether (**9d**) as an off-white solid. (24.7 mg, 55%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 6H), 3.72 (s, 3H), 3.75 (s,2H), 5.23 (s, 2H), 6.69 (t, *J*=57 Hz, 1H), 6.89 (d, *J*=9 Hz, 1H), 7.45-7.48 (m, 1H), 7.49-7.53 (m, 2H), 7.58 (d, *J*=6 Hz, 1H), 7.60 (s, 1H). The product was pushed forward without additional purification or characterization.



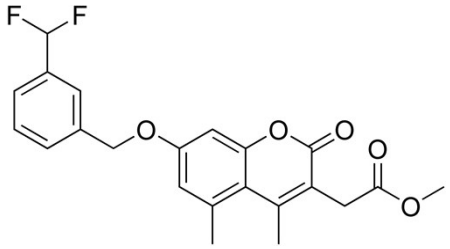
[7-(3-Bromo-benzyloxy)-4,5-dimethyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9e). Using general procedure 2, the 7-hydroxycoumarin (**7b**) (303 mg, 1.155 mmol) was converted to the benzyl ether (**9e**) as an off-white solid. (448 mg, 90%). ^{1}H NMR (500MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.52 (s, 3H), 3.71 (s, 3H), 3.74 (s, 2H), 5.09 (s, 2H), 6.61 (s, 1H), 6.81 (s, 1H), 7.30 (t, *J*=8 Hz, 1H), 7.38 (d, *J*=8 Hz, 1H), 7.52 (d, *J*=8 Hz, 1H), 7.60 (s, 1H). The product was pushed forward without additional purification or characterization.



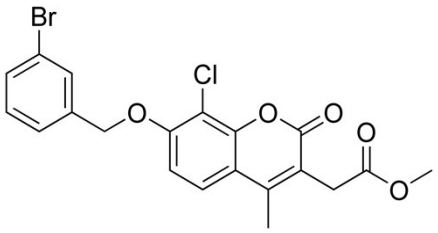
(7-(3-Iodo-benzyloxy)-4,5-dimethyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9f). Using general procedure 2, the 7-hydroxycoumarin (**7b**) (100mg, 0.381 mmol) was converted to the benzyl ether (**9f**) as an off-white solid. (155 mg, 85%). ^{1}H NMR (500MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.52 (s, 3H), 3.71 (s, 3H), 3.74 (s, 2H), 5.06 (s, 2H), 6.61(s, 1H), 6.80 (s, 1H), 7.16 (t, *J*=8 Hz, 1H), 7.40 (d, *J*=8 Hz, 1H), 7.72 (d, *J*=8 Hz, 1H), 7.81 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{21}H_{19}IO_5$ [M+H] 478.0, found [M+H] 478.8.



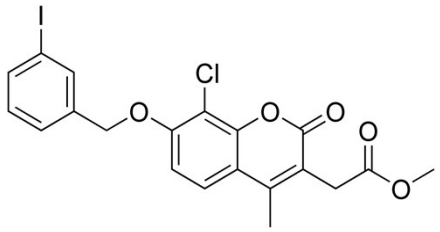
[4,5-Dimethyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl]-acetic acid methyl ester (9g). Using general procedure 2, the 7-hydroxycoumarin (**7b**) (82.5 mg, 0.315 mmol) was converted to the benzyl ether (**9g**) as an off-white solid. (79.4 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (s, 3H), 2.51 (s, 3H), 3.71 (s, 3H), 3.74 (s, 2H), 5.18 (s, 2H), 6.64 (s, 1H), 6.82 (s, 1H), 7.57 (d, *J*=8 Hz, 1H), 7.63-7.66 (m, 2H), 7.72 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{22}H_{19}F_3O_5$ [M+H] 420.1, found [M+H] 420.1.



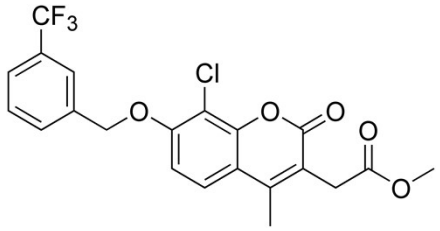
[7-(3-Difluoromethyl-benzyloxy)-4,5-dimethyl-2-oxo-2H-chromen-3-yl] acetic acid methyl ester (9h). Using general procedure 2, the 7-hydroxycoumarin (**7b**) (82.5mg, 0.315 mmol) was converted to the benzyl ether (**9h**) as an off-white solid. (98.7 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (s, 3H), 2.51 (s, 3H), 3.71 (s, 3H), 3.74 (s, 2H), 5.17 (s, 2H), 7.52-7.56 (m, 3H), 7.61 (s, 1H). The product was pushed forward without additional purification or characterization.



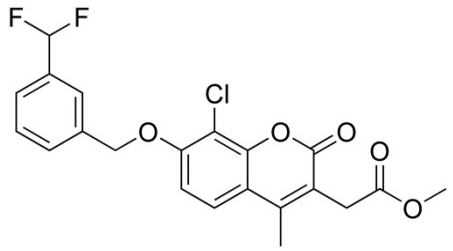
[7-(3-Bromo-benzyloxy)-8-chloro-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9i). Using general procedure 2, the 7-hydroxycoumarin (**7c**) (68.5 mg, 0.242 mmol) was converted to the benzyl ether (**9i**) as an off-white solid. (61mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.72 (s, 3H), 3.75 (s, 2H), 5.24 (s, 2H), 6.92 (d, *J*=9 Hz, 1H), 7.28-7.31 (m, 1H) (under solvent peak), 7.42 (d, *J*=8 Hz, 1H), 7.48-7.51 (m, 2H), 7.62 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{20}H_{16}BrClO_5$ assuming ^{79}Br and ^{37}Cl stable isotopes [M+H] 451.0, found [M+H] 450.5.



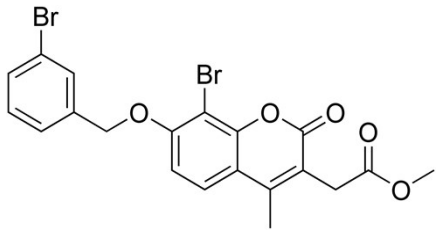
[8-Chloro-7-(3-iodo-benzyloxy)4-methyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (9j). Using general procedure 2, the 7-hydroxycoumarin (**7c**) (60 mg, 0.212 mmol) was converted to the benzyl ether (**9j**) as an off-white solid. (66.4 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.73 (s, 3H), 3.75 (s, 2H), 5.21 (s, 2H), 6.92 (d, *J*=9 Hz, 1H), 7.15 (t, *J*=8 Hz, 1H), 7.45 (d, *J*=8 Hz, 1H), 7.49 (d, *J*=9 Hz, 1H), 7.70 (d, *J*=8 Hz, 1H), 7.82 (s, 1H). The crude material was pushed forward without further purification or characterization.



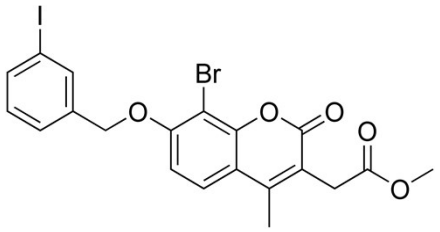
[8-chloro-4-methyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl]-acetic acid methyl ester (9k). Using general procedure 2, the 7-hydroxycoumarin (**7c**) (68.5 mg, 0.242 mmol) was converted to the benzyl ether (**9k**) as an off-white solid. (58.3mg, 54.6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.73 (s, 3H), 3.75 (s, 2H), 5.31 (s, 2H), 6.95 (d, *J*=9 Hz, 1H), 7.51 (d, *J*=9 Hz, 1H), 7.56 (t, *J*=8 Hz, 1H), 7.63 (d, *J*=8 Hz, 1H), 7.71 (d, *J*=8 Hz, 1H), 1H), 7.74 (s, 1H). The crude material was pushed forward without additional purification or characterization.



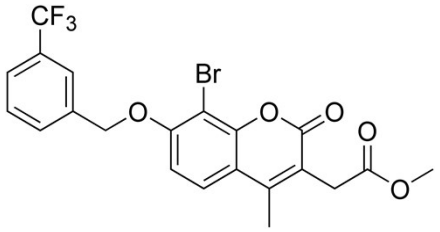
[8-Chloro-7-(3-difluoromethyl-benzyloxy)4-methyl-2-oxo-2H-chromen-3-yl)-acetic acid methyl ester (9L). Using general procedure 2, the 7-hydroxycoumarin (**7c**) (67 mg, 0.237 mmol) was converted to the benzyl ether (**9L**) as an off-white solid. (76.6 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.73 (s, 3H), 3.75 (s, 2H), 5.31 (s, 2H), 6.69 (t, *J*=56 Hz, 1H), 6.95 (d, *J*=9 Hz, 1H), 7.49-7.53 (m, 3H), 7.62 (s, 2H). The crude material was pushed forward without additional purification or characterization.



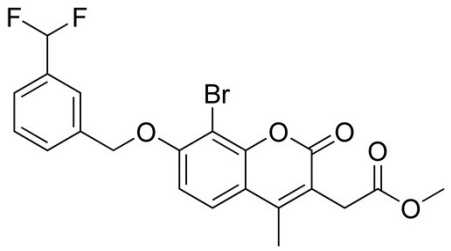
[8-Bromo-7-(3-bromo-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9m). Using general procedure 2, 7-hydroxycoumarin (**7d**) (23.4 mg, 0.072 mmol) was converted to the benzyl ether (**9m**) as an off-white solid. (20.5 mg, 57.8%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.72 (s, 3H), 3.75 (s, 2H), 5.25 (s, 2H), 6.89 (d, *J*=9 Hz, 1H), 7.29-7.31 (m, 1H), 7.43 (d, *J*=8 Hz, 1H), 7.49 (d, , *J*=10 Hz, 1H), 7.55 (d, *J*=9 Hz, 1H), 7.64 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{20}H_{16}Br_2O_5$ assuming two ^{79}Br stable isotopes [M+H] 494.9, found [M+H] 494.5.



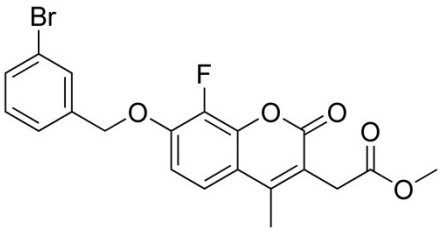
[8-Bromo-7-(3-iodo-benzyloxy)4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9n). Using general procedure 2, the 7-hydroxycoumarin (**7d**) (30 mg, 0.092 mmol) was converted to the benzyl ether (**9n**) as an off-white solid (21.2 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.72 (s, 3H), 3.75 (s, 2H), 5.22 (s, 2H), 6.89 (d, *J*=9 Hz, 1H), 7.153 (t, *J*=8 Hz, 1H), 7.47 (d, *J*=8 Hz, 1H), 7.55 (d, *J*=9 Hz, 1H), 7.69 (d, *J*=9 Hz, 1H), 7.84 (s, 1H). The crude material was pushed forward without additional purification or characterization.



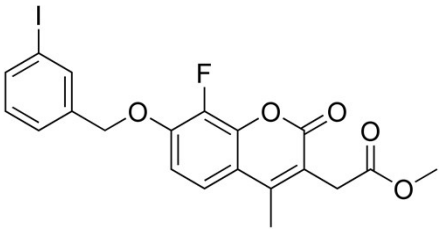
[8-Bromo-4-methyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl]-acetic acid methyl ester (9o). Using general procedure 2, the 7-hydroxycoumarin (**7d**) (82 mg, 0.251 mmol) was converted to the benzyl ether (**9o**) as an off-white solid. (87.7 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.73 (s, 3H), 3.75 (s, 2H), 5.32 (s, 2H), 6.92 (d, *J*=9 Hz, 1H), 7.55-5.59 (m, 2H), 7.63 (d, *J*=8 Hz, 1H), 7.72 (d, *J*=8 Hz, 1H), 7.76 (s, 1H). The crude material was pushed forward without additional purification or characterization.



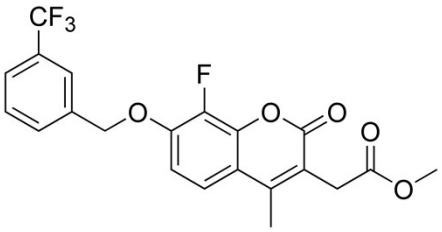
[8-Bromo-7-(3-difluoromethyl-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9p). Using general procedure 2, the 7-hydroxycoumarin (**7d**) (38 mg, 0.116 mmol) was converted to the benzyl ether (**9p**) as an off-white solid (32 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.73 (s, 3H), 3.75 (s, 2H), 5.31 (s, 2H), 6.69 (t, *J*=57 Hz, 1H), 6.92 (d, *J*=9, 1H), 7.51-7.52 (m, 2H), 7.55(d, *J*=9, 1H), 7.63-7.64 (m, 2H). The crude material was pushed forward without additional purification or characterization.



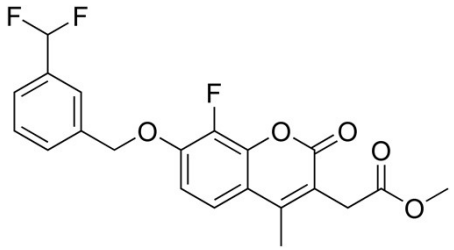
[7-(3-Bromo-benzyloxy)-8-fluoro-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9q). Using general procedure 2, the 7-hydroxycoumarin (**7e**) (30 mg, 0.113 mmol) was converted to the benzyl ether (**9q**) as an off-white solid (35.1 mg, 72%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.40 (s, 3H), 3.62 (s, 3H), 3.70 (s, 2H), 5.35 (s, 2H), 7.30 (dd, *J*=8,9 Hz, 1H), 7.38 (t, *J*=8 Hz, 1H), 7.49 (d, *J*=8 Hz, 1H), 7.57 (dt, *J*=1, 8 Hz, 1H), 7.63 (dd, *J*=2, 10 Hz, 1H), 7.71 (t, *J*=2 Hz, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{20}H_{16}BrFO_5$ assuming ^{79}Br stable isotope [M+H] 435.0, found [M+H] 435.1.



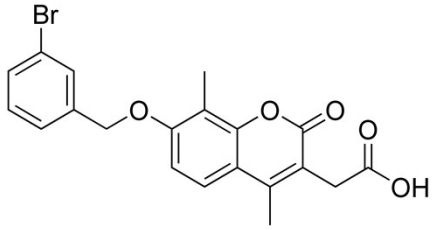
[8-Fluoro-7-(3-iodo-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9r). Using general procedure 2, the 7-hydroxycoumarin (**7e**) (30 mg, 0.312 mmol) was converted to the benzyl ether (**9r**) as an off-white solid (42.2 mg, 77%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.40 (s, 3H), 3.63 (s, 3H), 3.70 (s, 2H), 5.25 (s, 2H), 7.23 (t, *J*=8 Hz, 1H), 7.30 (dd, *J*=8,9 Hz, 1H), 7.51 (d, *J*=8 Hz, 1H), 7.63 (dd, *J*=2,9 Hz, 1H), 7.74 (dt, *J*=1,8 Hz, 1H), 7.88 (t, *J*=2 Hz, 1H). The crude material was pushed forward without additional purification or characterization. ESI-LCMS (low resolution) m/z calculated for $C_{20}H_{16}FIO_5$ [M+H] 483.0, found [M+H] 483.0.



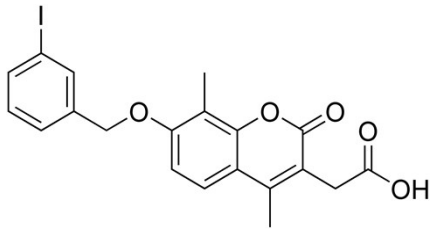
[8-Fluoro-4-methyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl]-acetic acid methyl ester (9s). Using general procedure 2, the 7-hydroxycoumarin (**7e**) (25.5 mg, 0.096 mmol) was converted to the benzyl ether (**9s**) as an off-white solid (38.1 mg, 94%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.40 (s, 3H), 3.63 (s, 3H), 3.70 (s, 2H), 5.45 (s, 2H), 7.33 (dt, *J*=8,10 Hz, 1H), 7.65 (dd, *J*=2,9 Hz, 1H), 7.68 (d, *J*=8 Hz, 1H), 7.75 (d, *J*=8 Hz, 1H), 7.81 (d, *J*=8 Hz, 1H), 7.87 (s, 1H). The crude material was pushed forward without additional purification or characterization.



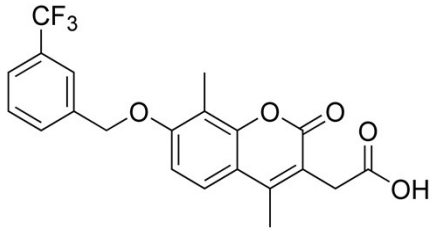
[7-(3-Difluoromethyl-benzyloxy)-8-fluoro-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid methyl ester (9t). Using general procedure 2, the 7-hydroxycoumarin (**7e**) (25.5 mg, 0.096 mmol) was converted to the benzyl ether (**9t**) as an off-white solid (35.1 mg, 72%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.41 (s, 3H), 3.63 (s, 3H), 3.71 (s, 2H), 5.42 (s, 2H), 7.09 (t, *J*=56 Hz, 1H), 7.34 (dt, *J*=8,10 Hz, 1H), 7.57-7.59 (m, 2H), 7.64 (dd, *J*=2, 9 Hz, 1H), 7.68 (d, *J*=5 Hz, 1Hz), 7.71 (s, 1H). The material was pushed forward without additional purification or chacterization.



[7-(3-Bromo-benzyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl] acetic acid (4a). Using general procedure 3, the methyl ester (**9a**) (100 mg, 0.232 mmol) was hydrolyzed to the carboxylic acid (**4a**) as an off-white solid (58.4 mg, 60.4%). ^{1}H NMR (500 MHz, acetone-*d6*): δ 2.33 (s, 3H), 2.44 (s, 3H), 3.72 (s, 2H), 5.32 (s, 2H), 7.12 (d, *J*=9 Hz, 1H), 7.39 (t, *J*=8 Hz, 1H), 7.53-7.55 (m, 2H), 7.65 (d, *J*=9 Hz, 1H), 7.72 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 7.6, 14.5, 32.1, 69.2, 108.4, 113.2, 114.3, 117.0, 12.1, 123.5, 126.2, 130.6, 130.9, 139.9, 148.9, 151.4, 158.6, 161.0, 170.8. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}BrO_5$ assuming ^{79}Br stable isotope [M+H] 417.0332, found [M+H] 417.0330. The material matched the compound as reported previously.[1]

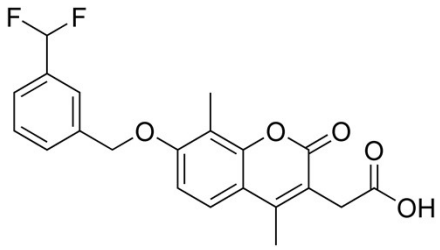


[7-(3-Iodo-benzyloxy)-4,8-dimethyl-2-oxo--2H-chromen-3-yl] acetic acid (4b). Using general procedure 3, the methyl ester (**9b**) (150 mg, 0.314 mmol) was hydrolyzed to the carboxylic acid (**4b**) as an off-white solid (53.8 mg, 36%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.24 (s, 3H), 2.29 (s, 3H), 5.23 (s, 2H), 7.08 (d, *J*=9 Hz, 1H), 7.22 (t, *J*=8 Hz, 1H), 7.50 (d, *J*=8 Hz, 1H), 7.60 (d, J=9 Hz, 1H), 7.71 (d, *J*=8 Hz, 1H), 7.87 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 8.5, 15.5, 33.1, 69.2, 95.2, 109.3, 112.9, 114.2, 117.0, 124.1, 127.1, 131.2, 136.1, 137.0, 139.8, 149.6, 151.0, 158.5, 161.4, 172.0. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}IO_5$ [M+H] 465.0194, found [M+H] 465.0194.

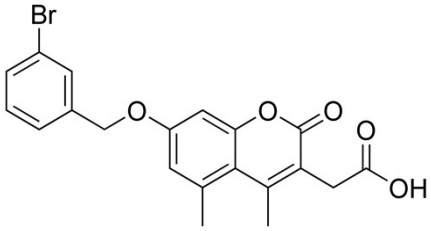


[4,8-dimethyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl] acetic acid (4c). Using general procedure 3, the methyl ester (**9c**) (37 mg, 0.088 mmol) was hydrolyzed to the carboxylic acid (**4c**) as an off-white solid (29.1 mg, 81%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.25 (s, 3H), 2.28 (s, 3H), 5.36 (s, 2H), 7.11 (d, *J*=9 Hz, 1H), 7.60 (d, *J*=9 Hz, 1H), 7.66-7.71 (m 1H)7.81 (d, *J*=8 Hz, 1H), 7.86 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 7.7, 14.5, 68.6, 108.1, 111.9, 114.4, 122.6, 123.5, 123.7 (q, *J* = 84 Hz), 128.8 (q, *J* = 31 Hz), 129.3, 131.0, 138.1,

150.1, 156.9, 161.0, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{21}H_{17}F_3O_5$ [M+H] 407.1101, found [M+H] 407.1099.

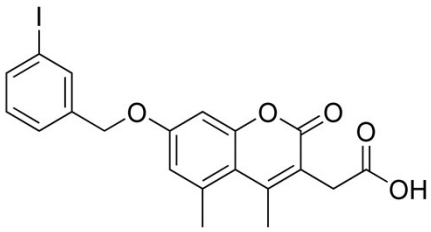


[7-(3-Difluoromethyl-benzyloxy)-4,8-dimethyl-2-oxo-2H-chromen-3-yl] acetic acid (4d). Using general procedure 3, the methyl ester (**9d**) (300 mg, 2.417 mmol) was hydrolyzed to the carboxylic acid (**4d**) as an off-white solid (267 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.26 (s, 3H), 2.37 (s, 3H), 3.57 (s, 2H), 5.35 (s, 2H), 7.08 (t, *J*=56 Hz, 1H), 7.15 (d, *J*=9 Hz, 1H), 7.56-7.57 (m, 3H), 7.66-7.69 (m, 2H). ^{13}C NMR (125 MHz, acetone-d_6): δ 8.6, 15.6, 33.3, 69.8, 109.3, 113.0, 113.4, 115.3 (t, J = 76 Hz), 117.3, 124.2, 124.8 (t, *J* = 6 Hz), 125.7 (t, *J* = 6 Hz), 129.6, 130.3, 134.8 (t, *J* = 22 Hz), 138.2, 149.5, 151.2, 158.6, 161.4, 172.0. ESI-LCMS (high resolution) m/z calculated for $C_{21}H_{18}F_2O_5$ [M+H] 389.1195, found [M+H] 389.1194.

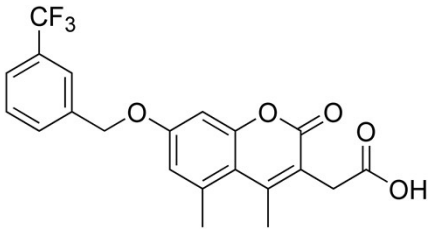


[7-(3-Bromo-benzyloxy)-4,5-dimethyl-2-oxo-2H-chromen-3-yl] acetic acid (4e). Using general procedure 3, the methyl ester (**9e**) (501 mg, 1.162 mmol) was hydrolyzed to the carboxylic acid (**4e**) as an off-white solid (168.6 mg, 35%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (s, 3H), 2.59 (s, 3H), 3.77 (s, 2H), 5.10 (s, 2H), 6.63 (d, *J*=9 Hz, 1H), 6.82 (s, 1H), 7.24-7.32 (m, 1H), 7.36-7.53 (m, 2H), 7.59 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 19.7, 20.8, 32.0, 70.3, 108.5, 109.3, 103.9, 118.4, 122.0, 127.1, 130.6, 131.0, 131.2, 139.3, 142.6, 149.7, 153.9, 156.8,

160.4, 170.7. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}BrO_5$ assuming ^{79}Br stable isotope [M+Na] 439.0157, found [M+Na] 439.0175. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}BrO_5$ assuming ^{81}Br stable isotope [M+Na] 441.0136, found [M+Na] 441.0158.

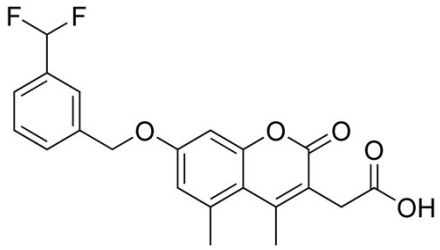


[7-(3-Iodo-benzyloxy)-4,5-dimethyl-2-oxo--2H-chromen-3-yl] acetic acid (4f). Using general procedure 3, the methyl ester (**9f**) (183 mg, 0.383 mmol) was hydrolyzed to the carboxylic acid (**4f**) as an off-white solid (56 mg, 42%). ^1H NMR (500 MHz, $CDCl_3$): δ 2.41 (s, 3H), 2.56 (s, 3H), 3.77 (s, 2H), 5.07 (s, 2H), 6.62 (s, 1H), 6.81 (s, 1H), 7.16 (t, *J*=8 Hz, 1H), 7.4 (d, *J*=8 Hz, 1H), 7.72 (d, *J*=8 Hz, 1H), 7.80 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 20.8, 22.2, 33.0, 70.7, 94.7, 109.1, 111.0, 117.1, 127.0, 127.2, 130.7, 137.0, 137.8, 143.1, 151.7, 154.1, 156.9, 162.2, 173.5. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}IO_5$ [M+H] 465.0194, found [M+H] 465.0177.

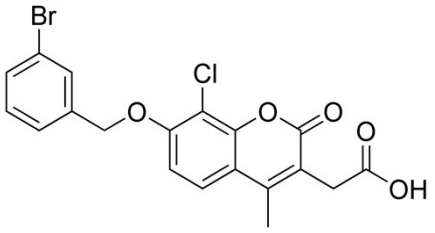


[4,5-Dimethyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl] acetic acid (4g). Using general procedure 3, the methyl ester (**9g**) (79.7 mg, 0.190 mmol) was hydrolyzed to the carboxylic acid (**4g**) as an off-white solid (65 mg, 90%). ^1H NMR (500 MHz, DMSO-d_6): δ 2.39 (s, 3H), 2.44 (s, 3H), 3.55 (s, 2H), 5.33 (s, 2H), 6.86 (s, 1H), 6.98 (s, 1H), 7.68 (t, *J*=8 Hz, 1H),

7.75 (d, *J*=8, 1H), 7.85 (d, *J*=8 Hz, 1H), 7.92 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 19.7, 20.8, 32.0, 70.7, 108.5, 109.3, 109.9, 115.0 (t, J = 236 Hz), 125.3, 125.5, 125.5, 129.2, 130.6, 135.1 (t, *J* = 24 Hz), 137.5, 142.6, 149.7, 153.9, 156.9, 160.4, 170.2. ESI-LCMS (high resolution) m/z calculated for $C_{21}H_{17}F_3O_5$ [M+H] 406.1028, found [M+H] 406.1085.

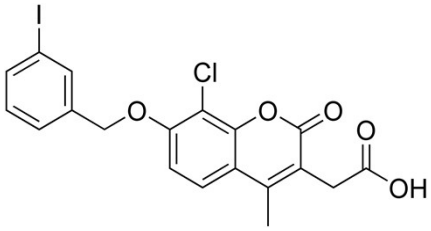


[7-(3-Difluoromethyl-benzyloxy)-4,5-dimethyl-2-oxo-2H-chromen-3-yl] acetic acid (4h). Using general procedure 3, the methyl ester (**9h**) (60 mg, 0.149 mmol) was hydrolyzed to the carboxylic acid (**4h**) as an off-white solid (58.7 mg, quantitative). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H), 2.44 (s, 3H), 3.32 (s, 2H), 5.29 (s, 2H), 6.97 (t, *J*=60 Hz, 1H), 6.85 (s, 1H), 7.08 (s, 1H), 7.57-7.58 (m, 2H), 7.70 (d, *J*=6 Hz, 1H), 7.73 (s, 1H). ESI-LCMS (high resolution) m/z calculated for $C_{21}H_{18}F_2O_5$ [M+H] 389.1195, found [M+H] 389.1202.

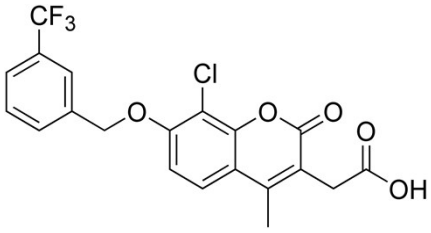


[7-(3-Bromo-benzyloxy)-8-chloro-4-methyl -2-oxo-2H-chromen-3-yl] acetic acid **(4i).** Using general procedure 3, the methyl ester (**9i**) (24.7 mg, 0.055 mmol) was hydrolyzed to the carboxylic acid (**4i**) as an off-white solid (16 mg, 67%). ^{1}H NMR (500 MHz, acetone-d6): δ 2.47 (s, 3H), 3.73 (s, 2H), 5.42 (s, 2H), 7.28 (d, *J*=9 Hz, 1H), 7.40 (t, *J*=8 Hz, 1H), 7.54-7.56 (m, 2H), 7.51 (s, 1H), 7.79 (d, *J*=9 Hz, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 16.1, 33.6, 71.2, 110.9, 116.7, 119.3, 123.5, 125.8, 127.6, 131.6, 132.0, 132.5, 140.5, 150.1, 150.8, 157.6, 161.5, 172.0.

ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}BrClO_5$ assuming ^{79}Br stable isotope [M+Na] 458.9611, found [M+Na] 458.9634. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}BrClO_5$ assuming ^{81}Br stable isotope [M+Na] 460.9590, found [M+Na] 460.9638.

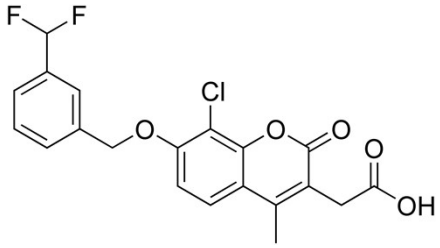


[8-Chloro-7-(3-iodo -benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl] acetic acid (4j). Using general procedure 3, the methyl ester (**9j**) (59.4 mg, 0.119 mmol) was hydrolyzed to the carboxylic acid (**4j**) as an off-white solid (16.1 mg, 28%). ^1H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H), 3.56-3.70 (m, 2H), 5.34 (s, 2H), 7.21-7.29 (m, 2H), 7.50 (d, *J*=8 Hz, 1H), 7.72 (d, *J*=8 Hz, 1H), 7.79 (d, *J*= 9 Hz, 1H), 7.77 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.6, 32.2, 69.9, 93.8, 109.6, 115.3, 118.0, 124.3, 126.7, 130.6, 136.2, 137.1, 139.1, 184.6, 149.5, 156.4, 160.1, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}ClIO_5$ assuming ^{35}Cl stable isotope [M+Na] 506.9472, found [M+Na] 506.9510. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}ClIO_5$ assuming ^{37}Cl stable isotope [M+Na] 508.9443, found [M+Na] 508.9483.

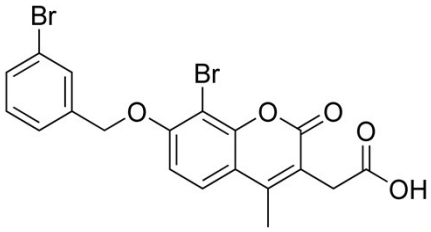


[8-Chloro-4-methyl -2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl] acetic acid (4k). Using general procedure 3, the methyl ester (**9k**) (26 mg, 0.059 mmol) was hydrolyzed to the carboxylic acid (**4k**) as an off-white solid (24 mg, 95%). ^1H NMR (500 MHz, acetone-*d6*): δ

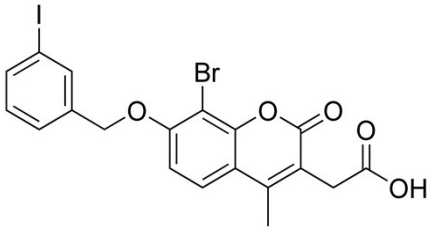
2.47 (s, 3H), 2.87 (bs, 1H), 3.73 (s, 2H), 5.52 (s, 2H), 7.32 (d, *J* = 9 Hz, 1H), 7,69-7.72 (m, 2H), 7.81 (d, *J*=9 Hz, 2H), 7.87 (d, *J*=8 Hz, 1H), 7.92 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 14.6, 32.2, 70.0, 109.3, 109.6, 115.4, 118.1, 123.9, 124.4, 124.8, 129.6, 130.2, 131.1, 138.0, 148.6, 149.5, 156.3, 160.1, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{14}ClF_3O_5$ assuming ^{35}Cl stable isotope [M+H] 427.0555, found [M+H] 427.0556. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{14}ClF_3O_5$ assuming ^{37}Cl stable isotope [M+H] 429.0526, found [M+H] 429.0528.



[8-Chloro-7-(3-difluoromethyl-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl] acetic acid (4L). Using general procedure 3, the methyl ester (**9L**) (45 mg, 0.106 mmol) was hydrolyzed to the carboxylic acid (**4L**) as an off-white solid (31.6 mg, 72.6%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.40 (s, 3H), 3.57 (s, 2H), 5.45 (s, 2H), 7.09 (t, *J*=56 Hz, 1H), 7.33 (d, *J*=9 Hz, 1H), 7.57-7.59 (m, 2H), 7.68 (d, *J*=7 Hz, 1H), 7.12 (s, 1H), 7.81 (d, *J*=9 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.7, 33.3, 70.5, 108.7, 110.5, 113.4, 115.27, 115.3 (t, *J* = 243 Hz), 118.2, 125.0 (t, *J* = 7 Hz), 125.3, 126.0 (t, *J* = 6 Hz), 130.7, 130.4, 134.9 (t, *J* = 21 Hz), 137.5, 149.1, 149.4, 156.3, 160.5, 171.8. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{15}ClF_2O_5$ assuming ^{35}Cl stable isotope [M+Na] 431.0474, found [M+H] 431.0484. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{15}ClF_2O_5$ assuming ^{37}Cl stable isotope [M+Na] 433.0445, found [M+H] 433.0459.

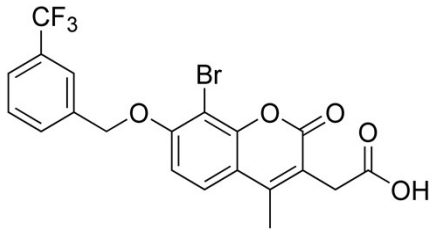


[8-Bromo-7-(3-bromo-benzyloxy)-4-methyl -2-oxo-2H-chromen-3-yl] acetic acid (4m). Using general procedure 3, the methyl ester (**9m**) (20.4 mg, 0.041 mmol) was hydrolyzed to the carboxylic acid (**4m**) as an off-white solid (17 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.44 (s, 3H), 3.80 (s, 2H), 5.25 (s, 2H), 6.913 (d, *J*=9 Hz, 1H), 7.29-7.31 (m, 1H), 7.43 (d, *J*= 10 Hz, 1H), 7.48-7.50 (m, 1H), 7.57 (d, *J*=10 Hz, 1H), 7,64 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 14.6, 32.2, 70.0, 109.6, 115.5, 118.0, 122.1, 125.4, 126.1, 130.1, 131.0, 139.2, 148.6, 150.6, 157.4, 160.2, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}Br_2O_5$ assuming two ^{79}Br stable isotope [M+H] 480.9281, found [M+H] 480.9259. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}Br_2O_5$ assuming one ^{79}Br and one ^{81}Br stable isotopes [M+H] 482.9260, found [M+H] 482.9239.

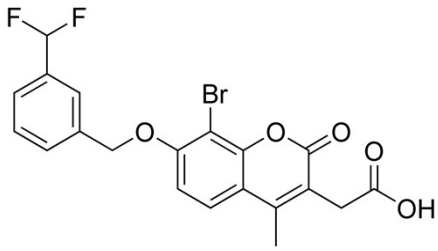


[8-Bromo-7-(3-iodo-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid (4n). Using general procedure 3, the methyl ester (**9n**) (77 mg, 0.142 mmol) was hydrolyzed to the carboxylic acid (**4n**) as an off-white solid (28.3 mg, 38%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.44 (s, 3H), 3.80 (s, 2H), 5.23 (s, 2H), 6.91 (d, *J*=9 Hz, 1H), 7.06 (s, 1H), 7.16 (t, *J*=8 Hz, 1H), 7.46-7.48 (m, 2H), 7.57 (d, *J*=9 Hz, 1H), 7.70 (d, J=9 Hz, 1H), 7.84 (s, 1H). ESI-LCMS (high

resolution) m/z calculated for $C_{19}H_{14}BrIO_5$ assuming ^{79}Br stable isotope [M+H] 528.9142, found [M+H] 528.9136. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}BrIO_5$ assuming ^{81}Br stable isotope [M+H] 530.9121, found [M+H] 530.9118.

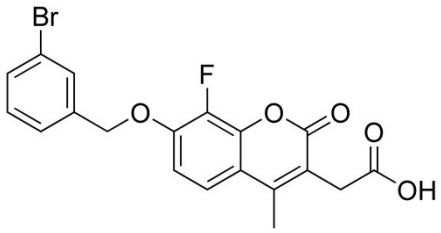


[8-Bromo-4-methyl -2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl] acetic acid (4o). Using general procedure 3, the methyl ester (**9o**) (61.8 mg, 0.127 mmol) was hydrolyzed to the carboxylic acid (**4o**) as an off-white solid (45.2 mg, 75%). ^{1}H NMR (500 MHz, acetone-d_6): δ 2.49 (s, 1H), 3.75 (s, 2H), 5.53 (s, 2H), 7.29 (d, *J*=9 Hz, 1H), 7.69-7.74 (m, 3H), 7.86-7.89 (m, 2H), 7.95 (s, 1H). ESI-LCMS (low resolution) m/z calculated for $C_{20}H_{14}BrF_3O_5$ assuming ^{79}Br stable isotope [M+H] 471.0, found [M+H] 470.8.

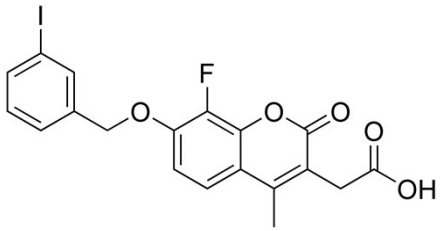


[8-Bromo-7-(3-difluoromethyl-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid (4p). Using general procedure 3, the methyl ester (**9p**) (30 mg, 0.064 mmol) was hydrolyzed to the carboxylic acid (**4p**) as an off-white solid (27 mg, 95%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.40 (s, 3H), 3.60 (s, 2H), 5.45 (s, 2H), 7.08 (t, *J*= 56 Hz, 1H), 7.29 (d, *J*=9 Hz, 1H), 7.57-1.60 (m, 2H), 7.68 (d, *J*=8, 1H), 7.72 (s, 1H), 7.85 (d, *J*=9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.6, 32.7, 52.5, 107.5, 112.2, 114.6, 116.8, 123.9, 149.2, 154.4, 160.6, 171.1. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{15}BrF_2O_5$ assuming ^{79}Br stable isotope [M+H]

453.0144, found [M+H] 453.0126. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{15}BrF_2O_5$ assuming ^{81}Br stable isotope [M+H] 455.0123, found [M+H] 455.0108.

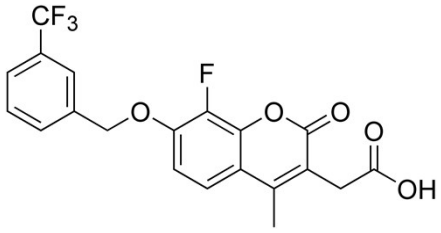


[7-(3-Bromo-benzyloxy)-8-fluoro-4-methyl-2-oxo-2H-chromen-3-yl] acetic acid (4q). Using general procedure 3, the methyl ester (**9q**) (34.1 mg, 0.078 mmol) was hydrolyzed to the carboxylic acid (**4q**) as an off-white solid (19.2 mg, 58%). ^1H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H), 3.59 (s, 2H), 5.35 (s, 2H), 7.30 (t, *J*=9 Hz, 1H), 7.39 (t, *J*=8 Hz, 1H), 7.50 (d, *J*=8 Hz, 1H), 7.57 (dt, J=10, 1H), 7.62 (dd, *J*=2,9 Hz, 1H), 7.71 (t, *J*=2 Hz, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 14.6, 32.2, 70.2, 110.6, 115.5, 118.2, 120.3, 122.1, 126.5, 130.5, 130.6, 131.2, 138.5, 139.1, 140.4, 141.7 (d, *J* = 9 Hz), 148.5, 148.7, 159.7, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}BrFO_5$ assuming ^{79}Br stable isotope [M+H] 421.0082, found [M+H] 421.0076. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}BrFO_5$ assuming ^{81}Br stable isotope [M+H] 423.0061, found [M+H] 423.0059.

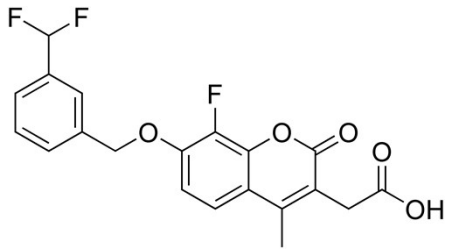


[8-Fluoro-7-(3-iodo-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid (4r). Using general procedure 3, the methyl ester (**9r**) (39.4 mg, 0.082 mmol) was hydrolyzed to the

carboxylic acid (**4r**) as an off-white solid (21 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H), 3.59 (s, 2H), 5.31 (s, 2H), 7.23 (t, J=8 Hz, 1H), 7.29 (t, *J*=9 Hz, 1H), 7.51 (d, *J*=8 Hz, 1H), 7.62 (dd, *J*=2,9 Hz, 1H), 7.73 (d, *J*=8 Hz, 1H), 7.88 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 14.6, 32.2, 70.1, 93.8, 110.6, 115.4, 118.2, 120.2 (d, *J* = 5 Hz), 127.0, 130.6, 136.5, 137.2, 139.1, 141.7 (d, *J* = 9 Hz), 148.6 (d, *J* = 11 Hz), 159.7, 170.4. ESI-LCMS (high resolution) m/z calculated for $C_{19}H_{14}FIO_5$ [M+H] 468.9943, found [M+H] 468.9944.



[8-Fluoro-4-methyl-2-oxo-7-(3-trifluoromethyl-benzyloxy)-2H-chromen-3-yl] acetic acid (4s). Using general procedure 3, the methyl ester (**9s**) (36.8 mg, 0.087 mmol) was hydrolyzed to the carboxylic acid (**4s**) as an off-white solid (34 mg 97%). ^{1}H NMR (500 MHz, DMSO): δ 2.39 (s, 1H), 3.58 (s, 2H), 5.45 (s, 2H), 7.33 (dt, *J*=8,9 Hz, 1H), 7.63 (dd, *J*=2,10 Hz, 1H), 7.68 (d, *J*=8 Hz, 1H), 7.75 (d, *J*=8 Hz, 1H), 7.81 (d, *J*=8 Hz, 1H), 7.88 (s, 1H). ^{13}C NMR (125 MHz, acetone-d_6): δ 14.6, 33.3, 70.4, 110.6, 115.5, 118.3, 120.3, 124.2, 124.3, 129.6 130.2, 131.5, 137.9, 141.8, 148.5, 159.7, 170.5. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{14}F_4O_5$ [M+Na] 433.0675, found [M+H] 433.0680.



[8-Fluoro-7-(3-difluoromethyl-benzyloxy)-4-methyl-2-oxo-2H-chromen-3-yl]-acetic acid (4t). Using general procedure 3, the methyl ester (**9t**) (36.5 mg, 0.099 mmol) was hydrolyzed

to the carboxylic acid (**4t**) as an off-white solid (33 mg, 95%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.39 (s, 3H), 3.60 (s, 2H), 5.41 (s, 2H), 7.08 (t, *J*=56 Hz, 1H), 7.32 (t, *J*=8 Hz, 1H), 7.57-7.58 (m, 2H), 7.62 (d, *J*=10 Hz, 1H), 7.67 (d, *J*=5 Hz, 1H), 7.70 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.7, 33.5, 70.7, 111.2, 115.4 (t, J = 250 Hz), 118.5, 121.2, 125.4 (d, J = 7 Hz), 126.0, 127.5, 129.7, 130.8, 134.9 (t, *J* = 22 Hz), 137.4, 138.1, 140.0, 141.4 (d, J = 9 Hz), 146.1, 148.5 (d, J = 8 Hz), 160.1, 171.7. ESI-LCMS (high resolution) m/z calculated for $C_{20}H_{17}F_3O_5$ [M+H] 393.0945, found [M+H] 393.0961.

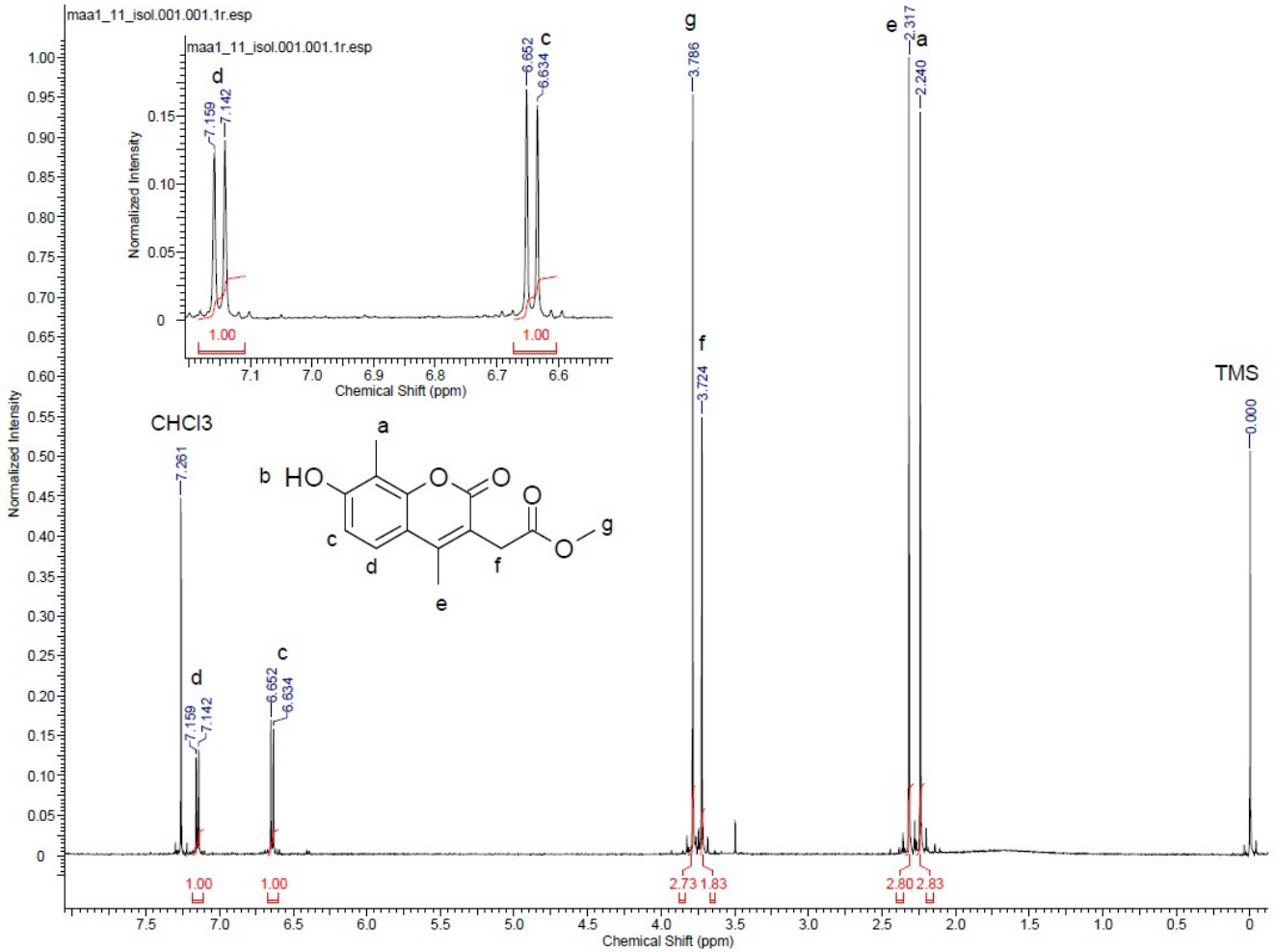


Figure S1. ^{1}H-NMR of intermediate **7a** (500 MHz, $CDCl_3$).

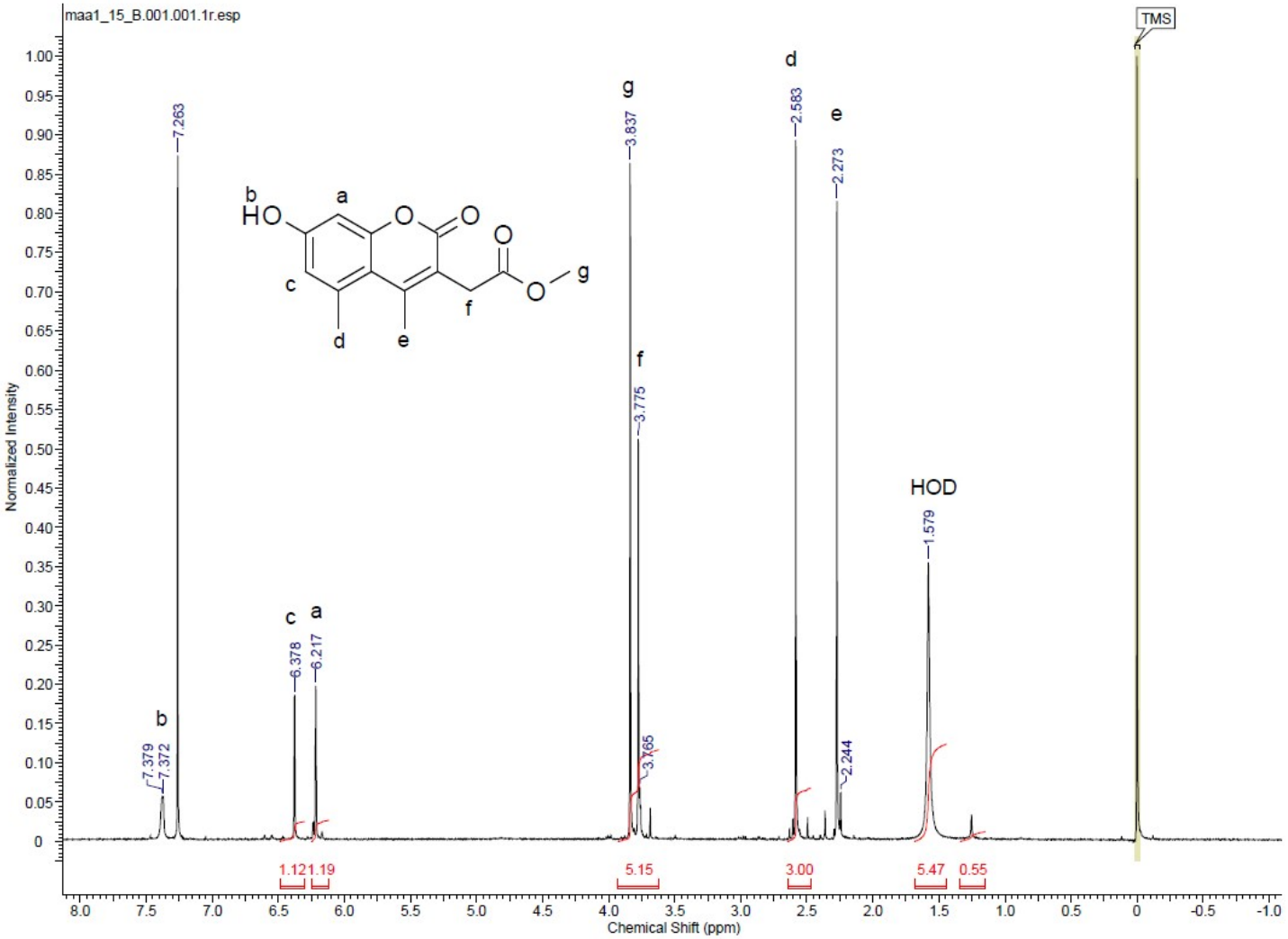


Figure S2. ^{1}H-NMR of intermediate **7b** (500 MHz, $CDCl_3$).

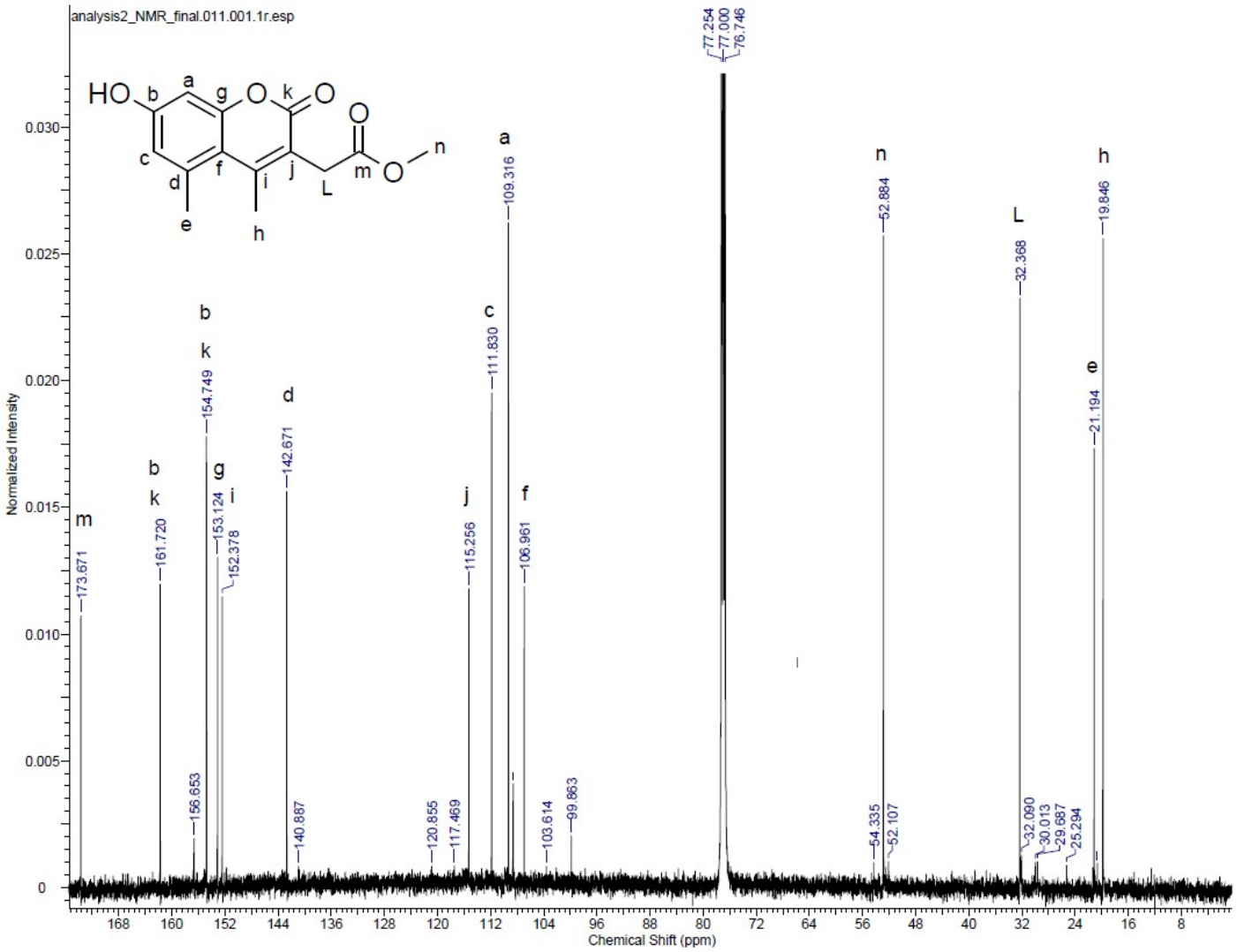


Figure S3. ^{13}C-NMR of intermediate **7b** (500 MHz, $CDCl_3$).

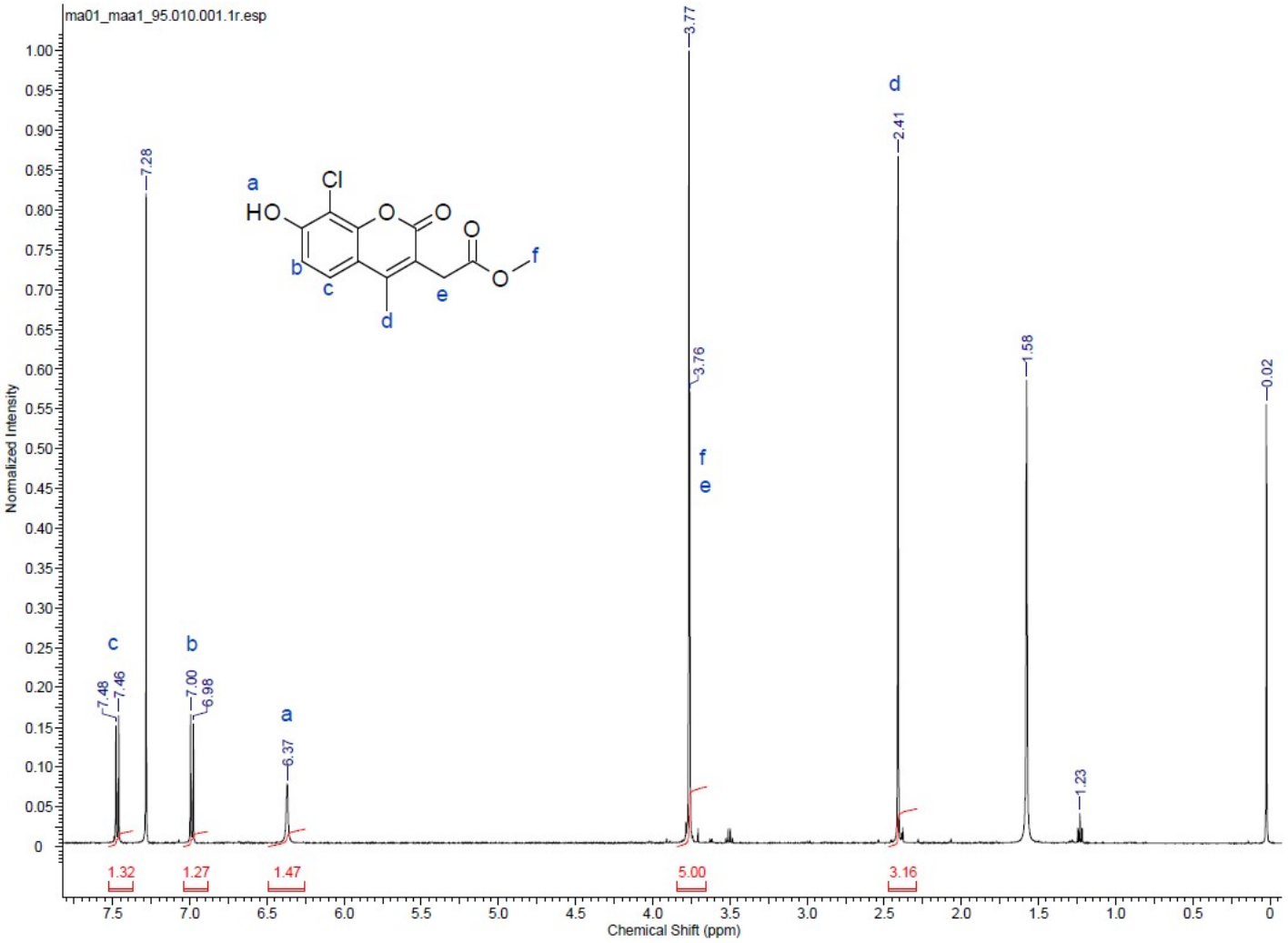


Figure S4. ^{1}H-NMR of intermediate **7c** (500 MHz, $CDCl_3$).

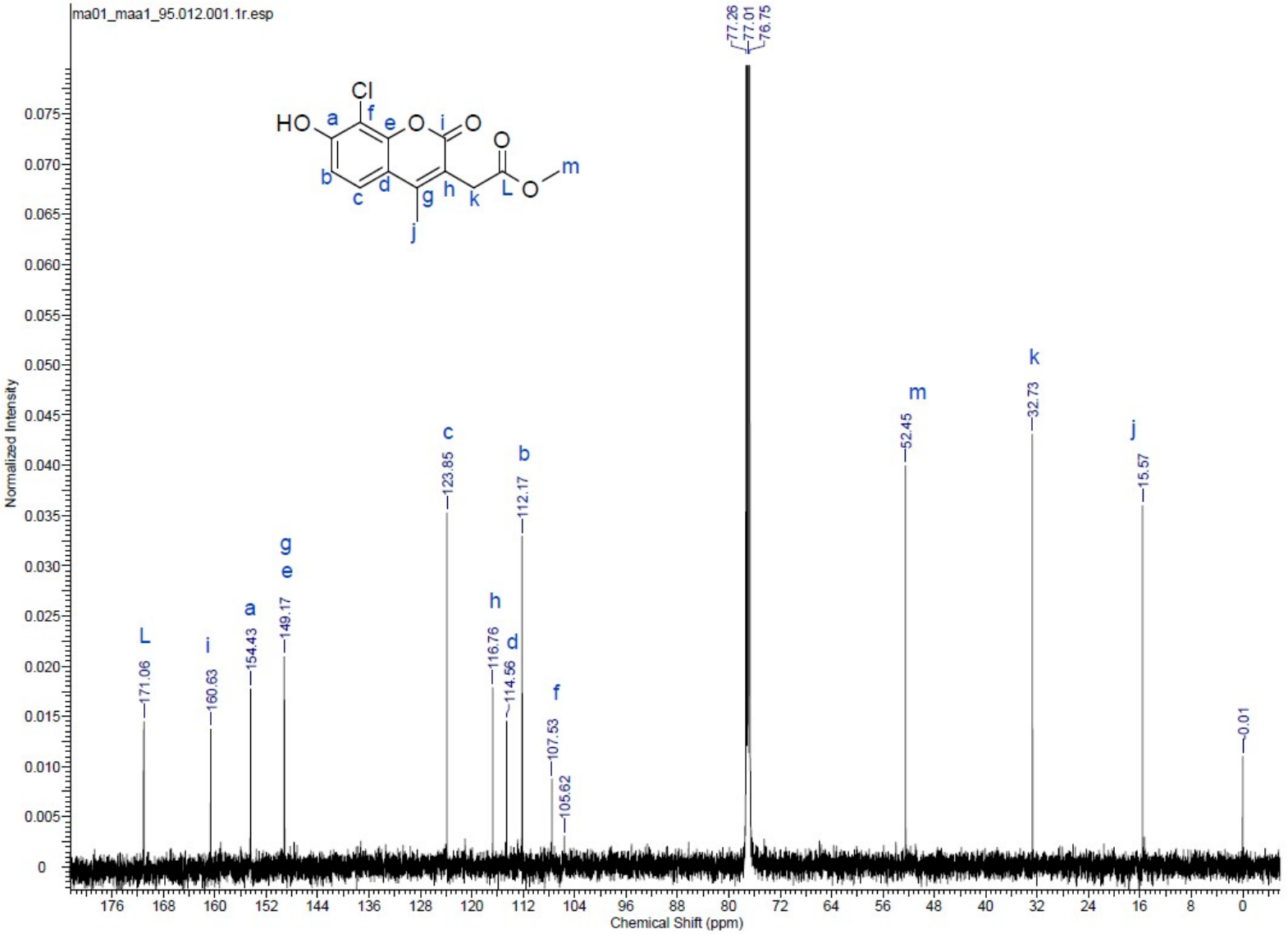


Figure S5. ^{13}C-NMR of intermediate **7c** (500 MHz, $CDCl_3$).

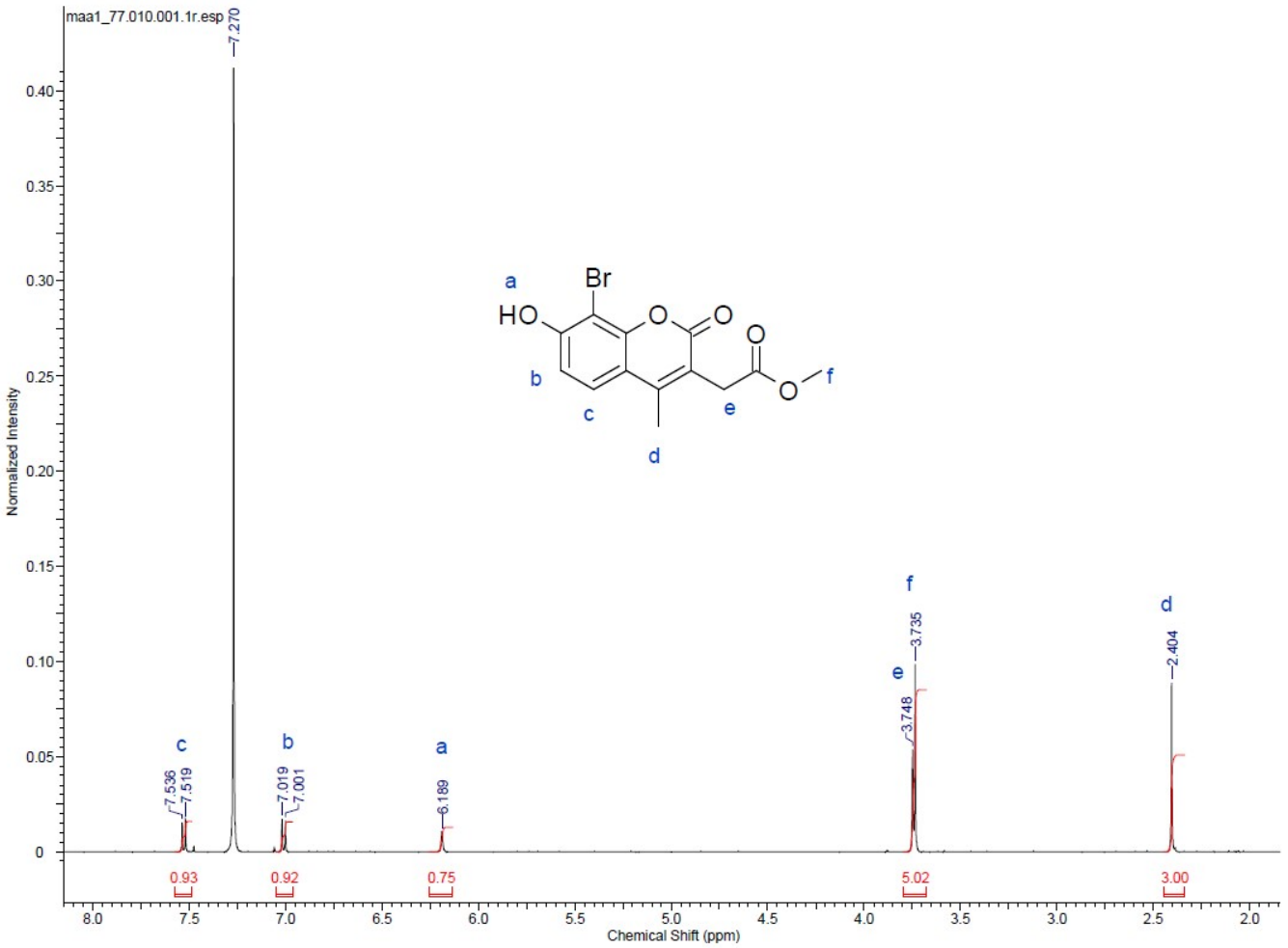


Figure S6. ^{1}H-NMR of intermediate **7d** (500 MHz, $CDCl_3$).

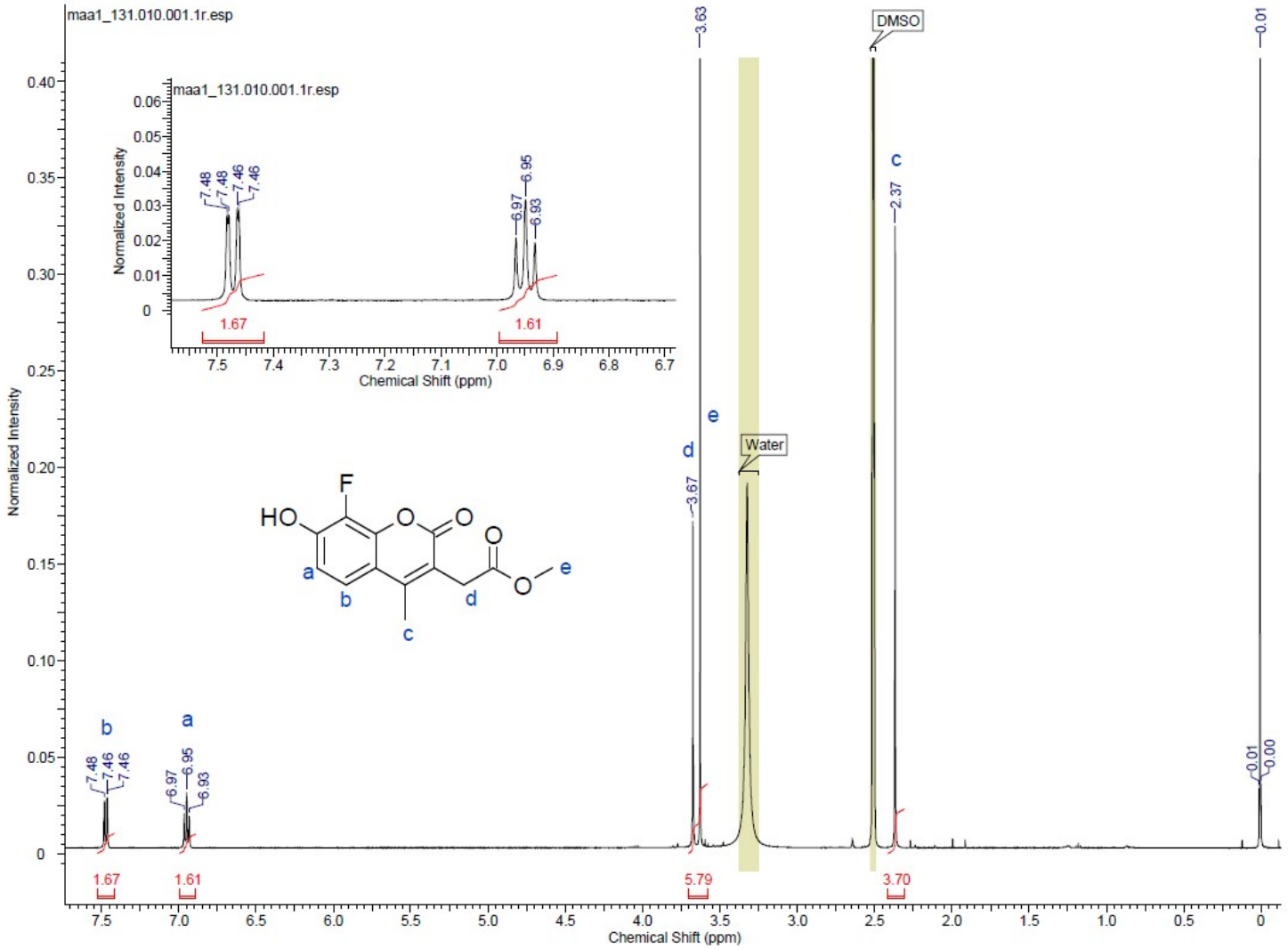


Figure S7. ^{1}H-NMR of intermediate **7e** (500 MHz, DMSO-d6).

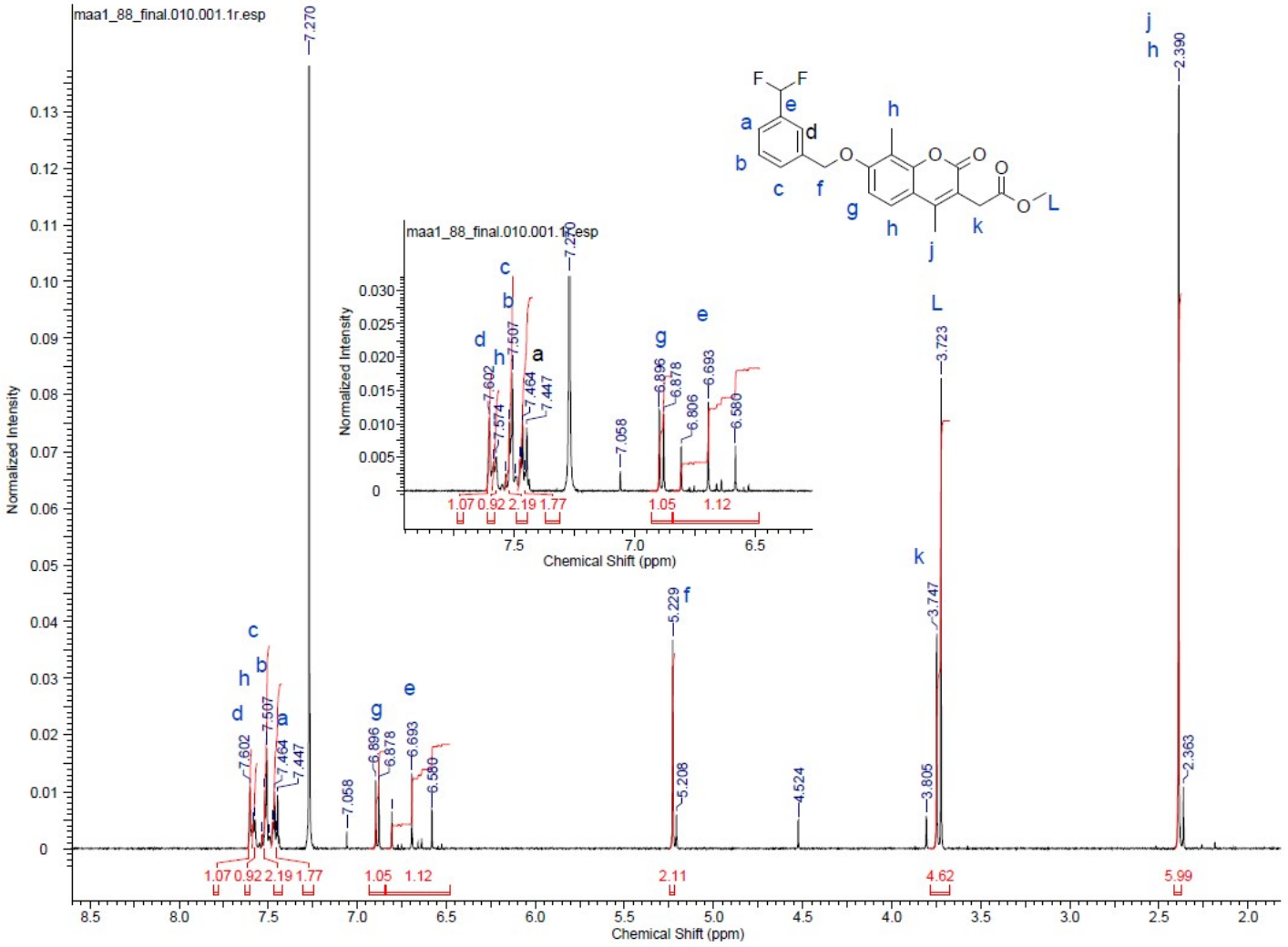


Figure S8. ^{1}H-NMR of intermediate **9d** (500 MHz, CDCl3).

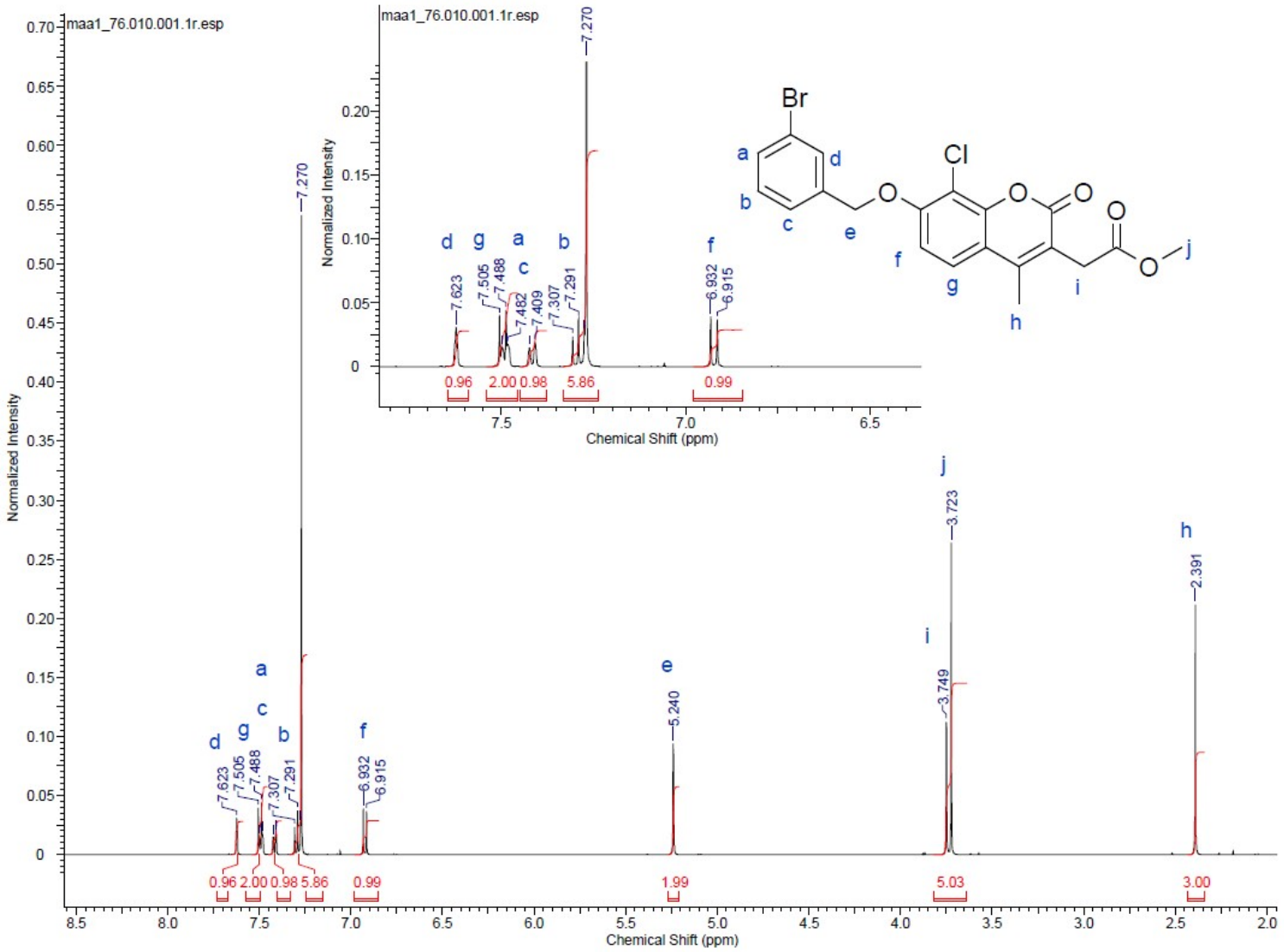


Figure S9. ^{1}H-NMR of intermediate **9i** (500 MHz, CDCl3).

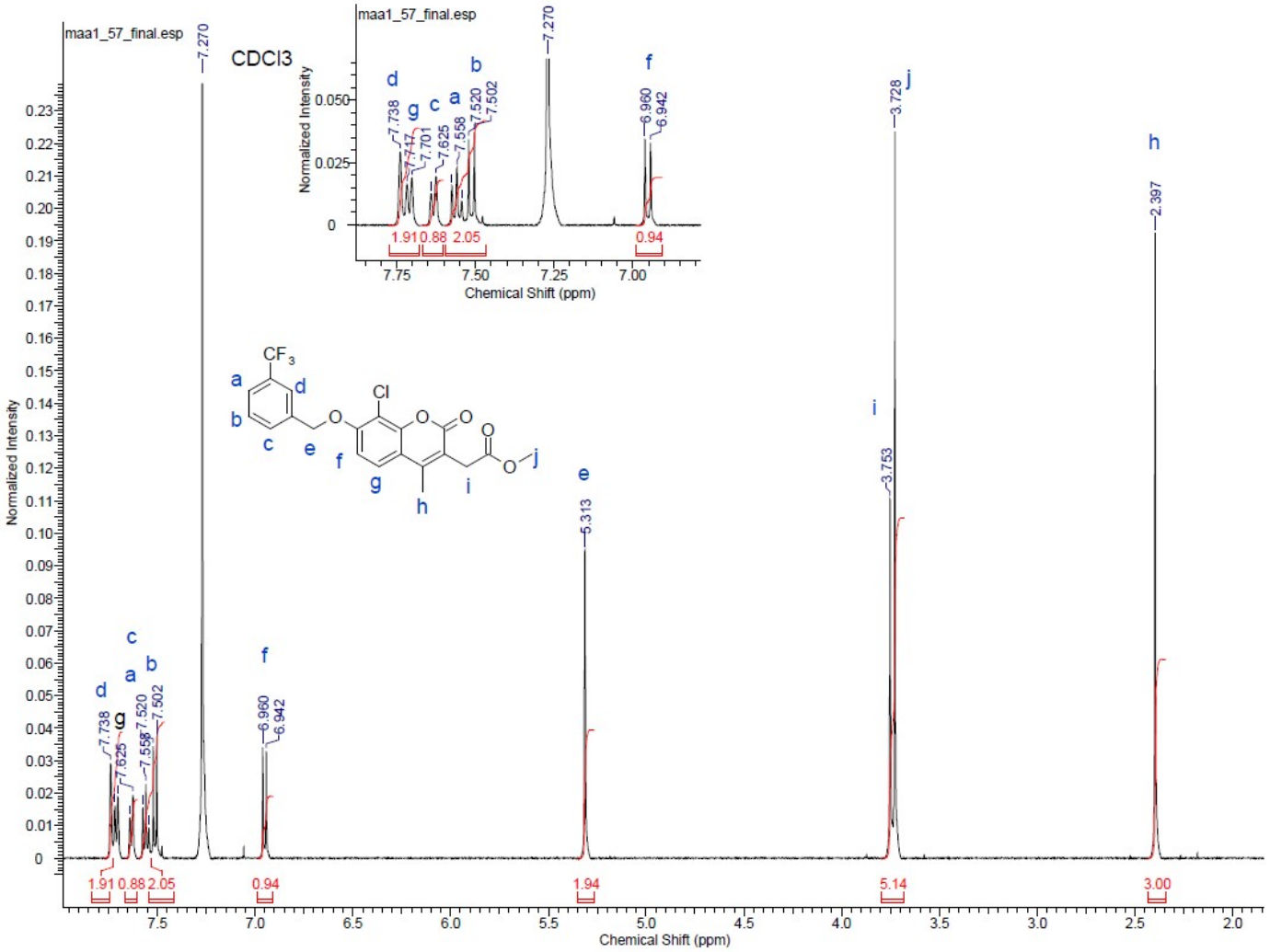


Figure S10. ^{1}H-NMR of intermediate **9k** (500 MHz, CDCl3).

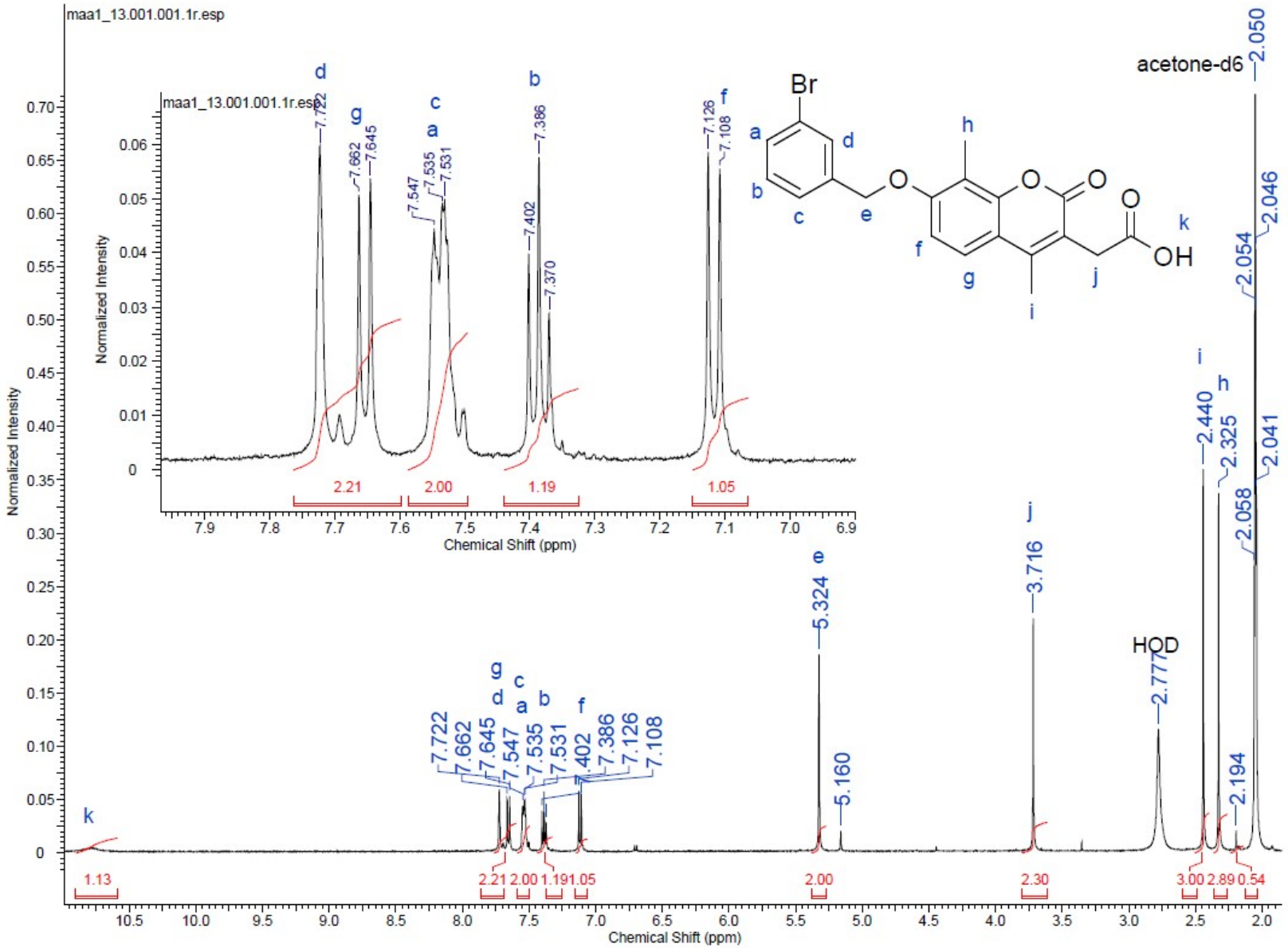


Figure S11. ^{1}H-NMR of inhibitor candidate **4a** (500 MHz, acetone-*d6*).

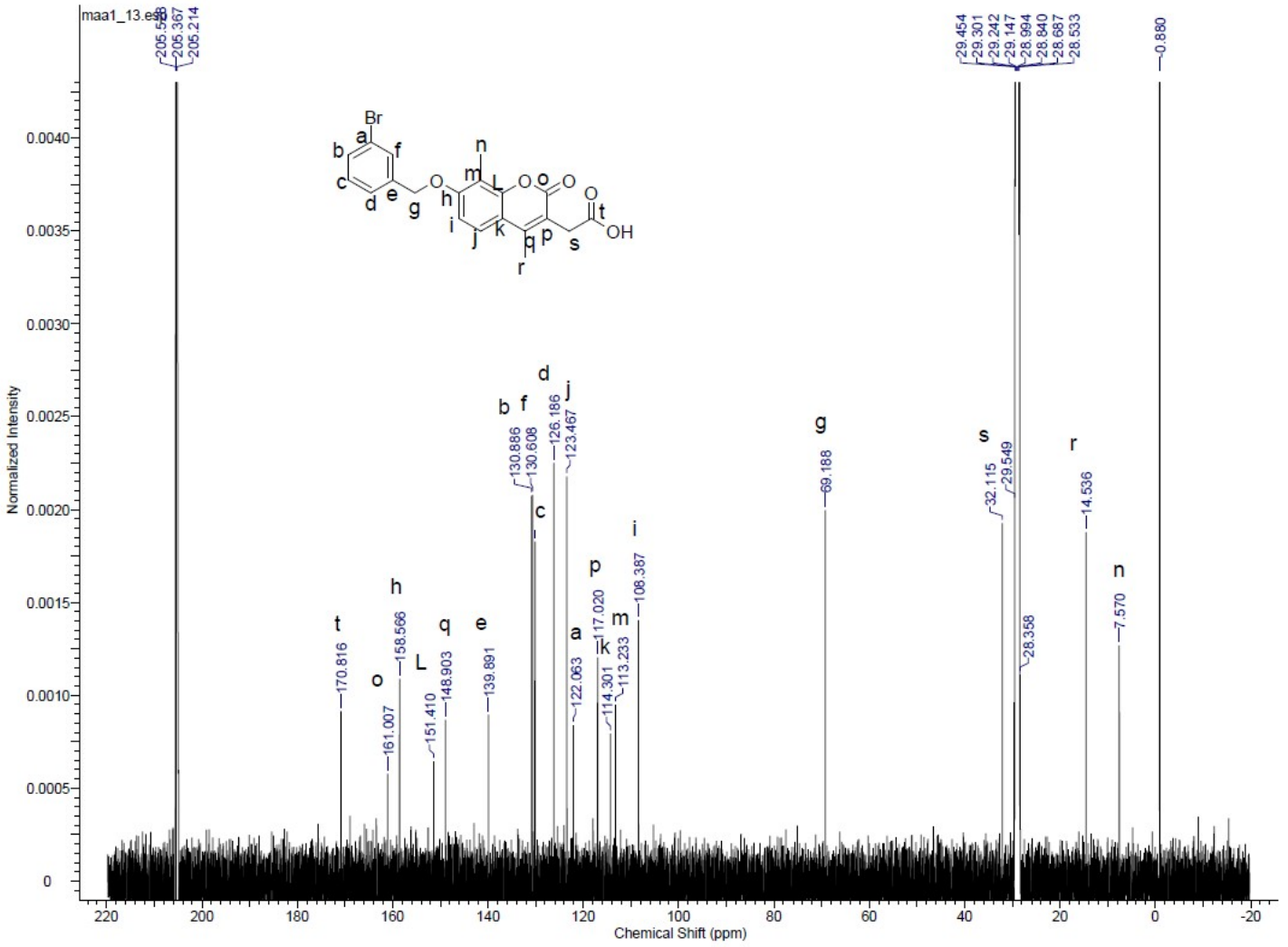


Figure S12. ^{13}C-NMR of inhibitor candidate **4a** (125 MHz, acetone-*d6*).

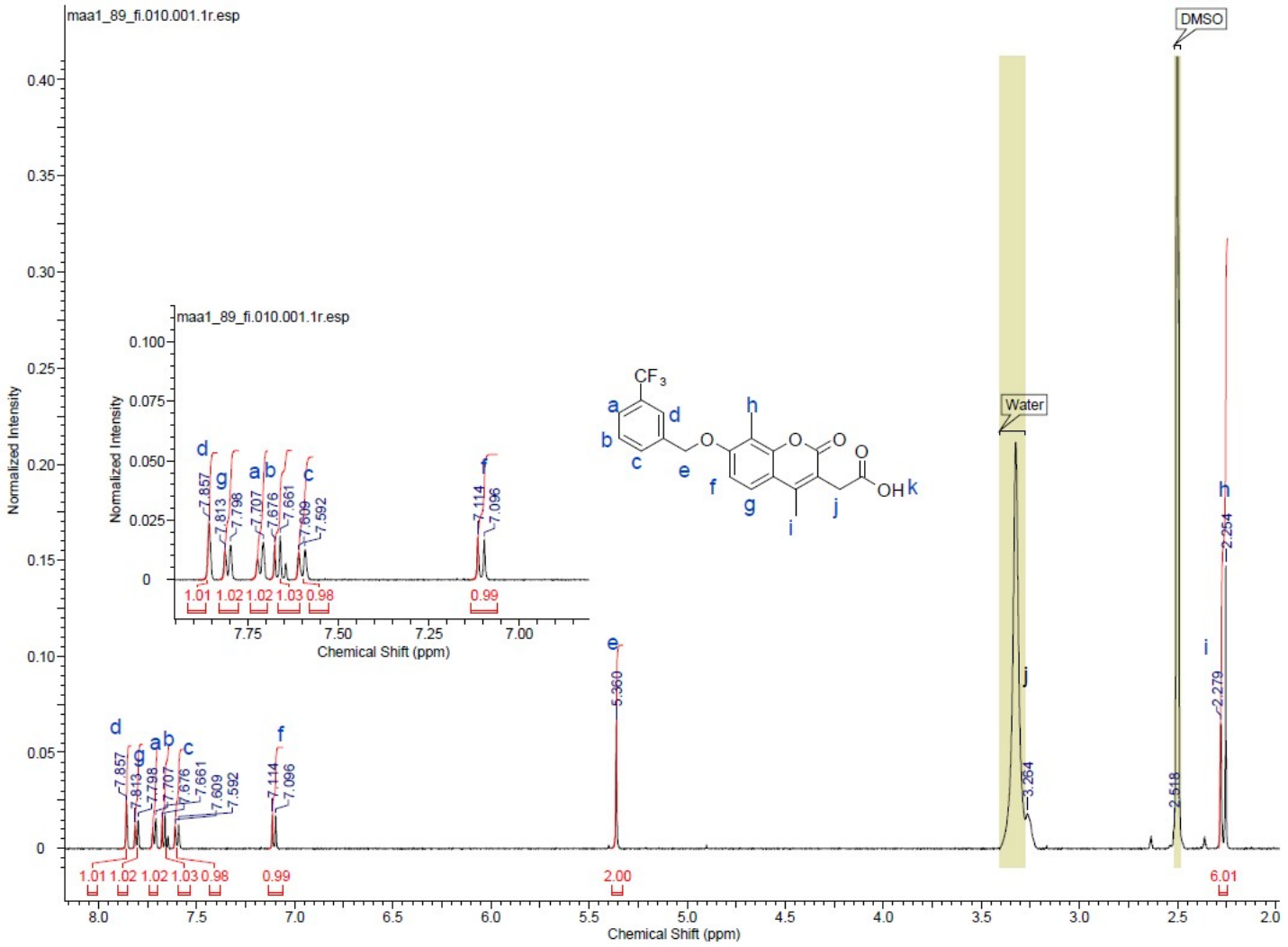


Figure S13. ^{1}H-NMR of inhibitor candidate **4c** (500 MHz, DMSO-*d6*).

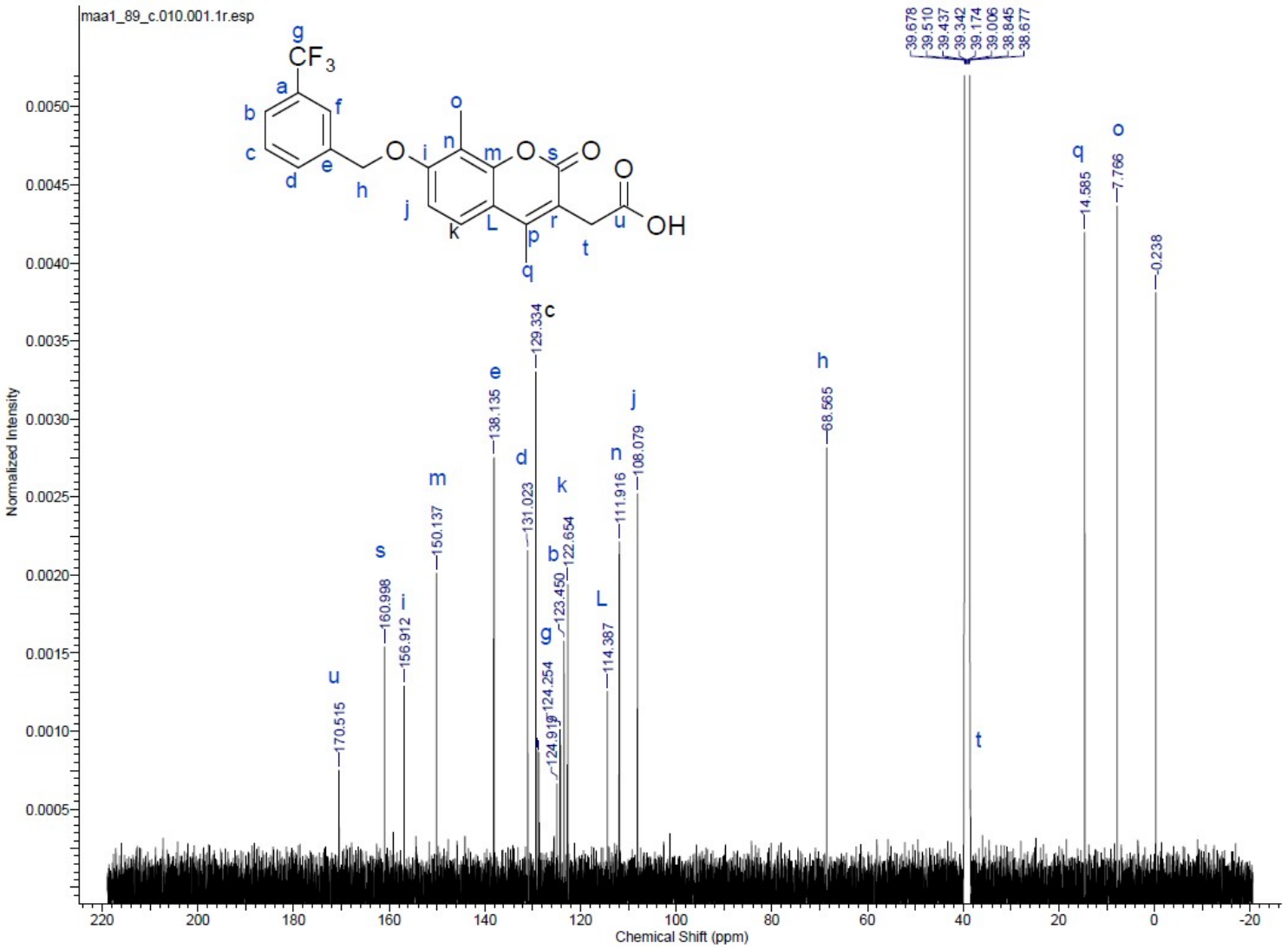


Figure S14. ^{13}C-NMR of inhibitor candidate **4c** (125 MHz, DMSO-*d6*).

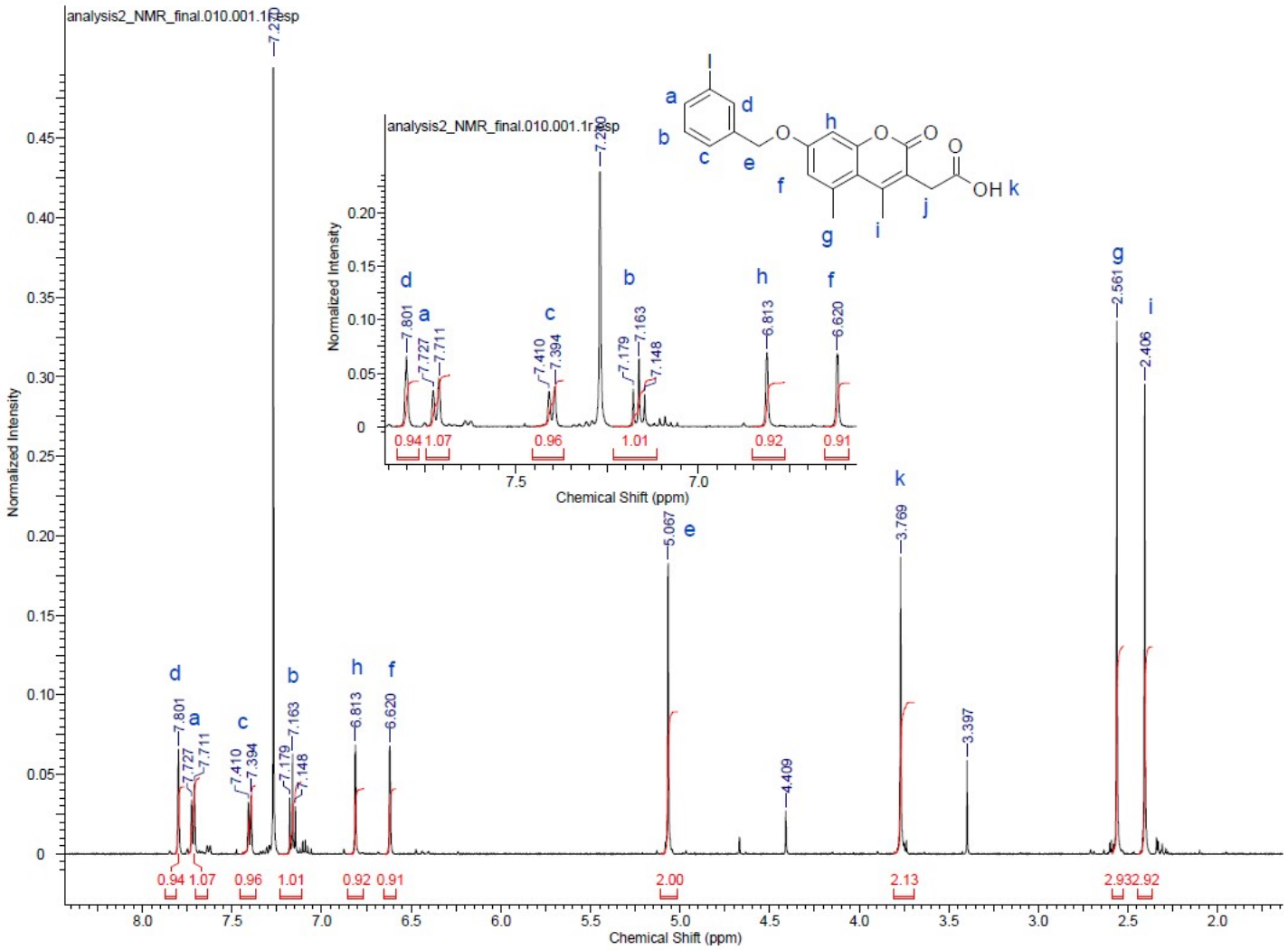


Figure S15. ^{1}H-NMR of inhibitor candidate **4f** (500 MHz, $CDCl_3$).

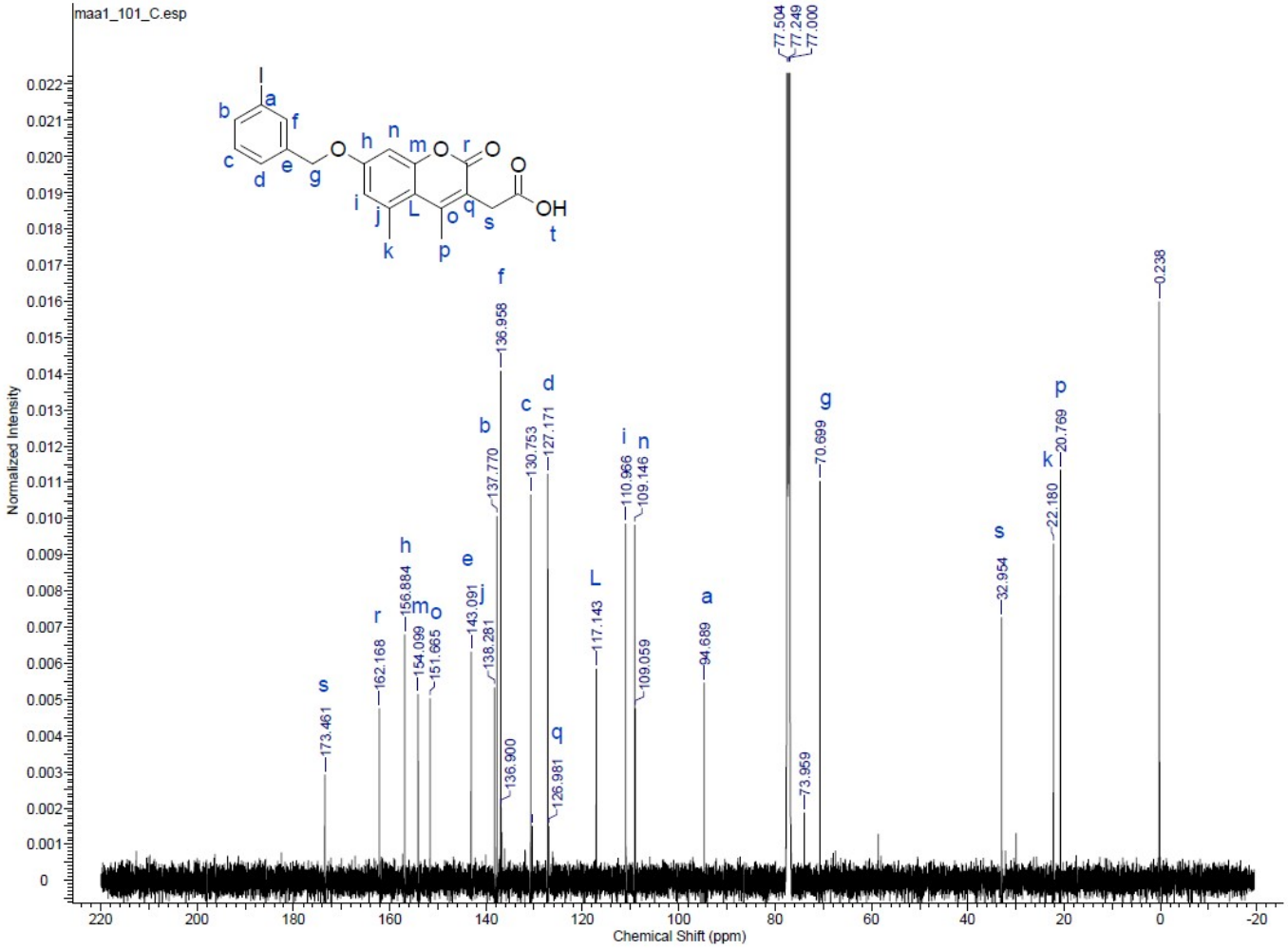


Figure S16. ^{13}C-NMR of inhibitor candidate **4f** (125 MHz, $CDCl_3$).

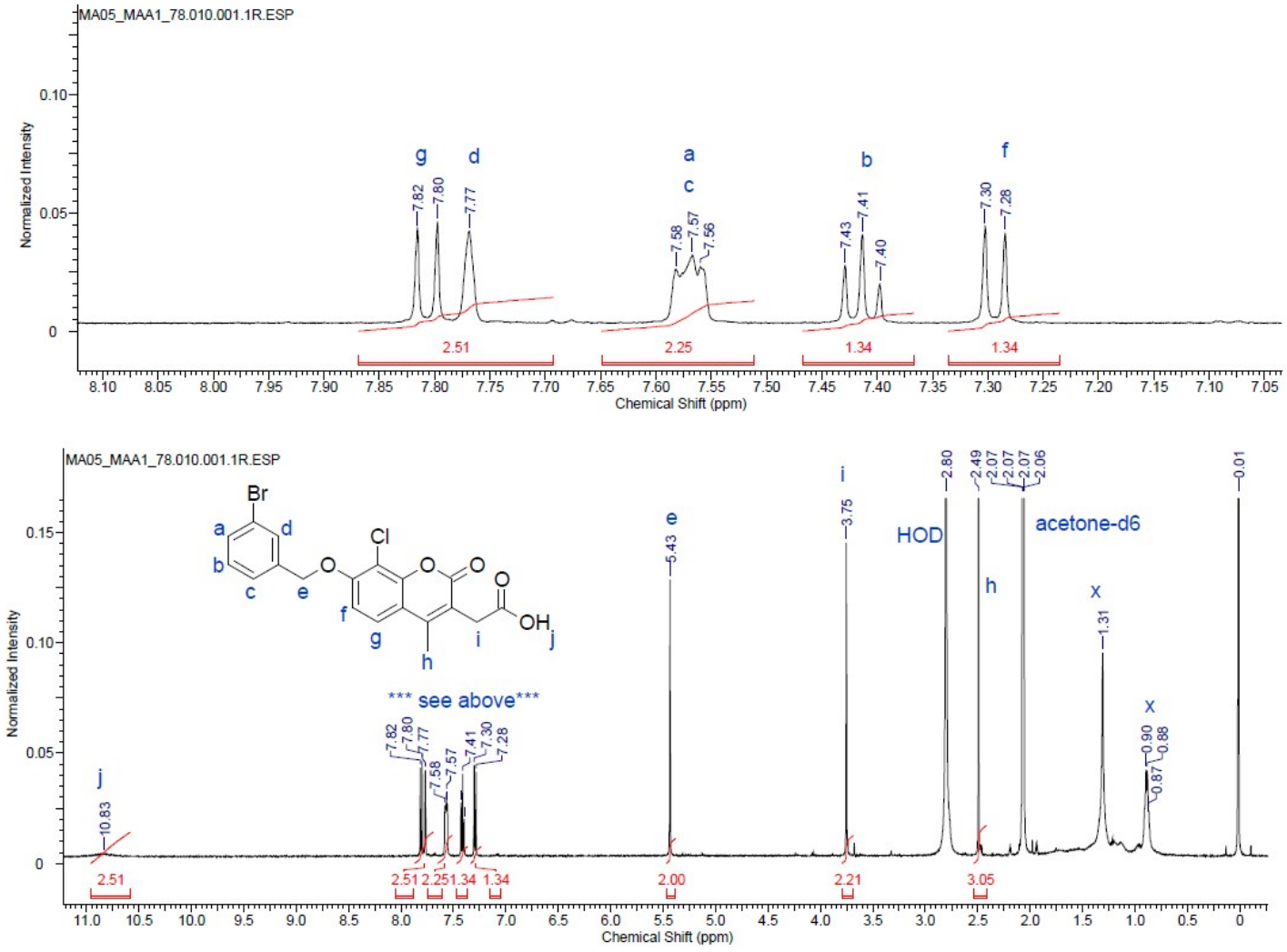


Figure S17. ^{1}H-NMR of inhibitor candidate **4i** (500 MHz, acetone-d6).

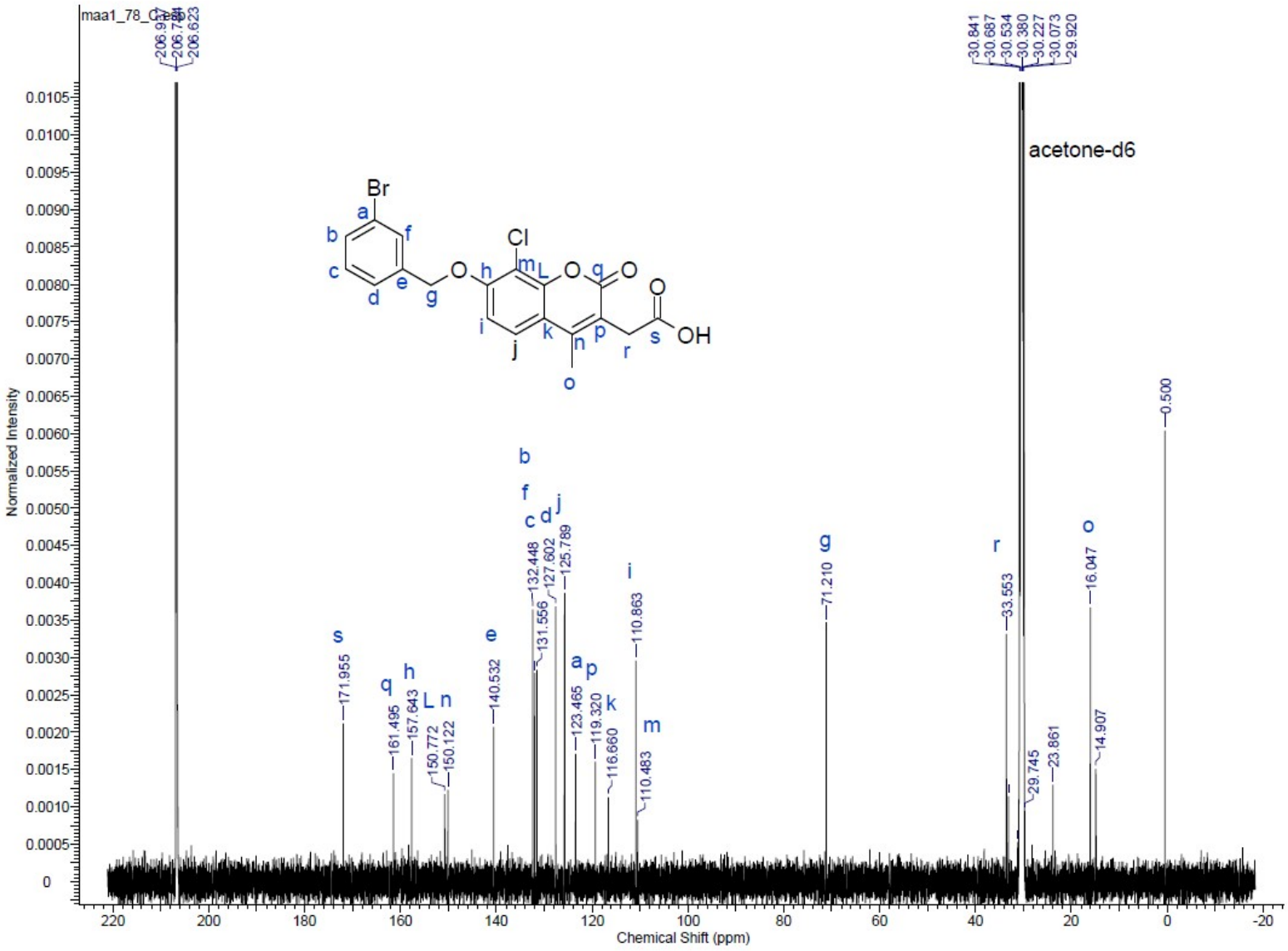


Figure S18. ^{13}C-NMR of inhibitor candidate **4k** (125 MHz, acetone-d6).

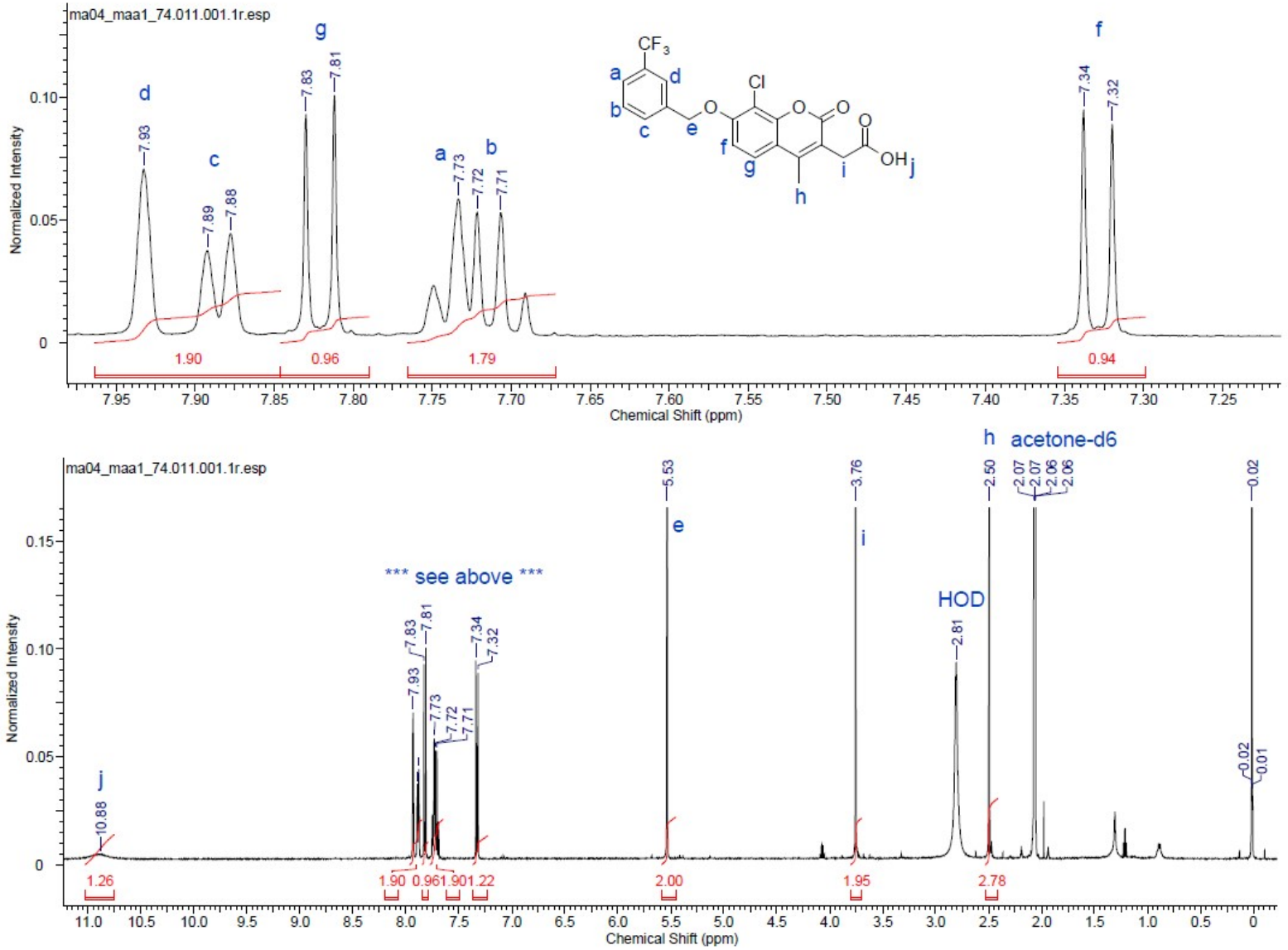


Figure S19. ^{1}H-NMR of inhibitor candidate **4k** (500 MHz, acetone-d6).

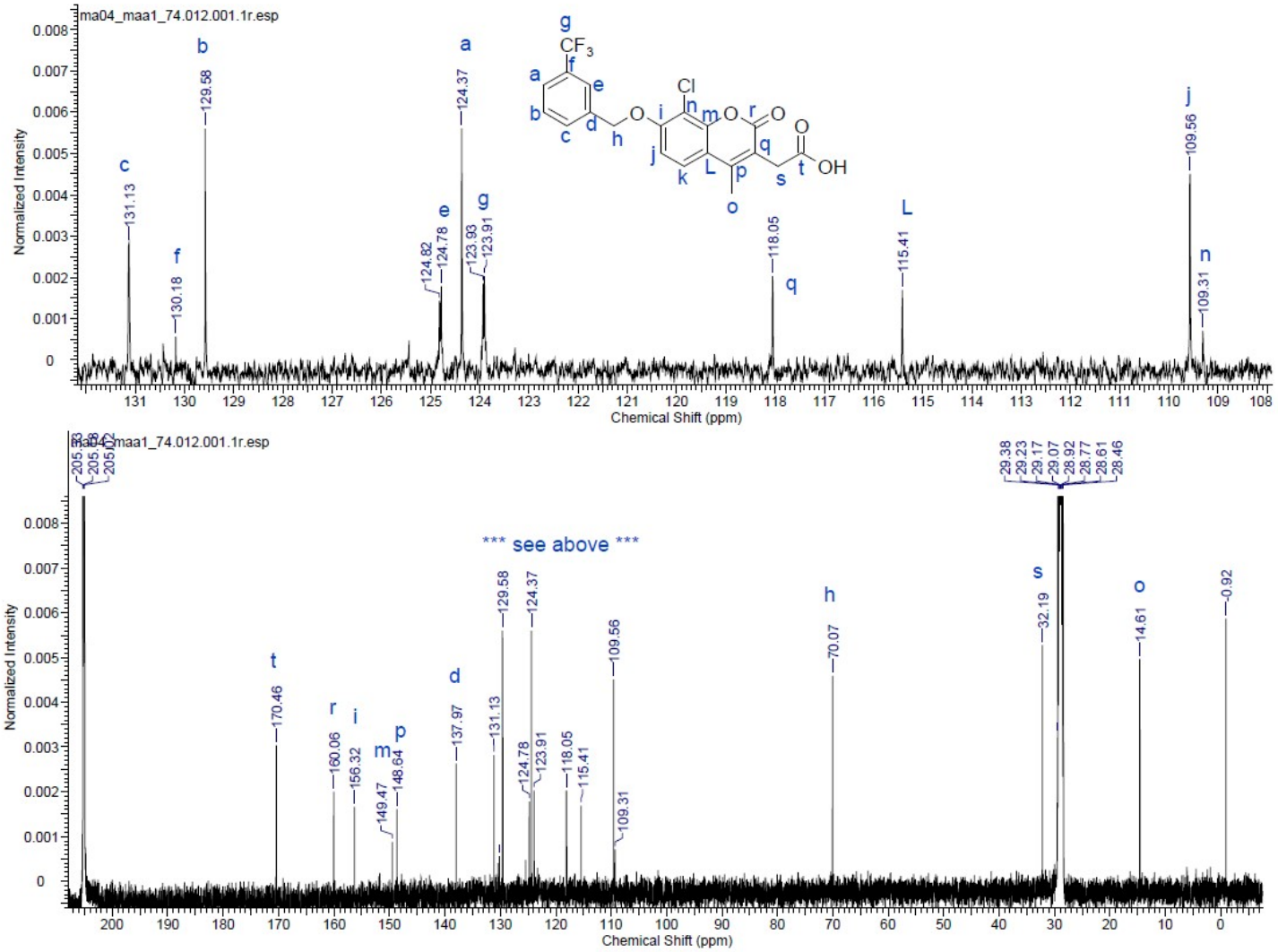


Figure S20. ^{13}C-NMR of inhibitor candidate **4k** (125 MHz, acetone-d6).

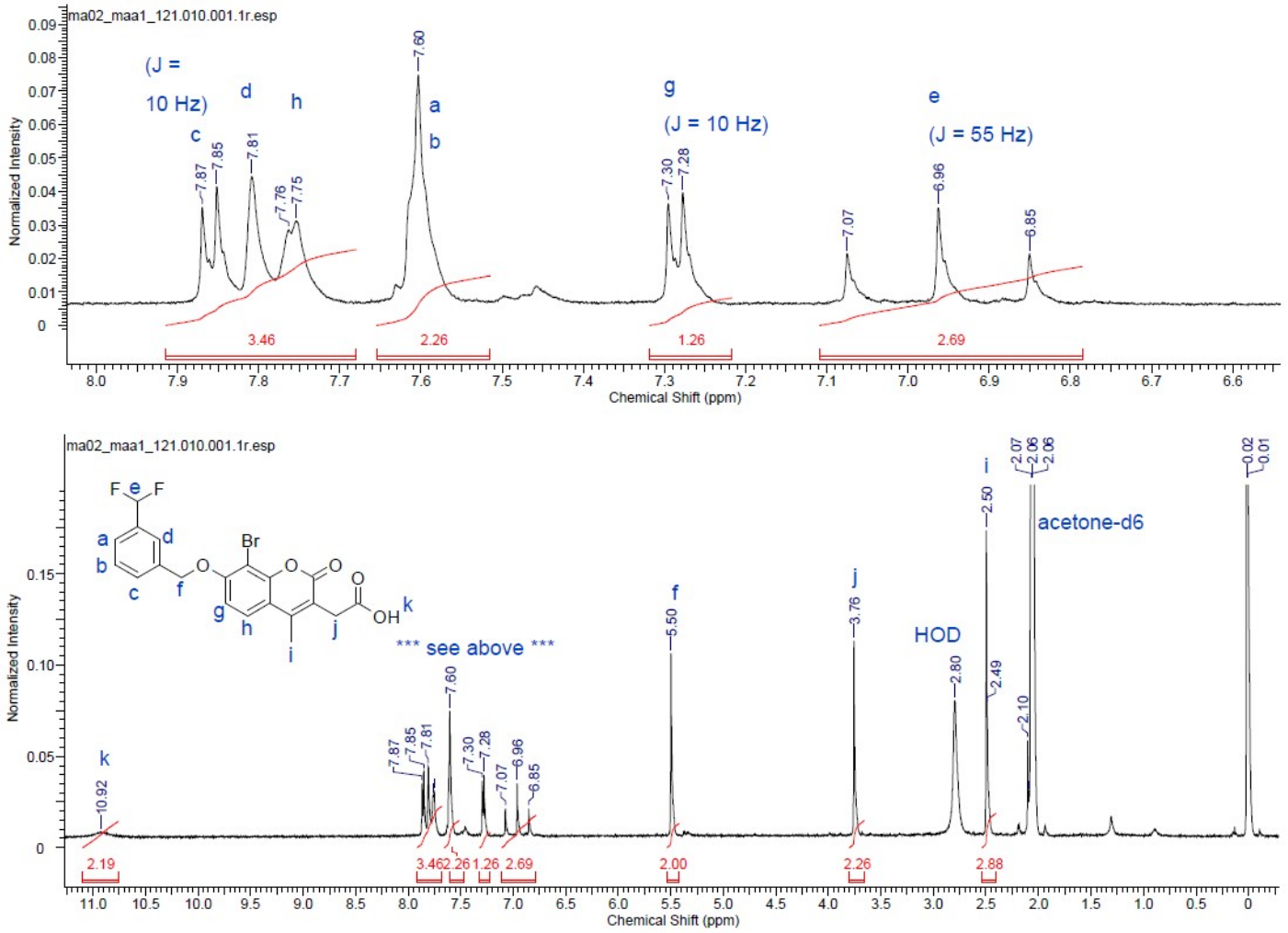


Figure S21. ^{1}H-NMR of inhibitor candidate **4p** (500 MHz, acetone-d6).

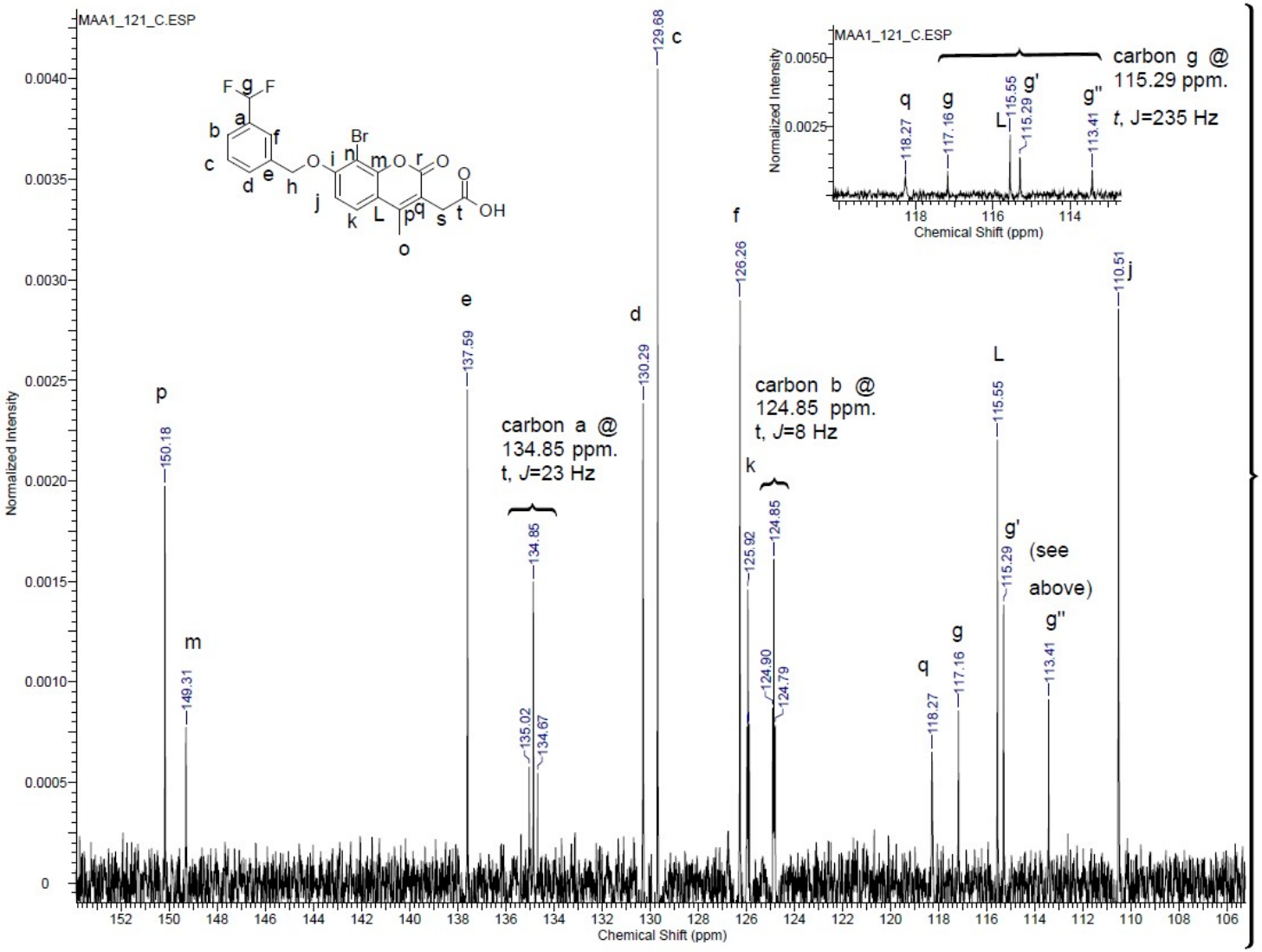


Figure S22. ^{13}C-NMR of inhibitor candidate **4p** (125 MHz, DMSO-d6).

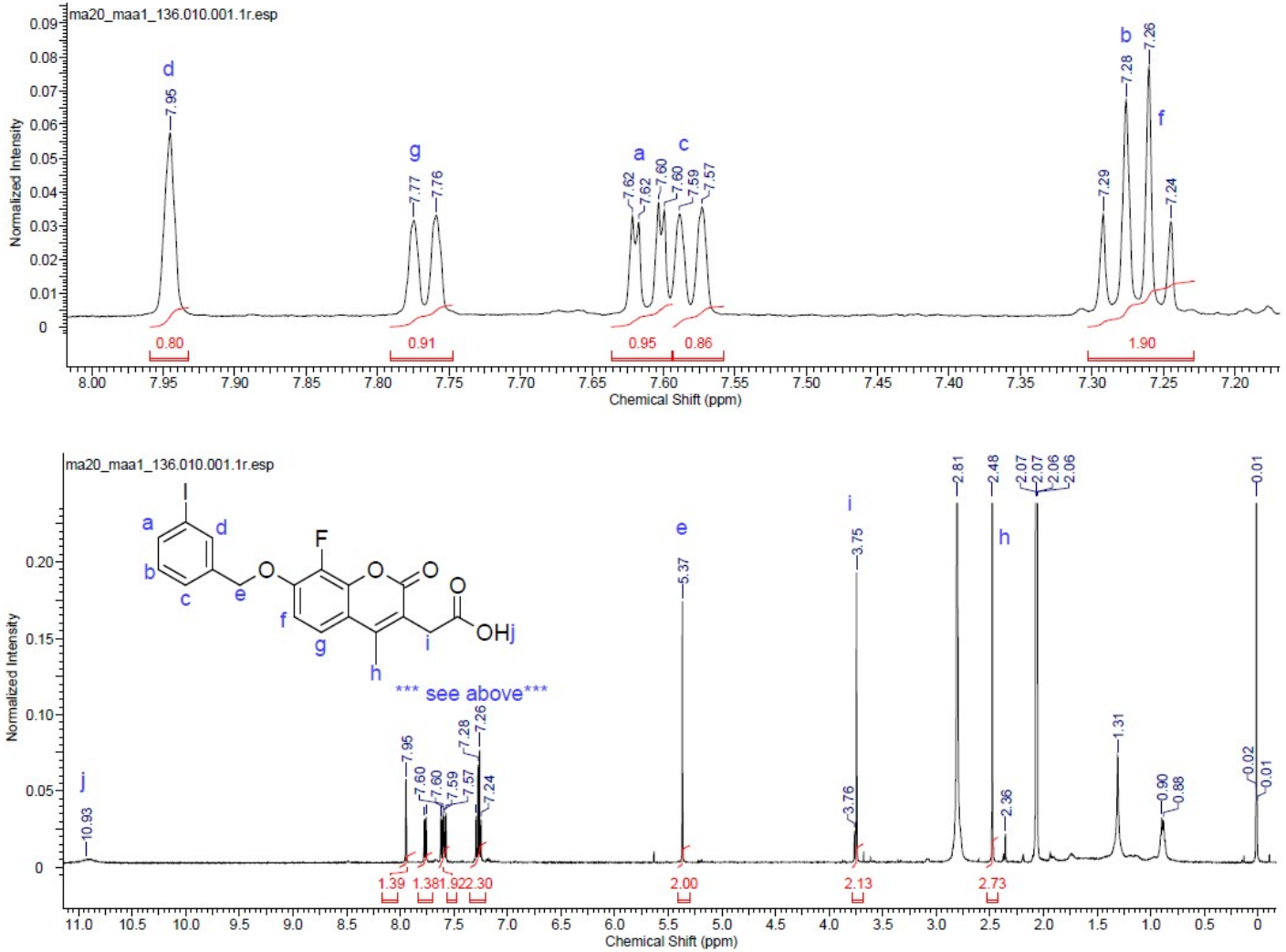


Figure S23. ^{1}H-NMR of inhibitor candidate **4r** (500 MHz, acetone-d6).

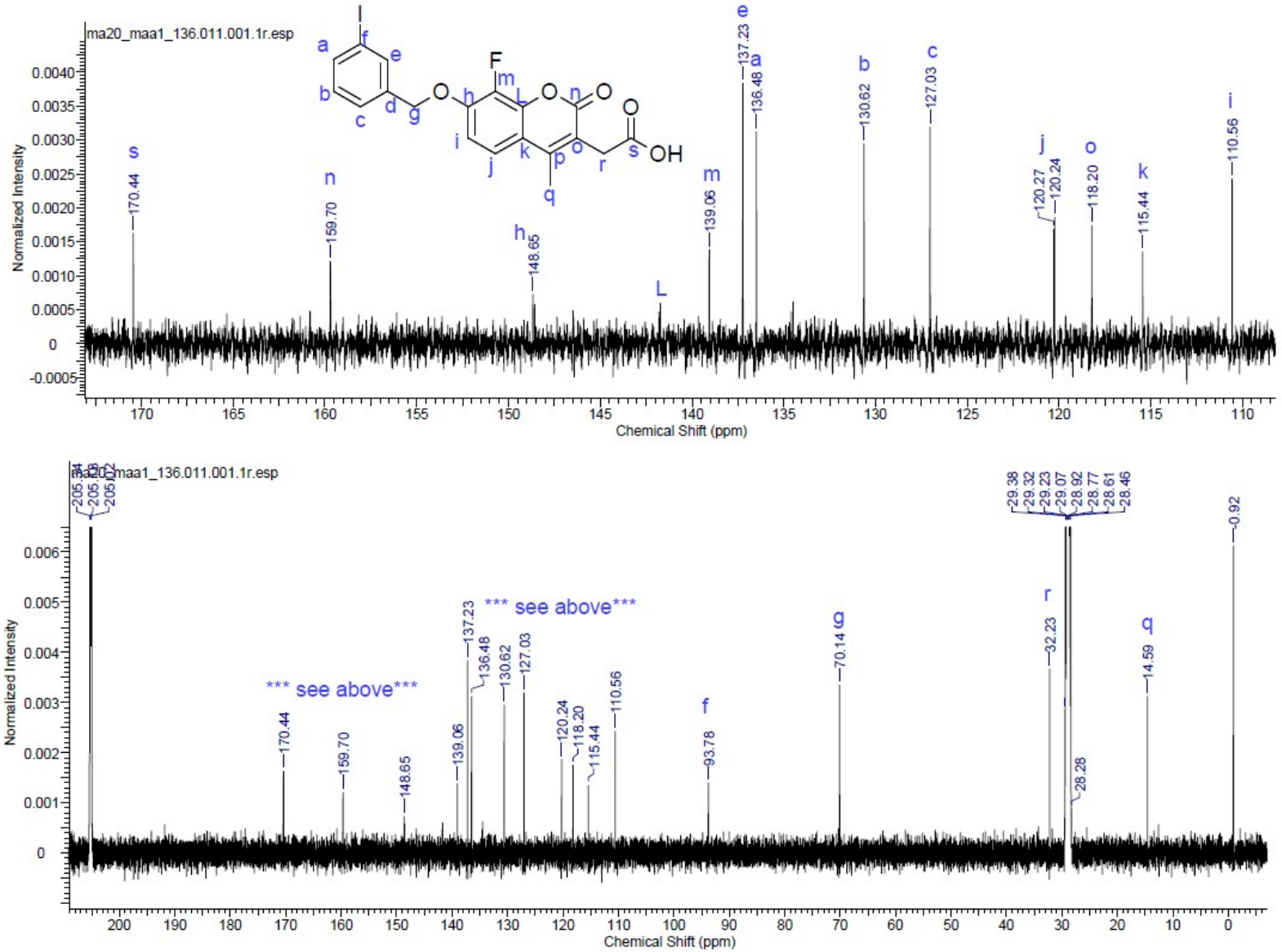


Figure S24. ^{13}C-NMR of inhibitor candidate **4r** (125 MHz, acetone-d6).

References

(1) Lee, S.; Cil, O.; Haggie, P. M.; Verkman, A. S. 4,8-Dimethylcoumarin Inhibitors of Intestinal Anion Exchanger Slc26a3 (Downregulated in Adenoma) for Anti-Absorptive Therapy of Constipation. *J. Med. Chem.* **2019**, *62* (17), 8330–8337. https://doi.org/10.1021/acs.jmedchem.9b01192.

(2) Haggie, P. M.; Cil, O.; Lee, S.; Tan, J.-A.; Rivera, A. A.; Phuan, P.-W.; Verkman, A. S. SLC26A3 Inhibitor Identified in Small Molecule Screen Blocks Colonic Fluid Absorption and Reduces Constipation. *JCI Insight* **2018**, *3* (14), 121370. https://doi.org/10.1172/jci.insight.121370.