Supplementary Material for:

Synthesis, structure-activity relationship and binding mode analysis of 4-thiazolidinone derivatives as novel inhibitors of human dihydroorotate dehydrogenase

Fanxun Zeng,[a#] Tiantian Qi,[b#] Chunyan Li,[a] Tingfang Li,[a] Honglin Li,[b] Shiliang Li,[ab*] Lili Zhu[b*] and Xiaoyong Xu[ac*]

[a] *Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China*

[b] *Shanghai Key Laboratory of New Drug Design, State Key Laboratory of Bioreactor Engineering, School of Pharmacy, East China University of Science & Technology, Shanghai 200237, China*

[c] *Shanghai Collaborative Innovation Center for Biomanufacturing Technology, 130 Meilong Road, Shanghai 200237, China*

[#] Authors contributed equally to this work

* Corresponding author. Tel.: +86-21-64250213; fax: +86-21-64250213. *E-mail:* slli403@163.com.

* Corresponding author. Tel.: +86-21-64253379; fax: +86-21-64250213. *E-mail:* zhulfl@ecust.edu.cn.

* Corresponding author. Tel.: +86-21-64252945; fax: +86-21-64252603. *E-mail:* xyxu@ecust.edu.cn.

Table of Contents

Figure S1

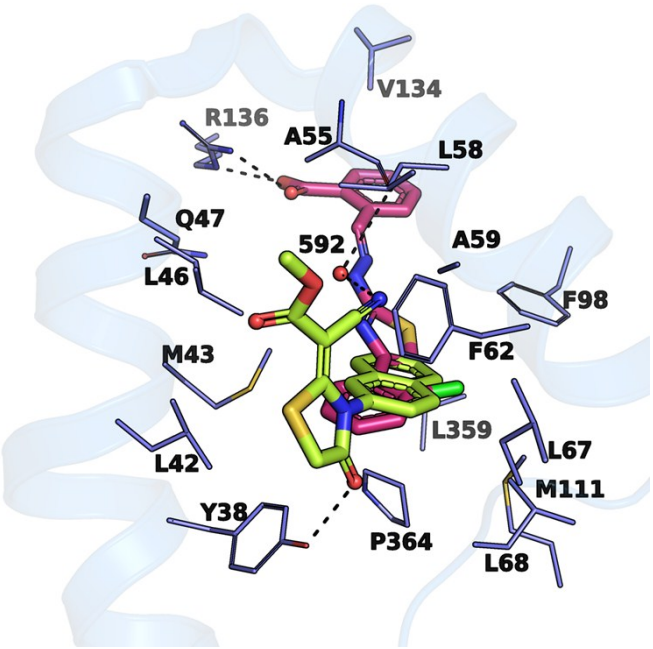


Figure S1. Comparison of the binding difference between the representative compound **31** and the reported DHODH inhibitor in the X-ray crystal structure of 4LS1. Compound **31** is presented as yellow-green sticks, while the ligand of 4LS1 is shown in pink. Key residues are shown as thin blue sticks labeled with residue numbers. Hydrogen bonds are displayed as black dashes. Which are necessary for maintaining the bioactivities of the compounds in this series

Materials and General Methods

Unless otherwise noted, reagents and solvents were obtained from commercial sources and used without further purification. Melting points (Mp) were recorded on Büchi B540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. ^{1}H NMR, ^{19}F NMR and ^{13}C NMR spectra were recorded on Bruker AM-400 (^{1}H at 400 MHz, ^{13}C at 100 MHz, ^{19}F at 376 MHz) spectrometer with DMSO-d_6 or $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are reported in δ (parts per million). High-resolution electron mass spectra (ESI-TOF) were performed on a Micromass LC-TOF spectrometer. High Resolution Mass Spectrometry (HRMS) EI were recorded under electron impact (70 eV) condition using a MicroMass GCT CA

055 instrument. Analytical thin-layer chromatography (TLC) was performed on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, coupling constant (Hz) and integration.

In Vitro Enzyme Assay

Expression and purification of recombined *h*DHODH were followed the protocols of previously published literatures.[1-2] The DHODH inhibition assays were carried out using the DCIP-based assay.[2-3] The assay buffer contained 50 mM HEPES pH 8.0, 150 mM KCl, 0.1% Triton X-100, 100 μM UQ_0 and 120 μM DCIP. The dihydroorotate was added to a final concentration of 500 μM to initiate the reaction. Brequinar was measured as the positive control. Inhibition rate was calculated from $(1-V_i/V_0) \times 100$. For the determination of the IC_{50} values, eight to nine different concentrations were applied. Each inhibitor concentration point was tested in triplicate. IC_{50} values were calculated using the sigmoidal fitting option of the program Origin 8.0.

General Procedure for the Synthesis of Intermediates

Synthesis of aryl isothiocyanates

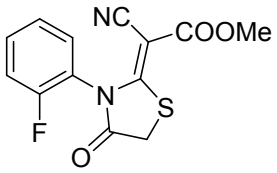
A mixture of DABCO (15 mmol), aromatic amine (5 mmol), and carbon disulfide (25 mL) in acetone (5 mL) was stirred overnight at room temperature. The precipitated solid was filtered. To a mixture of the solid and chloroform (20 mL) at 0 °C, was added dropwise a solution of triphosgene (2 mmol) in chloroform (10 mL) over 30 min. The reaction mixture was allowed to warm to room temperature and stirred overnight. After the resulting mixture was filtered, the filtrate was concentrated under reduced pressure and purified by column chromatography (100% PE) in 70%-95% yield as white solid or colorless oil.

Synthesis of 4-isothiocyanatobenzoic acid

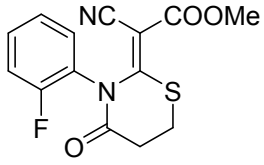
4-Aminobenzoic acid (5mmol) was slowly added to a solution of TCDI (6 mmol) and TEA (5.5 mmol) in DCM (7.5 mL) at 0 °C. The mixture was stirred for 2h at 0 °C and then added dropwise to 4M aqueous HCl (9 mL). The precipitation was filtered and washed with 1M aqueous HCl (1 mL×2). The resulting sold was dried to afford 4-isothiocyanatobenzoic acid in yield of 90%.

General Procedure for Target Compounds 5-11 and 13-31

Appropriate active methylene compound (2 mmol) followed by a solution of aryl isothiocyanate (2 mmol) in anhydrous DMF (2 mL) were added to a cold suspension of powdered KOH (4 mmol) in dry DMF (2 mL). The mixture was stirred at room temperature for 0.5 h, then cooled again to 0°C, treated with a solution of corresponding halogenated compound (3 mmol) in anhydrous DMF (2 mL) and stirred at room temperature overnight. The mixture was poured into ice-cold water, and the resulting precipitate was filtered off, dried, and crystallized from DCM-EtOH to give compounds **5-11 and 13-31** in yield of 48%–80%.

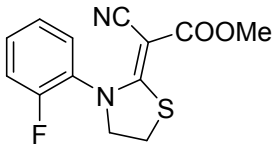


methyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (5): Mp 169.3-169.5 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.65–7.55 (m, 2H), 7.43 (t, J = 9.0 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 4.20 (ABq, J_{gem} = 18.8 Hz, 2H), 3.73 (s, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -122.55 – -122.67 (m). HRMS (ES+) calcd for $C_{13}H_9FN_2O_3S$ $(M+Na)^+$, 315.0216; found, 315.0218.

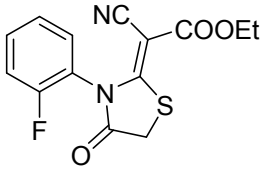


methyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxo-1,3-thiazinan-2-ylidene)acetate (6): Mp 222.0-222.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (dd, J_1 = 13.2 Hz, J_2 =

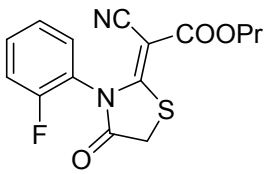
7.2 Hz, 1H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.31–7.19 (m, 2H), 3.80 (s, 3H), 3.23–3.17 (m, 2H), 3.15–3.10 (m, 2H). ^{19}F NMR (376 MHz, $CDCl_3$): δ -116.13 – -116.15 (m). HRMS (ES+) calcd for $C_{14}H_{12}N_2O_3FS$ $(M+H)^+$, 307.0553; found, 307.0554.



methyl (Z)-2-cyano-2-(3-(2-fluorophenyl)thiazolidin-2-ylidene)acetate (7): Mp 153.0-153.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.41 (m, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 9.2 Hz, 1H), 4.20–4.09 (m, 2H), 3.75 (s, 3H), 3.27 (t, *J* = 7.6 Hz, 2H). ^{19}F NMR (376 MHz, $CDCl_3$): δ -120.52 – -120.57 (m). HRMS (ES+) calcd for $C_{13}H_{11}N_2O_2FS$ $(M+Na)^+$, 301.0423; found, 301.0430.

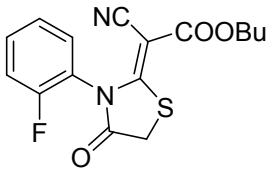


ethyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (8): Mp 137.2-137.8 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.66–7.55 (m, 2H), 7.43 (t, *J* = 9.2 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 4.20 (ABq, J_{gem} = 18.8 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 1.20 (t, *J* = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -122.55 – -122.73 (m). HRMS (ES+) calcd for $C_{14}H_{11}FN_2O_3S$ $(M+Na)^+$, 329.0372; found, 329.0371.

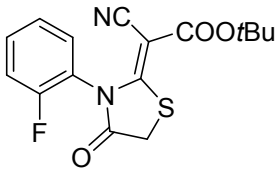


propyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (9): Mp 131.9-132.1 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.65–7.55 (m, 2H), 7.43 (t, *J* = 9.0 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 4.20 (ABq, J_{gem} = 18.4 Hz, 2H), 4.10 (d, *J* =

7.2 Hz, 2H), 1.63–1.55 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -122.62 – -22.70 (m). HRMS (ES+) calcd for $C_{15}H_{13}FN_2O_3S$ $(M+Na)^+$, 343.0529; found, 343.0533.



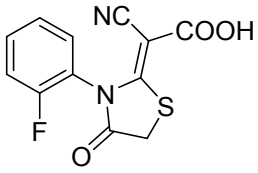
butyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (10): Mp 128.6-128.9 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.65–7.55 (m, 2H), 7.43 (t, J = 9.0 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 4.19 (ABq, J_{gem} = 18.4 Hz, 2H), 4.15 (t, J = 7.2 Hz, 2H), 1.59–1.52 (m, 2H), 1.35–1.26 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -122.63 – -122.71 (m). HRMS (ES+) calcd for $C_{16}H_{15}FN_2O_3S$ $(M+Na)^+$, 357.0685; found, 357.0690.



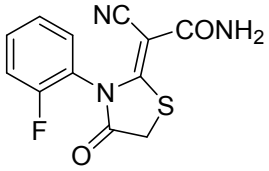
tert-butyl (Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (11): Mp 200.9-201.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61–7.55 (m, 1H), 7.34–7.25 (m, 3H), 3.88 (ABq, J_{gem} = 18.4 Hz, 2H), 1.50 (s, 9H). ^{19}F NMR (376 MHz, $CDCl_3$): δ -120.87 – -120.93 (m). HRMS (ES-) calcd for $C_{16}H_{14}N_2O_3FS$ $(M-H)^-$, 333.0709; found, 333.0708.

Synthesis of Compound 12

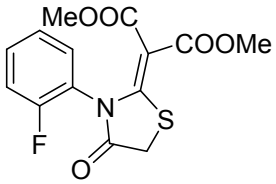
To a solution of compound **11** (1 mmol) in DCM (10 mL) was added a mixture of TFA (1.5 mL) and DCM (5 mL). The mixture was stirred at room temperature until the reaction was complete as indicated by TLC. The solvent was evaporated under reduced pressure. The residual solid was further crystallized from DCM-MeOH to afford the compound **12** in yield of 85%.



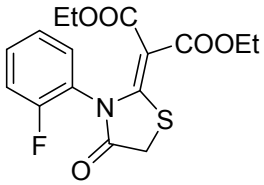
(Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetic acid (12): Mp 228.1-228.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.32 (s, 1H), 7.65–7.54 (m, 2H), 7.42 (t, J = 8.8 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 4.14 (ABq, J_{gem} = 18.8 Hz, 2H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -122.69 – -122.75 (m). HRMS (ES-) calcd for $C_{12}H_6N_2O_3FS$ $(M-H)^-$, 277.0083; found, 277.0087.



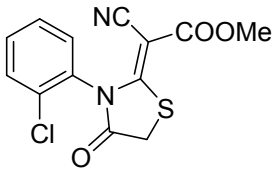
(Z)-2-cyano-2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetamide (13): Mp 182.5-182.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68–6.87 (m, 6H), 4.04 (ABq, J_{gem} = 18.0 Hz, 2H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -122.69 (s). HRMS (ES+) calcd for $C_{12}H_8FN_3O_2S(M+Na)^+$, 300.0219; found, 300.0220.



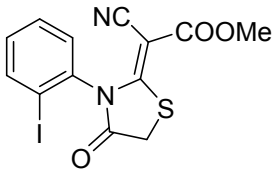
dimethyl 2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)malonate (14): Mp 168.4-169.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58–7.52 (m, 1H), 7.41–7.34 (m, 2H), 7.32–7.28 (m, 1H), 4.10 (ABq, J_{gem} = 18.0 Hz, 2H), 3.63 (s, 3H), 2.98 (s, 3H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -119.61 – -119.68 (m). HRMS (ES+) calcd for $C_{14}H_{12}NO_5FS$ $(M+Na)^+$, 348.0318; found, 348.0322.



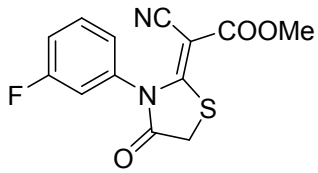
diethyl 2-(3-(2-fluorophenyl)-4-oxothiazolidin-2-ylidene)malonate (15): Mp 151.4-152.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.43 (m, 1H), 7.26–7.18 (m, 3H), 4.20 (q, J = 7.2 Hz, 2H), 3.85 (ABq, J_{gem} = 17.6 Hz, 2H), 3.52–3.35 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H), 1.08 (t, J = 7.2 Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$): δ -117.80 – -117.88 (m). HRMS (ES+) calcd for $C_{16}H_{16}NO_5FS$ $(M+Na)^+$, 376.0631; found, 376.0624.



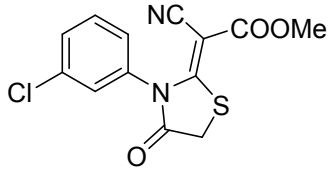
methyl (Z)-2-(3-(2-chlorophenyl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (16): Mp 158.2-158.3 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.67–7.57 (m, 3H), 7.50 (td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 4.22 (ABq, J_{gem} = 18.8 Hz, 2H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.19, 171.63, 165.62, 133.31, 133.24, 132.41, 132.18, 130.28, 128.85, 112.28, 76.40, 53.02, 32.28. HRMS (ES+) calcd for $C_{13}H_9ClN_2O_3S$ $(M+Na)^+$, 330.9920; found, 330.9927.



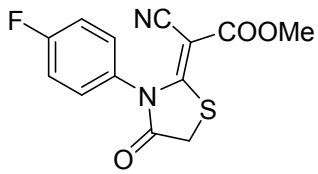
methyl (Z)-2-cyano-2-(3-(2-iodophenyl)-4-oxothiazolidin-2-ylidene)acetate (17): Mp 201.4-201.9 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.98 (d, J = 8.0 Hz, 1H), 7.56 (q, J = 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 4.20 (ABq, J_{gem} = 18.8 Hz, 2H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 173.06, 171.27, 165.72, 139.46, 137.68, 133.03, 131.45, 129.93, 112.29, 101.13, 76.62, 53.03, 32.47. HRMS (ES+) calcd for $C_{13}H_9IN_2O_3S$ $(M+Na)^+$, 400.9412; found, 400.9455.



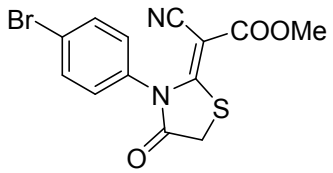
methyl (Z)-2-cyano-2-(3-(3-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (18): Mp 179.3-179.5 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.59–7.53 (m, 1H), 7.41 (t, J = 8.0 Hz, 2H), 7.30 (d, J = 7.6 Hz, 1H), 4.13–3.99 (m, 2H), 3.71 (s, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -111.83 (dt, J_1 = 10.2 Hz, J_2 = 2.6 Hz). HRMS (ES+) calcd for $C_{13}H_9FN_2O_3S$ $(M+Na)^+$, 315.0216; found, 315.0217.



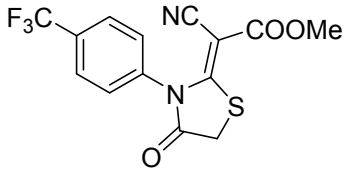
methyl (Z)-2-(3-(3-chlorophenyl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (19): Mp 194.3-195.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.67–7.41 (m, 4H), 4.06 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.78, 172.86, 165.82, 136.51, 133.81, 131.32, 131.11, 129.90, 128.89, 112.88, 76.24, 52.89, 32.67. HRMS (ES+) calcd for $C_{13}H_9ClN_2O_3S$ $(M+Na)^+$, 330.9920; found, 330.9925.



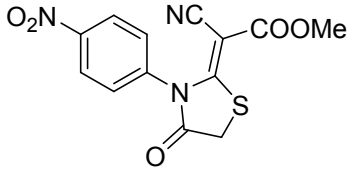
methyl (Z)-2-cyano-2-(3-(4-fluorophenyl)-4-oxothiazolidin-2-ylidene)acetate (20): Mp 188.4-188.8 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.48 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 2H), 7.34 (t, J = 8.0 Hz, 2H), 4.05 (s, 2H), 3.71 (s, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -110.70 – -110.77 (m). HRMS (ES+) calcd for $C_{13}H_9FN_2O_3S$ $(M+Na)^+$, 315.0216; found, 315.0216.



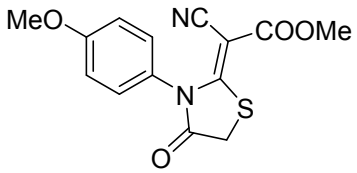
methyl (Z)-2-(3-(4-bromophenyl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (21): Mp 210.4-211.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, J = 7.2 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H), 4.06 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.81, 172.97, 165.85, 134.65, 132.83, 132.03, 124.33, 112.89, 76.20, 52.89, 32.68. HRMS (ES+) calcd for $C_{13}H_9BrN_2O_3S$ $(M+Na)^+$, 374.9415; found, 374.9422.



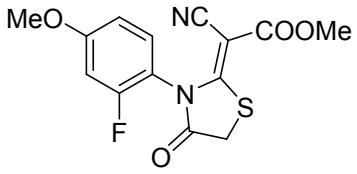
Methyl (Z)-2-cyano-2-(4-oxo-3-(4-(trifluoromethyl)phenyl)thiazolidin-2-ylidene) acetate (22): Mp 201.8-202.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92 (d, J = 7.6 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 4.09 (s, 2H), 3.72 (s, 3H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -61.18 (s). HRMS (ES+) calcd for $C_{14}HF_3N_2O_3S(M+Na)^+$, 365.0184; found, 365.0183.



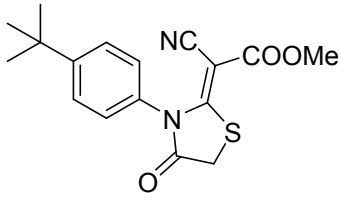
methyl (Z)-2-cyano-2-(3-(4-nitrophenyl)-4-oxothiazolidin-2-ylidene)acetate (23): Mp 189.7-190.5 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.40 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 4.09 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.68, 172.68, 165.67, 148.91, 140.96, 131.79, 125.00, 113.09, 76.23, 52.95, 32.89. HRMS (ES+) calcd for $C_{13}H_9N_3O_5S$ $(M+Na)^+$, 342.0161; found, 342.0165.



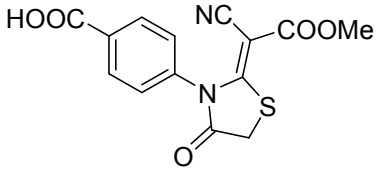
methyl (Z)-2-cyano-2-(3-(4-methoxyphenyl)-4-oxothiazolidin-2-ylidene)acetate (24): Mp 128.3-129.7 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.30 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 4.04 (s, 2H), 3.80 (s, 3H), 3.70 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.01, 173.67, 166.02, 161.09, 131.00, 127.81, 114.91, 112.72, 76.25, 55.91, 52.81, 32.51. HRMS (ES+) calcd for $C_{14}H_{12}N_2O_4S$ $(M+H)^+$, 305.0597; found, 305.0597.



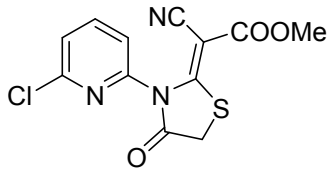
methyl (Z)-2-cyano-2-(3-(2-fluoro-4-methoxyphenyl)-4-oxothiazolidin-2-ylidene) acetate (25): Mp 145.6-145.9 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.44 (t, J = 8.8 Hz, 1H), 7.05 (dd, J_1 = 12.0 Hz, J_2 = 2.8 Hz, 1H), 6.91 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, Hz, 1H), 4.17 (ABq, J_{gem} = 18.8 Hz, 2H), 3.83 (s, 3H), 3.73 (s, 3H). ^{19}F NMR (376 MHz, DMSO-*d6*): δ -120.26 (t, J = 11.6 Hz). HRMS (ES+) calcd for $C_{14}H_{11}FN_2O_4S$ $(M+Na)^+$, 345.0322; found, 345.0321.



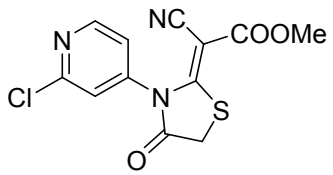
methyl (Z)-2-(3-(4-(tert-butyl)phenyl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (26): Mp 207.7-208.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.50 (dt, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 7.29 (dt, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 4.06 (s, 2H), 3.69 (s, 3H), 1.30 (s, 9H). ^{13}C NMR (100 MHz, DMSO): δ 173.46, 172.71, 165.50, 153.14, 132.18, 128.69, 126.02, 111.95, 75.91, 52.30, 34.56, 32.08, 30.99. HRMS (ES+) calcd for $C_{17}H_{18}N_2O_3S$ $(M+K)^+$, 369.0675; found, 369.0670.



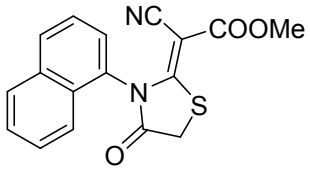
(Z)-4-(2-(1-cyano-2-methoxy-2-oxoethylidene)-4-oxothiazolidin-3-yl)benzoic acid (27): Mp 290.1-290.7. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 13.28 (s, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 4.08 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 173.30, 172.30, 166.57, 165.31, 138.53, 132.45, 130.22, 129.71, 112.32, 75.76, 52.38, 32.27. HRMS (EI) calc. for $C_{12}H_8N_2O_3S^+$ 318.0310; found 318.0312.



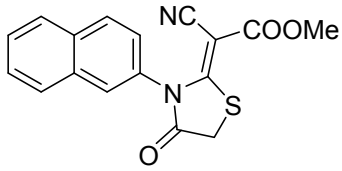
methyl (Z)-2-(3-(6-chloropyridin-2-yl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (28): Mp 158.9-159.4 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.61 (d, J = 5.2 Hz, 1H), 8.11 (d, J = 5.8 Hz, 1H), 7.34 (t, J = 5.2 Hz, 1H), 4.30 (d, J = 2.0 Hz, 2H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.58, 171.96, 163.21, 150.67, 149.29, 143.36, 120.47, 119.75, 113.11, 75.30, 52.87, 30.85. HRMS (ES+) calcd for $C_{12}H_9ClN_3O_3S$ $(M+H)^+$, 310.0053; found, 310.0045.



methyl (Z)-2-(3-(2-chloropyridin-4-yl)-4-oxothiazolidin-2-ylidene)-2-cyanoacetate (29): Mp 159.8-160.3°C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.64 (d, J = 5.2 Hz, 1H), 7.81 (s, 1H), 7.63 (d, J = 5.2 Hz, 1H), 4.10 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 173.33, 171.83, 165.53, 151.69, 151.19, 145.33, 125.57, 124.65, 113.21, 76.31, 52.97, 32.85. HRMS (ES+) calcd for $C_{12}H_9ClN_3O_3S$ $(M+H)^+$, 310.0053; found, 310.0044.



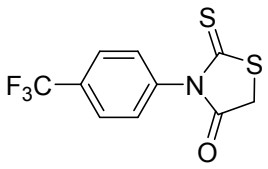
methyl (Z)-2-cyano-2-(3-(naphthalen-1-yl)-4-oxothiazolidin-2-ylidene)acetate (30): Mp 184.4-184.8 °C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.15 (d, J = 8.0 Hz, 1H), 8.08 (dd, J_1 = 5.4, J_2 = 4.0 Hz, 1H), 7.79–7.54 (m, 5H), 4.27 (ABq, J = 18.4 Hz, 2H), 3.67 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.07, 173.09, 165.82, 134.07, 131.68, 131.59, 130.92, 129.14, 128.63, 128.12, 127.15, 125.84, 121.95, 112.56, 76.45, 52.82, 32.79. HRMS (ES+) calcd for $C_{17}H_{13}N_2O_3S$ $(M+H)^+$, 325.0647; found, 325.0643.



methyl (Z)-2-cyano-2-(3-(naphthalen-2-yl)-4-oxothiazolidin-2-ylidene)acetate (31): Mp 269.2-270.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06–7.98 (m, 4H), 7.62 (pd, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 2H), 7.49 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1H), 4.13 (s, 2H), 3.70 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 173.65, 172.72, 165.42, 133.42, 132.58, 132.27, 129.18, 128.58, 128.38, 127.83, 127.42, 126.77, 126.34, 112.37, 75.91, 52.34, 32.22. HRMS (ES-) calcd for $C_{17}H_{11}N_2O_3S$ $(M-H)^-$, 323.0490; found, 323.0488.

Synthesis of Compound 32

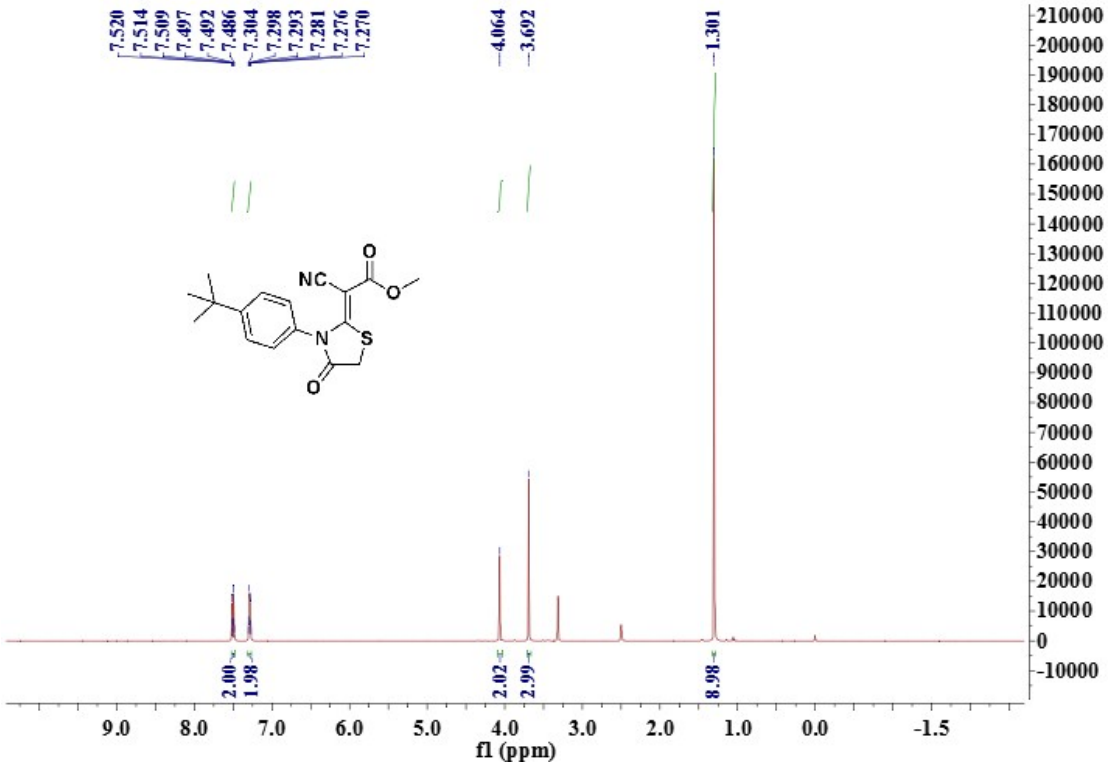
To a stirred solution of 2-mercaptoacetic acid (2 mmol) in 1, 4-dioxane (4 ml) was added triethylamine (2 mmol), isothiocyanate (2 mmol) at 0 °C. The mixture was heated to reflux for 24h. Then the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography to afford the pure product **32** in yield of 72%.



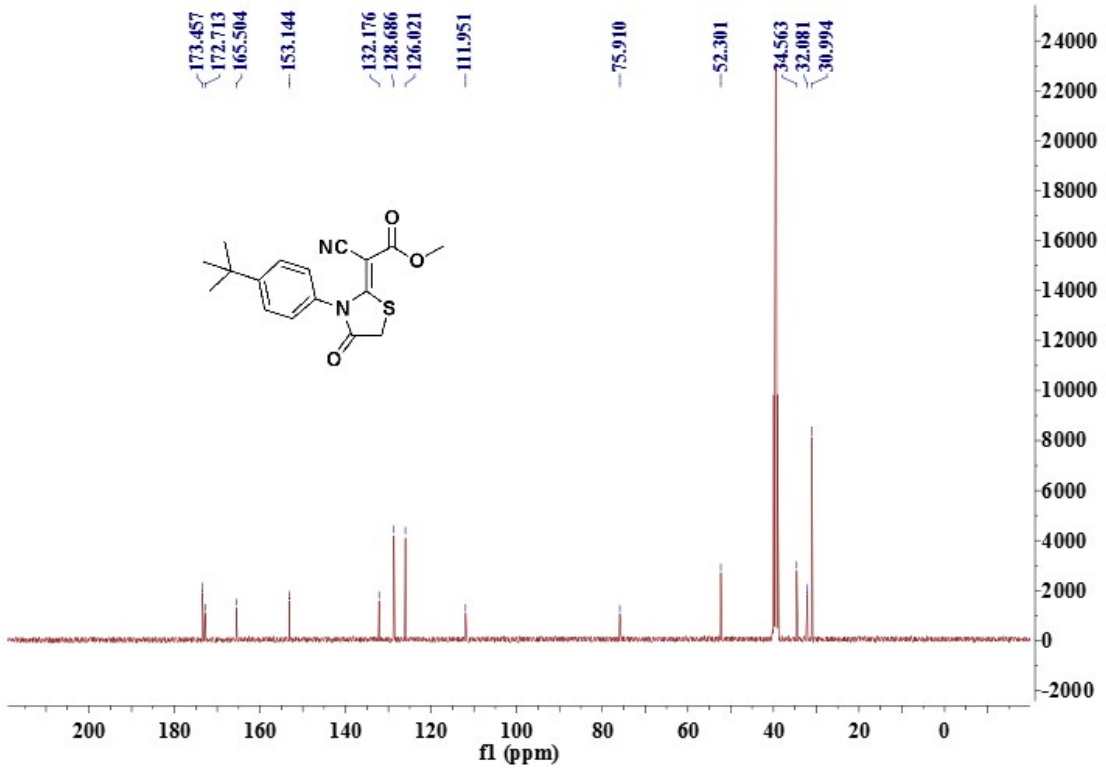
2-thioxo-3-(4-(trifluoromethyl)phenyl)thiazolidin-4-one (**32**)**:** Mp 140.7-141.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 4.40 (s, 2H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -61.16 (s). HRMS (EI) calc. for $C_{10}H_6F_3NOS_2^+$ 276.9843; found 276.9842.

^{1}H NMR, ^{13}C NMR and HRMS Spectra for Compounds 26 and 31

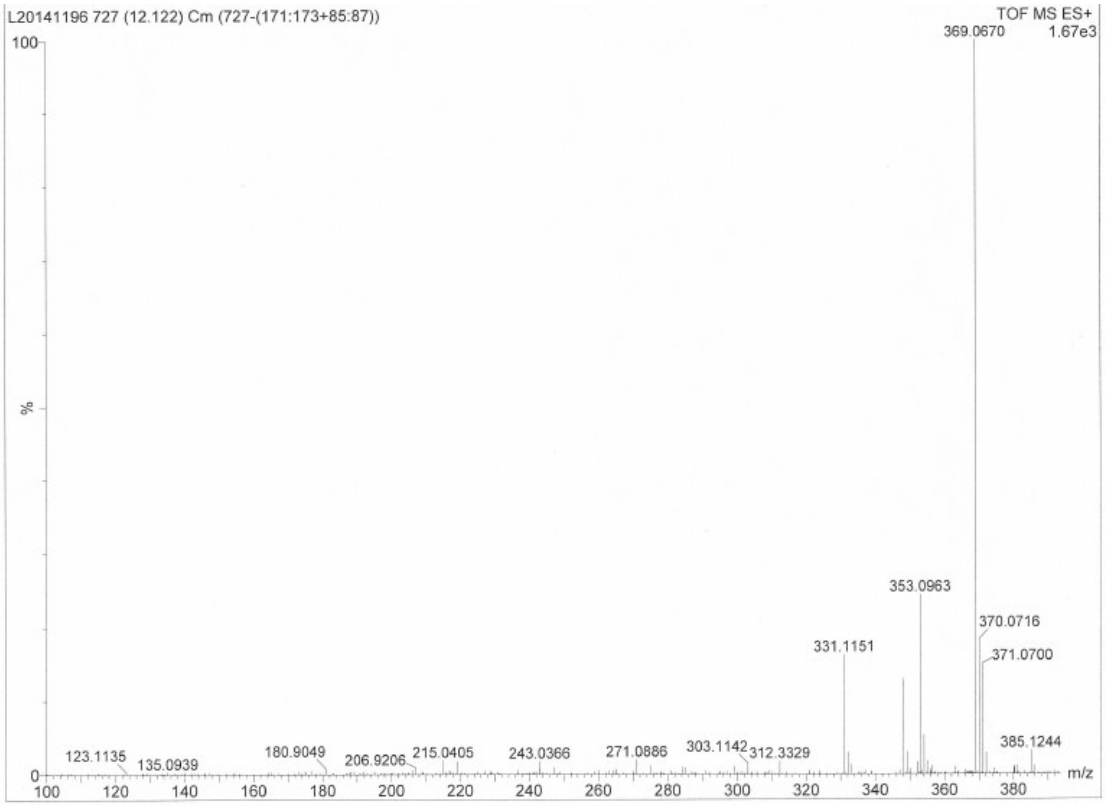
^{1}H NMR spectra for compound **26**



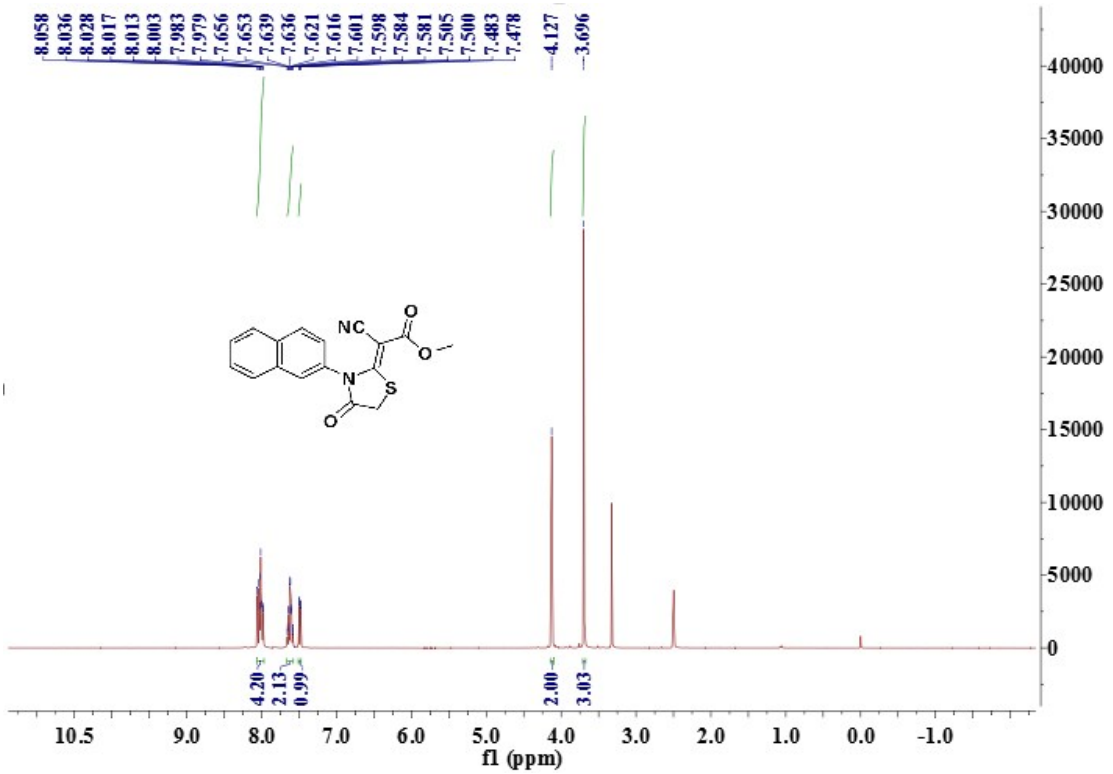
^{13}C NMR spectra for compound **26**



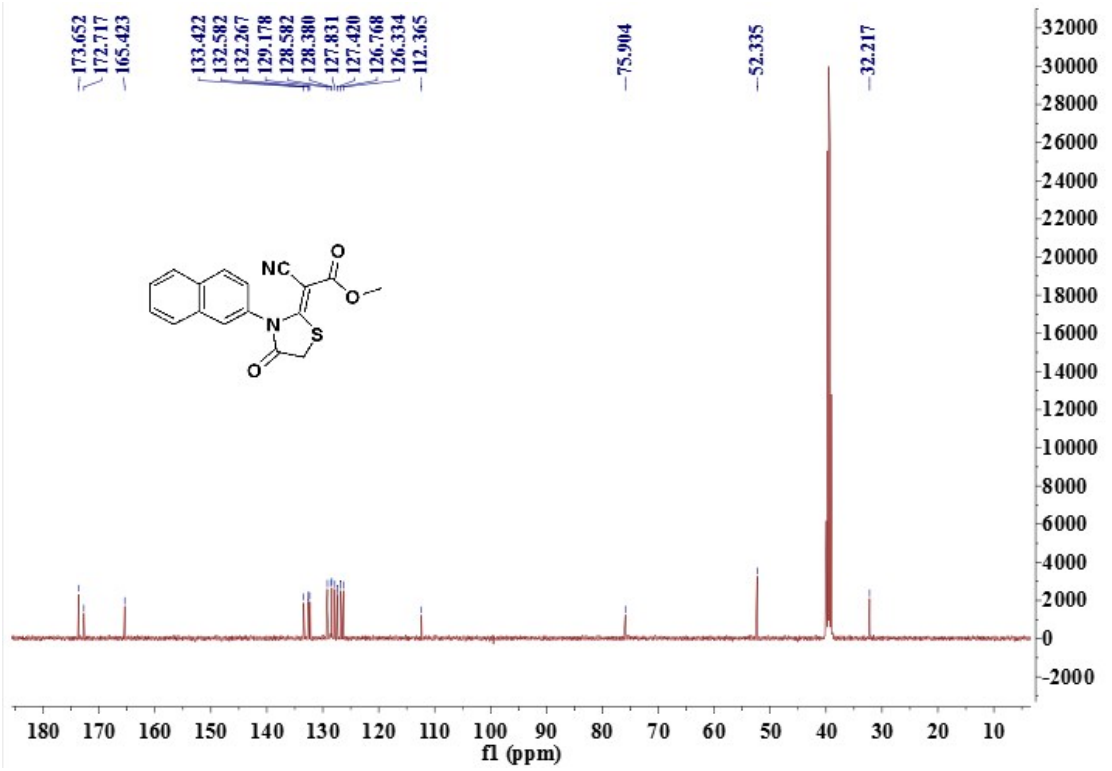
HRMS spectra for compound **26**



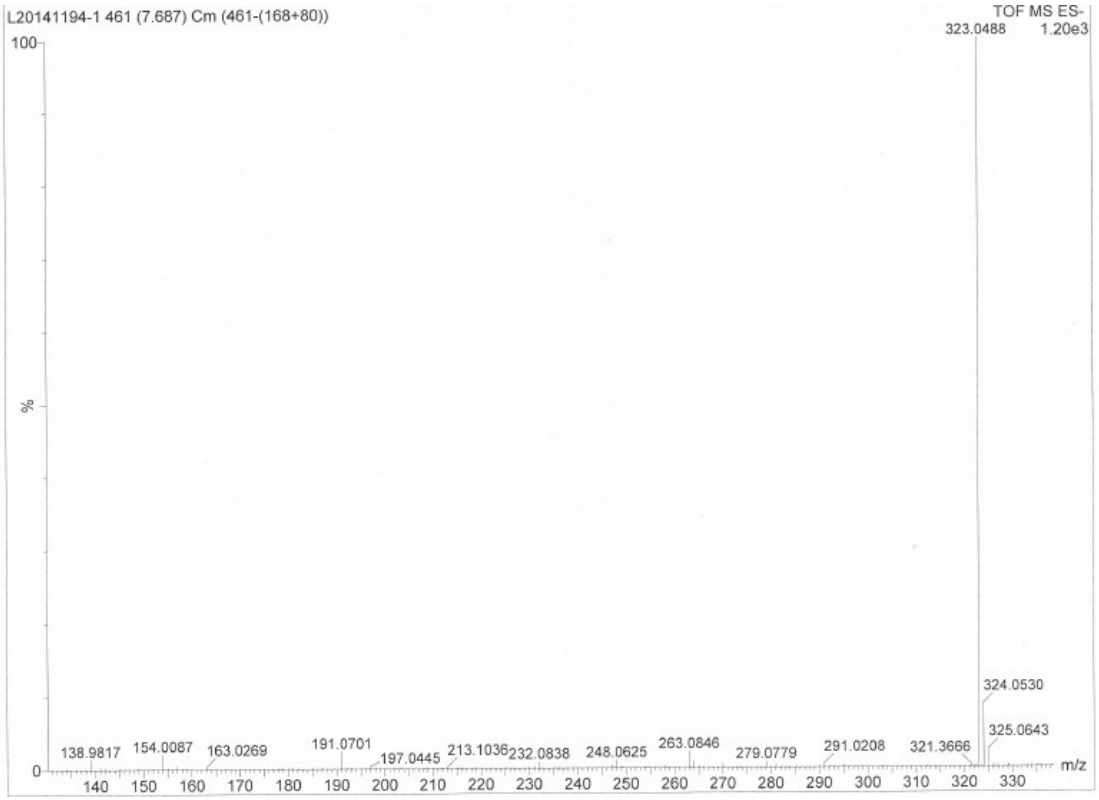
^{1}H NMR spectra for compound **31**



^{13}C NMR spectra for compound **31**



HRMS spectra for compound **31**



Reference:

1. Liu, S.; Neidhardt, E. A.; Grossman, T. H.; Ocain, T.; Clardy, J. *Structure* **2000**, *8*, 25.
2. Zhu, J.; Han, L.; Diao, Y.; Ren, X.; Xu, M.; Xu, L.; Li, S.; Li, Q.; Dong, D.; Huang, J. *J. Med. Chem.* **2015**, *58*, 1123.
3. Li, S.; Luan, G.; Ren, X.; Song, W.; Xu, L.; Xu, M.; Zhu, J.; Dong, D.; Diao, Y.; Liu, X.; Zhu, L.; Wang, R.; Zhao, Z.; Xu, Y.; Li, H. *Sci. Rep.* **2015**, *5*, 14836.