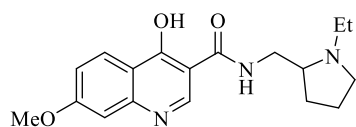


**Malonate-functionalised 4-hydroxyquinolin-2(1*H*)-ones:
a new subset of quinolinone derivatives**

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Nikolai D. Dagaev, Anton R. Izzi, Dmitry A. Skvortsov, Yuri K. Grishin,
Roman L. Antipin and Elena K. Beloglazkina**

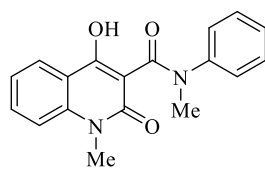
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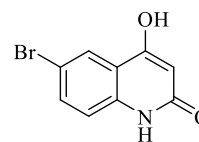
H1521

ameliorates the symptoms of lupus and prevents the development of glomerulonephritis
Int. Immunopharmacol. 2011, **11** (4), 435–443 [11]

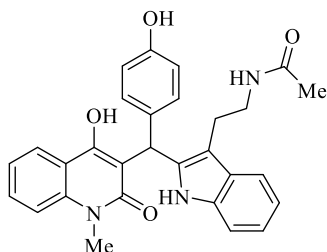


Linomide

immunostimulant, angiogenesis inhibitor

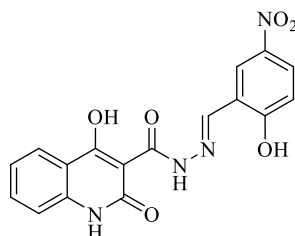


Neuronal Nitric Oxide Synthase (nNOS) selective Inhibitor
Chem. Pharm. Bull. 2001, **49** (10), 1372–1374 [10]

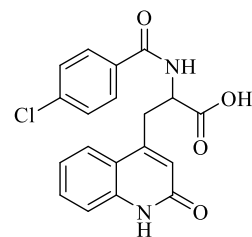


SF 2809 II

chymase and cathepsin G inhibitor
J. Antibiot. 2004, **57** (2), 83–88 [9]

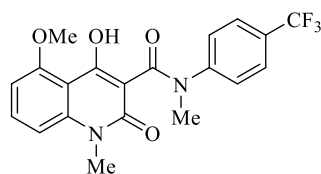


α -glucosidase inhibitor
Sci. Rep. 2023, **13**, 6304 [8]



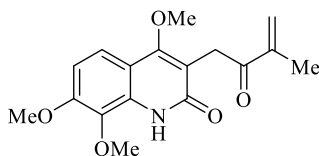
Rebamipide

mucosal protection, gastroduodenal ulcer healing, gastritis treatment



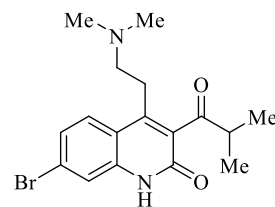
Tasquinimod

angiogenesis and metastasis inhibitor, immunomodulator



Dasycarine E

anti-inflammatory
Phytochemistry 2020, **172**, 112260 [7]



Flustramine Q

acetylcholinesterase inhibitor
Future Pharmacol. 2023, **3** (1), 38–47 [6]

Figure S1. Selected examples of biologically active quinolin-2(1H)-one derivatives

Synthesis

Control over the course of reactions and purity of chromatographic separation of substances was carried out by thin layer chromatography (TLC) on Merck TLC Silica gel 60 F254 plates. MN Kieselgel 60 0.04-0.063 mm/230-400 mesh ASTM silica gel was used for preparative chromatography. UV lamp (254, 365 nm), potassium permanganate solution, ninhydrin were used to show the stains.

^1H , ^{13}C NMR spectra were recorded on a Bruker Avance-400 instrument with operating frequencies of 400, 100 MHz. CDCl_3 , $\text{DMSO}-d_6$ were used as solvents. Chemical shifts are given in parts per million (ppm) on the δ scale and are calibrated on the residual peak of the deuterated solvent. The coupling constants J are given in Hertz [Hz]. The following abbreviations were used for the allocation of signal multiplicities: bs – broad singlet, s – singlet, d – doublet, dd – doublet of doublets, ddd – doublet of doublets of doublets, t – triplet, dt – doublet of triplets, td – triplet of doublets, tt – triplet of triplets, q – quartet, dq – doublet of quartets, qd – quartet of doublets, sept – septet, m – multiplet.

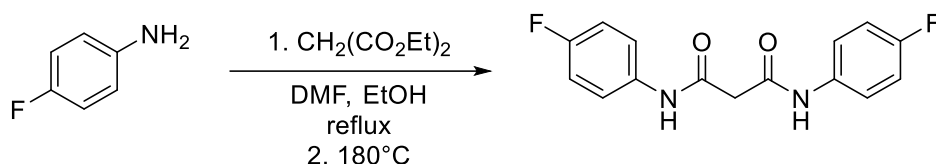
HRMS: the study was performed using a system consisting of a LC-30 Nexera liquid chromatograph (Shimadzu, Japan) equipped with a DGU-20A degasser, two LC-30AD chromatographic pumps, a CTO-20A column thermostat and an Orbitrap QExactive Plus high-resolution mass spectrometer (Thermo Scientific, USA) with a mass analyzer based on an orbital ion trap. Methanol was used as the sample solvent and mobile phase. We injected 0.2 μl of sample into a 0.2 ml/min stream of methanol without chromatographic separation directly into the ion source. The analysis time is 2 minutes. The high-resolution mass spectrometer was operated in the mode of recording positively and negatively charged ions under electrospray ionization (ESI) conditions. FullMS scanning mode. Ion source parameters: pressure of drying gas – 12, atomizing gas and curtain gas fluxes – 4 and 1 conventional units, desolvation line temperature – 320°C, voltage on the needle – 3.8 kV (ESI +) and 3.2 kV (ESI –), RF voltage on the S-lens – 55 uC. Mass spectra were recorded in the range of m/z 100–2500 with a spectral resolution value equal to 70000. Mass spectrometer control, data acquisition and primary data processing were performed using Xcalibur software (Thermo Scientific, USA).

Commercially available reagents were used without further purification. Solvents were purified and absolutized according to standard techniques.

Synthesis of compounds

Synthesis of N^1, N^3 -diphenylmalonamides

Synthesis of N^1, N^3 -bis(4-fluorophenyl)malonamide (1a)

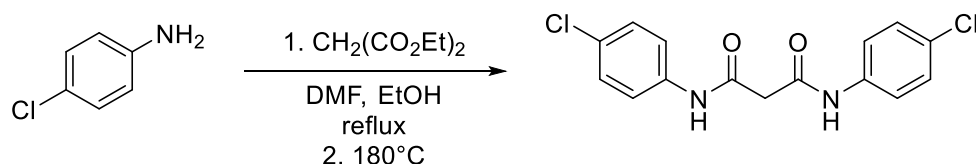


4-Fluoroaniline (4 ml, 42 mmol, 2.2 eq.), EtOH (15 ml), diethyl malonate (2.9 ml, 19 mmol, 1 eq.) and DMF (100 μ L) were placed in a round-bottomed 100 ml flask equipped with a magnetic stirrer. The reaction mixture was intensively mixed, gradually increasing the temperature in the oil bath to 180°C. First, the reaction mixture was heated with a vertical Liebig condenser installed, into which cold water was supplied. After that, the heating of the reaction mixture was continued, turning off the water supply to the Liebig condenser, thereby converting it into an air condenser. After a few hours, with a decrease in temperature, the solidification of the reaction mass and the formation of the product in the form of gray crystals were observed. Cold EtOH was added to the cooled crushed reaction mass, and precipitation of a gray product was observed. The flask was placed in the freezer for a while to complete the precipitation of the product, which was subsequently filtered, washed with a small amount of cold EtOH and dried in a drying cabinet at 100°C. As a result, a pure product was obtained in the form of a powder weighing 3.59 g with a yield of 65%.

^1H NMR (400 MHz, DMSO- d_6) δ 10.23 (s, 2H), 7.66 – 7.56 (m, 4H), 7.15 (t, J = 8.9 Hz, 4H), 3.44 (s, 2H).

The spectroscopic data correspond to those given earlier in the literature.[S1]

Synthesis of N^1, N^3 -bis(4-chlorophenyl)malonamide (1b)



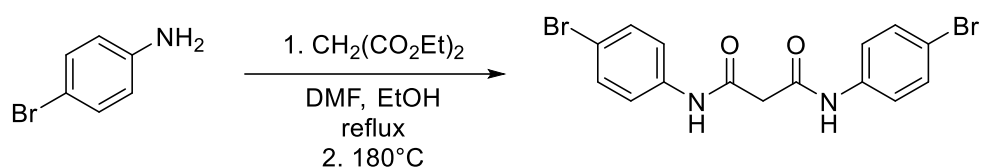
4-Chloroaniline (5.12 g, 40 mmol, 2.2 eq.), EtOH (15 ml), diethyl malonate (2.8 ml, 18 mmol, 1 eq.) and DMF (100 μ L) were placed in a 100 ml round bottom flask equipped with a magnetic stirrer. The reaction mixture was intensively mixed, gradually increasing the temperature in the oil bath to 180°C. First, the reaction mixture was heated with a vertical Liebig condenser installed, into which cold water was supplied. After that, the heating of the reaction mixture was continued, turning off

the water supply to the Liebig condenser, thereby converting it into an air condenser. After several hours, at lowering temperature, solidification of the reaction mass and formation of the product in the form of yellowish crystals were observed. Cold EtOH was added to the cooled crushed reaction mass and a light-yellow precipitate of the product was observed. The flask was placed in the freezer for a while to complete the precipitation of the product, which was subsequently filtered, washed with a small amount of cold EtOH and dried in a drying cabinet at 100°C. As a result, a pure product was obtained in the form of a powder weighing 4.31 g with a yield of 72%.

^1H NMR (400 MHz, DMSO- d_6) δ 10.32 (s, 2H), 7.69 – 7.54 (m, 4H), 7.44 – 7.28 (m, 4H), 3.46 (s, 2H).

The spectroscopic data correspond to those given earlier in the literature.[S1]

Synthesis of N^1,N^3 -bis(4-bromophenyl)malonamide (**1c**)



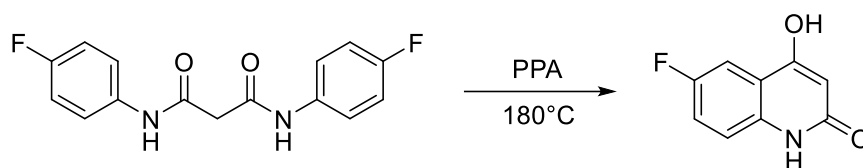
4-Bromoaniline (5.3 g, 31 mmol, 2.2 eq.), EtOH (15 ml), diethyl malonate (2.14 ml, 14 mmol, 1 eq.) and DMF (100 μ l) were placed in a 100 ml round bottom flask equipped with a magnetic stirrer. The reaction mixture was intensively mixed, gradually increasing the temperature in the oil bath to 180°C. First, the reaction mixture was heated with a vertical Liebig condenser installed, into which cold water was supplied. After that, the heating of the reaction mixture was continued, turning off the water supply to the Liebig condenser, thereby converting it into an air condenser. After a few hours, when the temperature was lowered, solidification of the reaction mass and formation of the product in the form of light gray crystals were observed. Cold EtOH was added to the cooled crushed reaction mass, and a light gray precipitate of the product was observed. The flask was placed in the freezer for a while to complete the precipitation of the product, which was subsequently filtered, washed with a small amount of cold EtOH and dried in a drying cabinet at 100°C. As a result, a pure product was obtained in the form of a powder weighing 2.98 g with a yield of 52%.

^1H NMR (400 MHz, DMSO- d_6) δ 10.31 (s, 2H), 7.57 (d, J = 9.0 Hz, 4H), 7.48 (d, J = 9.0 Hz, 4H), 3.47 (s, 2H).

The spectroscopic data are consistent with those reported earlier in the literature.[S2]

Synthesis of 4-hydroxyquinolin-2(1H)-ones

Synthesis of 6-fluoro-4-hydroxyquinolin-2(1H)-one (2a)

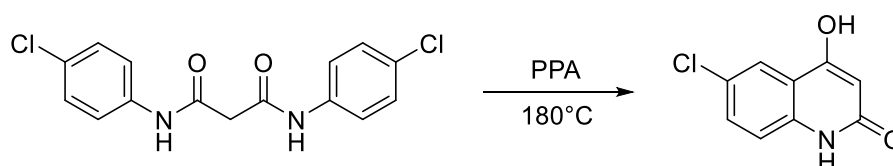


Dry powder *N*¹,*N*³-bis(4-fluorophenyl)malonamide **1a** (3.57 g, 12 mmol) was added to a 100 ml round bottom flask equipped with a magnetic stirrer. Polyphosphoric acid (PPA) was added in an amount sufficient to completely absorb the entire volume of malonamide in the flask. The resulting thick mass was left to stir vigorously under heating (oil bath temperature ~180°C) with a calcium chloride tube. After some time, ice water was added to the thick dark coloured reaction mass. The precipitated dark green precipitate was filtered, washed with water until the filtrate was neutral. The wet residue on the filter was dried in a drying cabinet at 100°C. As a result, the pure product was obtained as a powder weighing 2.09 g in quantitative yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.48 (bs, 1H), 11.30 (s, 1H), 7.45 (dd, *J* = 9.3, 2.9 Hz, 1H), 7.38 (td, *J* = 8.7, 3.0 Hz, 1H), 7.27 (dd, *J* = 9.0, 4.8 Hz, 1H), 5.77 (s, 1H).

The spectroscopic data are consistent with those reported earlier in the literature.[S1]

Synthesis of 6-chloro-4-hydroxyquinolin-2(1H)-one (2b)

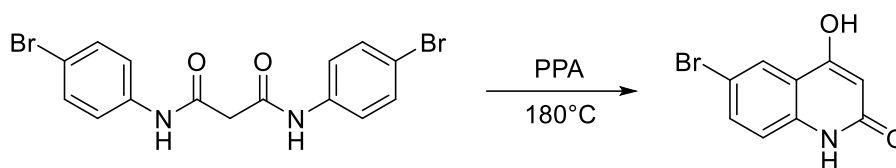


Dry powder *N*¹,*N*³-bis(4-chlorophenyl)malonamide **1b** (4.31 g, 13 mmol) was added to a 100 ml round bottom flask equipped with a magnetic stirrer. PPA was added in an amount sufficient to completely absorb the entire volume of malonamide in the flask. The resulting thick mass was left to stir vigorously under heating (oil bath temperature ~180°C) with a calcium chloride tube. After some time, ice water was added to the thick reaction mass of yellow-green colour. The precipitated light green precipitate was filtered, washed with water until the filtrate was neutral. The wet residue on the filter was dried in a drying cabinet at 100°C. As a result, the pure product was obtained as a powder weighing 2.53 g in quantitative yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.57 (bs, 1H), 11.34 (s, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.52 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 5.76 (s, 1H).

The spectroscopic data are consistent with those reported earlier in the literature.[S1]

Synthesis of 6-bromo-4-hydroxyquinolin-2(1H)-one (2c)



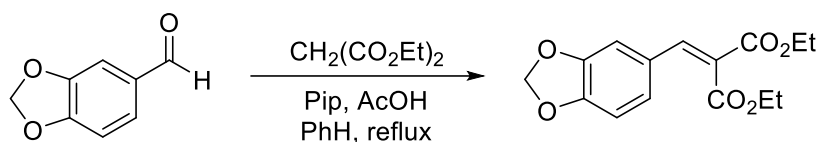
Dry powder *N*¹,*N*³-bis(4-bromophenyl)malonamide **1c** (2.4 g, 6 mmol) was added to a 100 ml round bottom flask equipped with a magnetic stirrer. PPA was added in an amount sufficient to completely absorb the entire volume of malonamide in the flask. The resulting thick mass was left to stir vigorously under heating (oil bath temperature ~180°C) with a calcium chloride tube. After some time, ice water was added to the thick reaction mass of dark green colour. The precipitated light green precipitate was filtered, washed with water until the filtrate was neutral. The wet residue on the filter was dried in a drying cabinet at 100°C. As a result, the pure product was obtained as a powder weighing 1.4 g in quantitative yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.56 (bs, 1H), 11.35 (s, 1H), 7.84 (d, *J* = 2.3 Hz, 1H), 7.63 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 5.74 (s, 1H).

The spectroscopic data are consistent with those reported earlier in the literature.[S3]

Synthesis of diethyl 2-(arylmethylene)malonates

Synthesis of diethyl 2-(benzo[d][1,3]dioxol-5-ylmethylene)malonate (3)

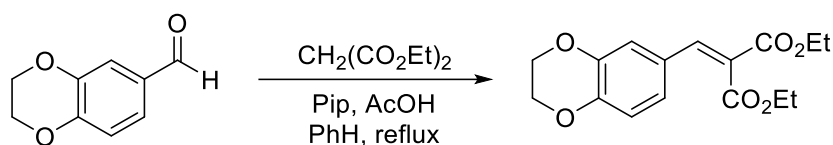


Benzo[d][1,3]dioxole-5-carbaldehyde (1.0 equiv.), diethyl malonate (1.0 equiv.), piperidine (0.05 equiv.) and acetic acid (0.05 equiv.) were dissolved in benzene and stirred at 120°C for 8 h. After cooling, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (EtOAc/hexane, 1:8) to give compound **3** as a yellow oil, with a yield of 75%.

¹H NMR (400 MHz, CDCl₃) δ 7.61 (s, 1H), 7.00 (dd, 1H, *J* = 8.1, 1.8 Hz), 6.95 (d, 1H, *J* = 1.8 Hz), 6.81 (d, 1H, *J* = 8.1 Hz), 6.01 (s, 2H), 4.36 (q, 2H, *J* = 7.1 Hz), 4.29 (q, 2H, *J* = 7.1 Hz), 1.33 (m, 6H, *J* = 7.1 Hz).

The spectroscopic data are consistent with those reported earlier in the literature.[S4]

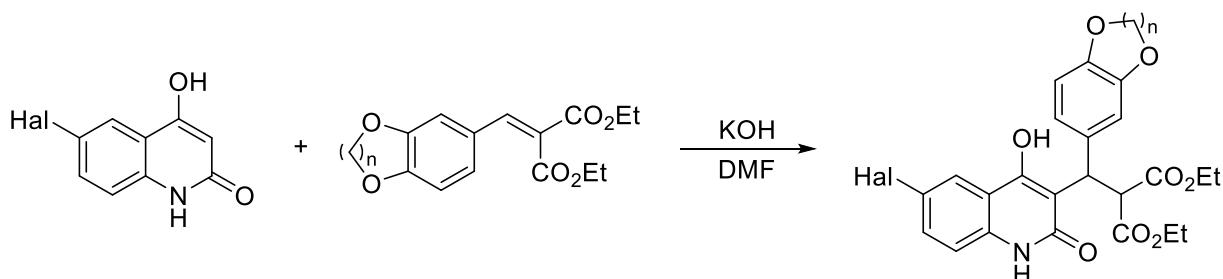
Synthesis of diethyl 2-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)methylene)malonate (**4**)



2,3-Dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde (1.0 equiv.), diethyl malonate (1.0 equiv.), piperidine (0.05 equiv.) and acetic acid (0.05 equiv.) were dissolved in benzene and stirred at 120°C for 8 h. After cooling, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (EtOAc/hexane, 1:8) to give the compound **4** as a yellow oil, with a yield of 80%.

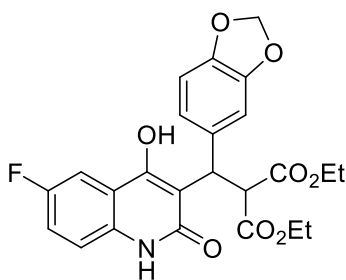
¹H NMR (400 MHz, CDCl₃) δ 7.59 (s, 1H), 7.00 (d, 1H, *J* = 2.2 Hz), 6.97 (dd, 1H, *J* = 8.4, 2.2 Hz), 6.84 (d, 1H, *J* = 8.4 Hz), 4.36 (q, 2H, *J* = 7.1 Hz), 4.30–4.23 (m, 6H), 1.33 (m, 6H, *J* = 7.1 Hz).

General procedure for the synthesis of malonates **5a–f**



Corresponding 6-halogeno-4-hydroxyquinolin-2(1*H*)-one (1.0 equiv.) was dissolved in DMF (10 ml per 1 mmol), and KOH (1.0 equiv.) was added. The mixture was stirred at room temperature for 30 min, then the corresponding malonate (1.0 equiv.) was added, and stirring was continued for 72 h. The solvent was removed under reduced pressure, and the residue was treated with 1 M HCl. The resulting precipitate was collected by filtration through a glass frit, washed with water, and purified by column chromatography (DCM/MeOH = 50:1) to afford the product.

Synthesis of diethyl ((benzo[*d*][1,3]dioxol-5-yl)(6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)methyl)malonate (**5a**)



The compound **5a** was obtained according to the general procedure in 55% yield.

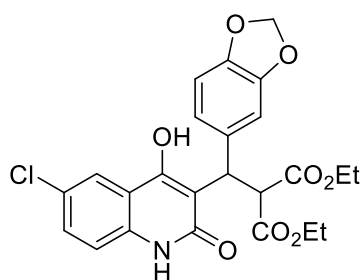
¹H NMR (400 MHz, DMSO-*d*₆) δ 11.38 (s, 1H), 10.71 (s, 1H), 7.70 (dd, 1H, *J* = 10.2, 2.8 Hz), 7.34 (td, 1H, *J* = 8.7, 2.8 Hz), 7.24 (dd, 1H, *J* = 9.0, 4.9 Hz), 7.08 (s, 1H), 6.94 (d, 1H, *J* = 8.2 Hz), 6.75 (d, 1H, *J* = 8.0 Hz), 5.91

(d, 2H, $J = 5.6, 1.0$ Hz), 5.19 (br s, 1H), 5.07 (d, 1H, $J = 12.0$ Hz), 4.02–3.86 (m, 4H), 0.96 (dt, 6H, $J = 21.2, 7.1$ Hz).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 168.2, 167.8, 162.8, 157.9 (d, $J = 237.5$ Hz), 157.1, 146.8, 145.7, 134.7, 134.4, 121.5, 118.4 (d, $J = 24.2$ Hz), 116.9 (d, $J = 7.6$ Hz), 115.8 (d, $J = 8.4$ Hz), 108.0 (d, $J = 25.0$ Hz), 107.9, 107.7, 100.7, 60.7, 60.6, 55.0, 53.4, 13.8, 13.7.

HRMS (ESI) (m/z): calcd for $\text{C}_{24}\text{H}_{22}\text{FNO}_8$ $[\text{M}+\text{H}]^+$ 472.1402, found 472.1407.

Synthesis of diethyl ((benzo[d][1,3]dioxol-5-yl)(6-chloro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)methyl)malonate (5b)



The compound **5b** was obtained according to the general procedure in 60% yield.

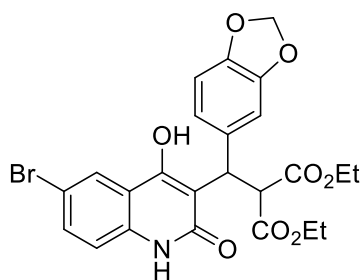
^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.47 (s, 1H), 10.83 (s, 1H), 8.10 (d, $J = 2.1$ Hz, 1H), 7.59 (dd, $J = 8.7, 2.2$ Hz, 1H), 7.16 (d, $J = 8.7$ Hz, 1H), 7.06 (s, 1H), 6.92 (d, $J = 8.1$ Hz, 1H), 6.76 (d, $J = 8.1$ Hz, 1H), 5.91 (d, $J = 5.4$

Hz, 2H), 5.15 (br s, 1H), 5.06 (d, $J = 12.1$ Hz, 1H), 4.00–3.88 (m, 4H), 0.95 (dt, $J = 21.9, 7.2$ Hz, 6H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 168.2, 167.7, 162.8, 149.6, 147.9, 146.7, 145.6, 136.4, 130.3, 126.6, 125.6, 125.3, 122.1, 116.9, 108.8, 108.1, 107.7, 100.7, 61.3, 60.8, 53.4, 43.4, 13.8, 13.7.

ESI-HRMS (m/z): calcd for $\text{C}_{24}\text{H}_{22}\text{ClNO}_8$ $[\text{M}+\text{H}]^+$ 488.1107, found 488.1103.

Synthesis of diethyl ((benzo[d][1,3]dioxol-5-yl)(6-bromo-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)methyl)malonate (5c)



The compound **5c** was obtained according to the general procedure in 58% yield.

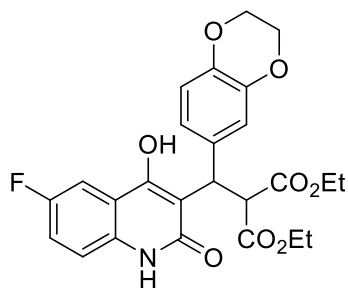
^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.47 (s, 1H), 10.83 (s, 1H), 7.96 (d, $J = 2.3$ Hz, 1H), 7.48 (dd, $J = 8.8, 2.3$ Hz, 1H), 7.22 (d, $J = 8.8$ Hz, 1H), 6.99 (s, 1H), 6.92 (d, $J = 7.9$ Hz, 1H), 6.76 (d, $J = 8.0$ Hz, 1H), 6.09 (s, 1H),

5.91 (d, $J = 5.7, 1.1$ Hz, 2H), 5.16 (br s, 1H), 5.06 (d, $J = 12.0$ Hz, 1H), 4.00–3.87 (m, 4H), 1.22 (t, $J = 7.1$ Hz, 1H), 0.95 (dt, $J = 21.8, 7.1$ Hz, 6H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 168.1, 167.7, 162.8, 146.8, 145.8, 145.7, 136.7, 134.7, 132.9, 125.1, 121.5, 117.2, 116.8, 112.9, 108.7, 108.6, 107.7, 100.7, 60.75, 60.7, 53.4, 42.5, 13.8, 13.7.

ESI-HRMS (m/z): calcd for $\text{C}_{24}\text{H}_{22}\text{BrNO}_8$ $[\text{M}+\text{H}]^+$ 532.0602, found 532.0596.

Synthesis of diethyl ((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)(6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)methyl)malonate (5d)



The compound **5d** was obtained according to the general procedure in 62% yield.

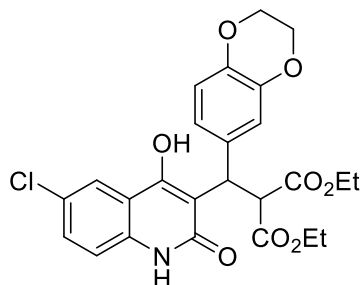
¹H NMR (400 MHz, DMSO-*d*₆) δ 11.38 (s, 1H), 10.71 (s, 1H), 7.70 (dd, *J* = 10.3, 2.8 Hz, 1H), 7.34 (td, *J* = 8.6, 2.8 Hz, 1H), 7.23 (dd, *J* = 9.0, 4.9 Hz, 1H), 6.98 (s, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 5.17

(br s, 1H), 5.03 (d, *J* = 11.9 Hz, 1H), 4.14 (s, 4H), 4.01–3.87 (m, 4H), 0.98 (t, *J* = 7.1 Hz, 3H), 0.91 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (100 MHz, DMSO-*d*₆) δ 169.1, 168.7, 163.4, 157.7 (d, *J* = 237.3 Hz), 157.8, 143.6, 142.6, 135.2, 134.8, 121.9, 119.2 (d, *J* = 24.9 Hz), 117.6 (d, *J* = 9.1 Hz), 116.7 (d, *J* = 8.3 Hz), 109.4, 109.0, 108.8 (d, *J* = 22.0 Hz), 64.9, 64.8, 61.5, 61.4, 54.1, 41.5, 14.6, 14.5.

ESI-HRMS (*m/z*) calcd for C₂₅H₂₄FNO₈ [M+H]⁺ 486.1559, found 486.1559.

Synthesis of diethyl ((6-chloro-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)methyl)malonate (5e)



The compound **5e** was obtained according to the general procedure in 54% yield.

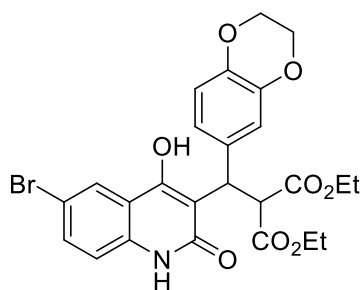
¹H NMR (400 MHz, DMSO-*d*₆) δ 11.45 (s, 1H), 10.79 (s, 1H), 7.96 (d, *J* = 2.4 Hz, 1H), 7.48 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 6.97 (s, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 5.15 (br s, 1H),

5.02 (d, *J* = 11.9 Hz, 1H), 4.14 (s, 4H), 4.01–3.87 (m, 4H), 0.98 (t, *J* = 7.1 Hz, 3H), 0.91 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (100 MHz, DMSO-*d*₆) δ 168.6, 168.2, 163.2, 157.2, 143.0, 142.4, 140.8, 136.8, 136.2, 134.3, 130.5, 125.6, 121.6, 119.6, 117.9, 117.2, 108.1, 64.4, 64.3, 61.2, 61.0, 53.7, 46.0, 14.2, 14.1.

ESI-HRMS (*m/z*) calcd for C₂₅H₂₄ClNO₈ [M+H]⁺ 502.1266, found 502.1263.

Synthesis of diethyl ((6-bromo-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)methyl)malonate (5f)



The compound **5f** was obtained according to the general procedure in 60% yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.45 (s, 1H), 10.79 (s, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 7.59 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 6.97 (s, 1H), 6.93–6.84 (m, 1H), 6.68 (d, *J* = 8.4 Hz, 1H), 5.14 (br s, 1H), 5.02

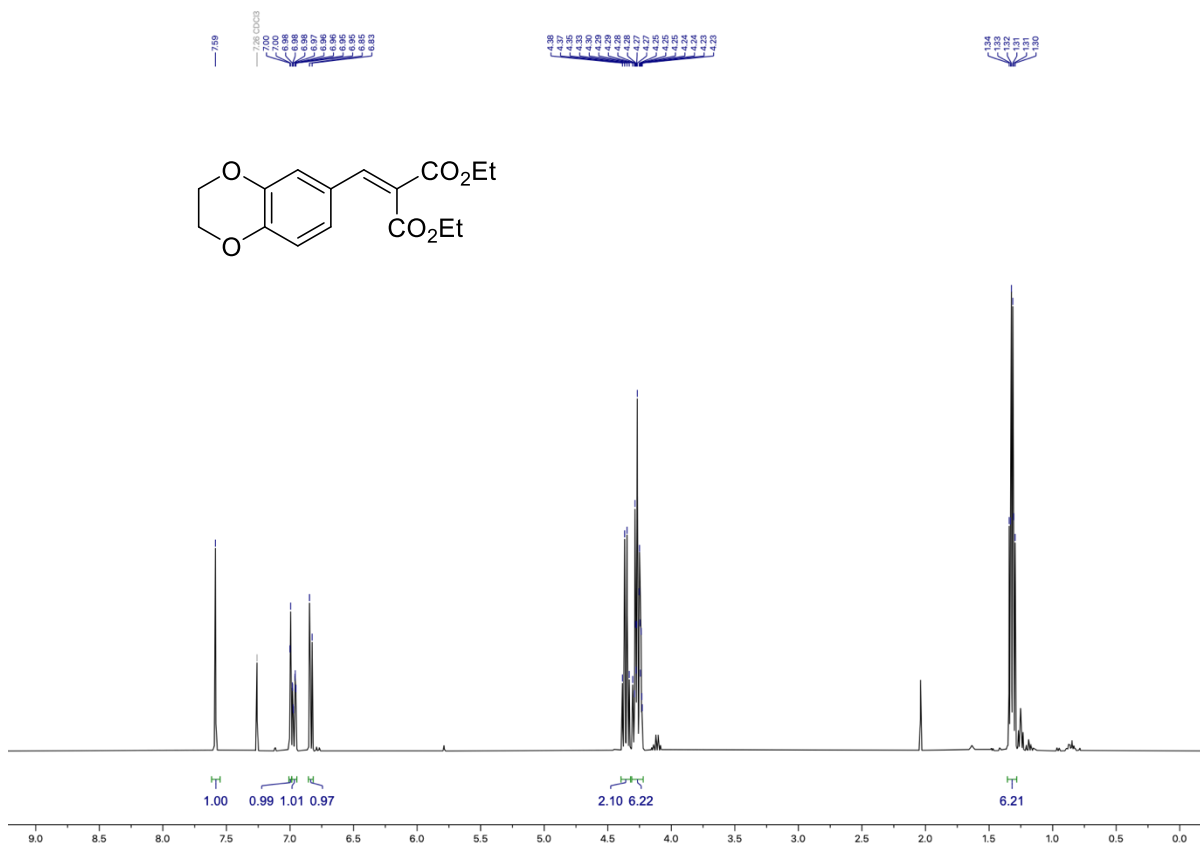
(d, *J* = 12.2 Hz, 1H), 4.14 (s, 4H), 4.00–3.87 (m, 4H), 0.98 (t, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (100 MHz, DMSO-*d*₆) δ 168.2, 167.8, 162.8, 156.6, 153.9, 142.6, 142.0, 136.7, 133.9, 132.9, 125.0, 121.1, 117.2, 116.8, 116.3, 113.3, 112.9, 64.0, 63.9, 60.7, 60.6, 53.3, 40.3, 13.8, 13.7.

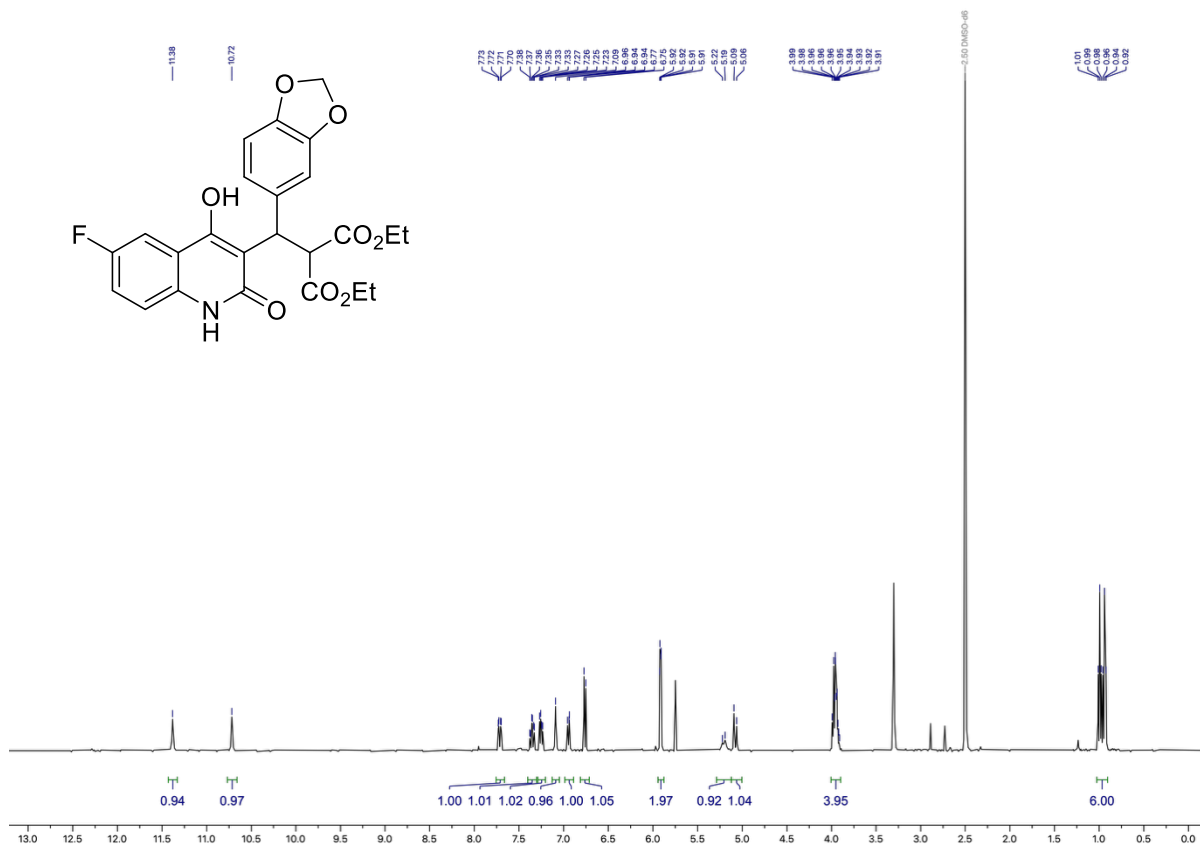
ESI-HRMS (*m/z*) calcd for C₂₅H₂₄BrNO₈ [M+H]⁺ 546.0758, found 546.0753.

NMR spectra of synthesised novel compounds

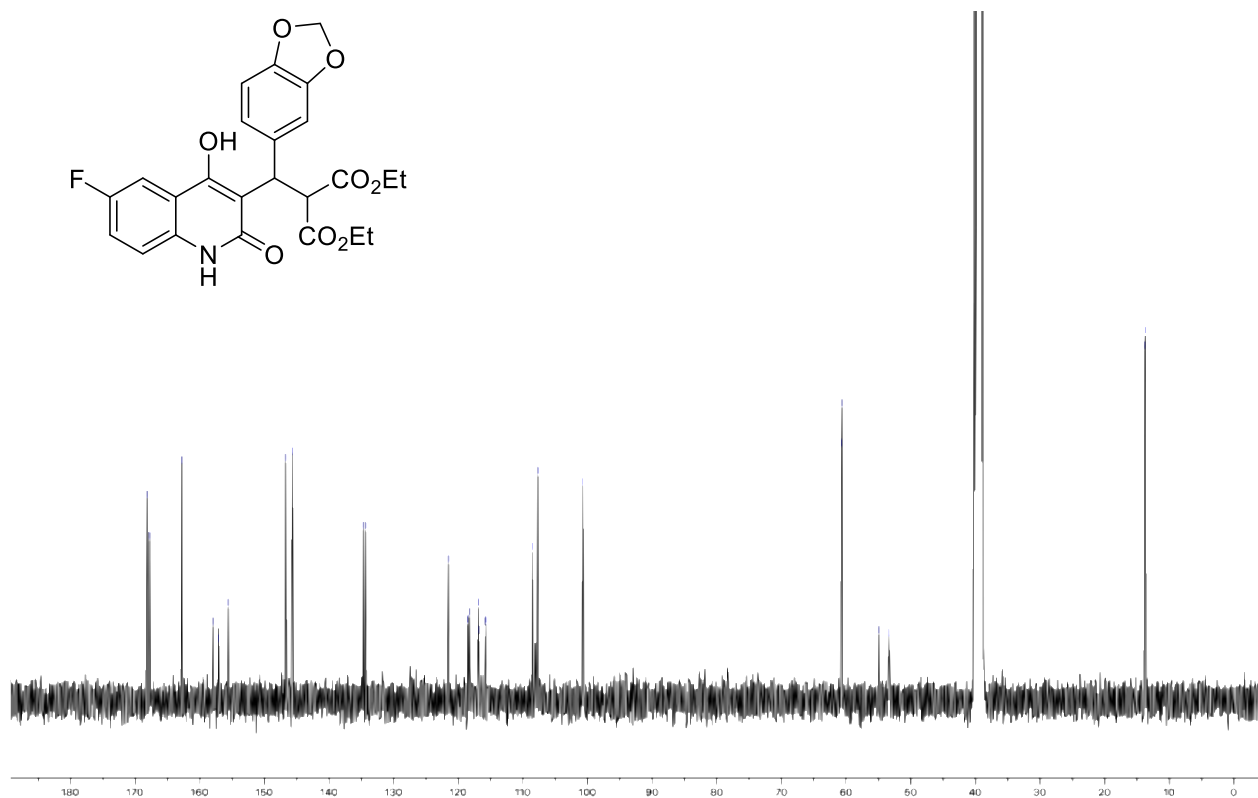
¹H NMR spectrum of compound 4 (400 MHz, CDCl₃)



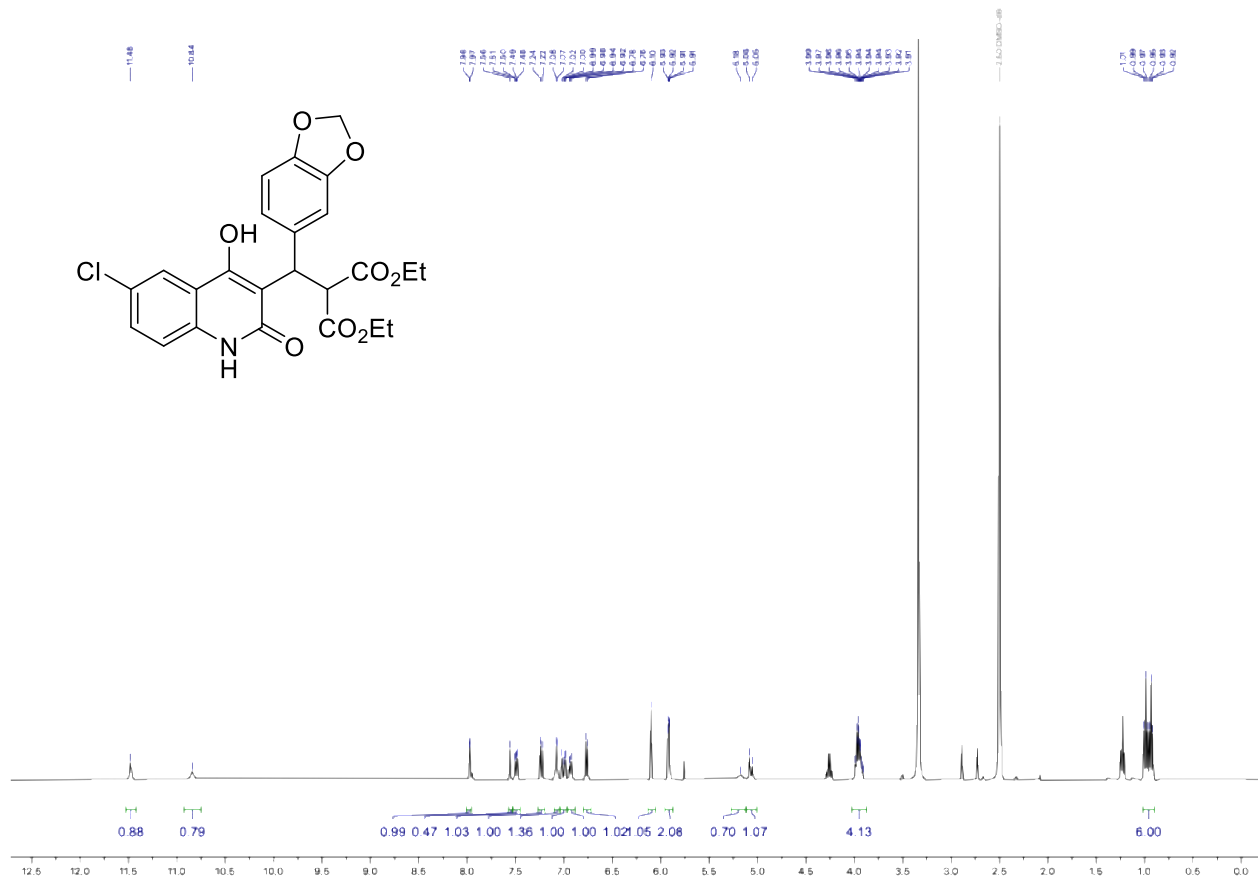
¹H NMR spectrum of compound 5a (400 MHz, DMSO-*d*₆)



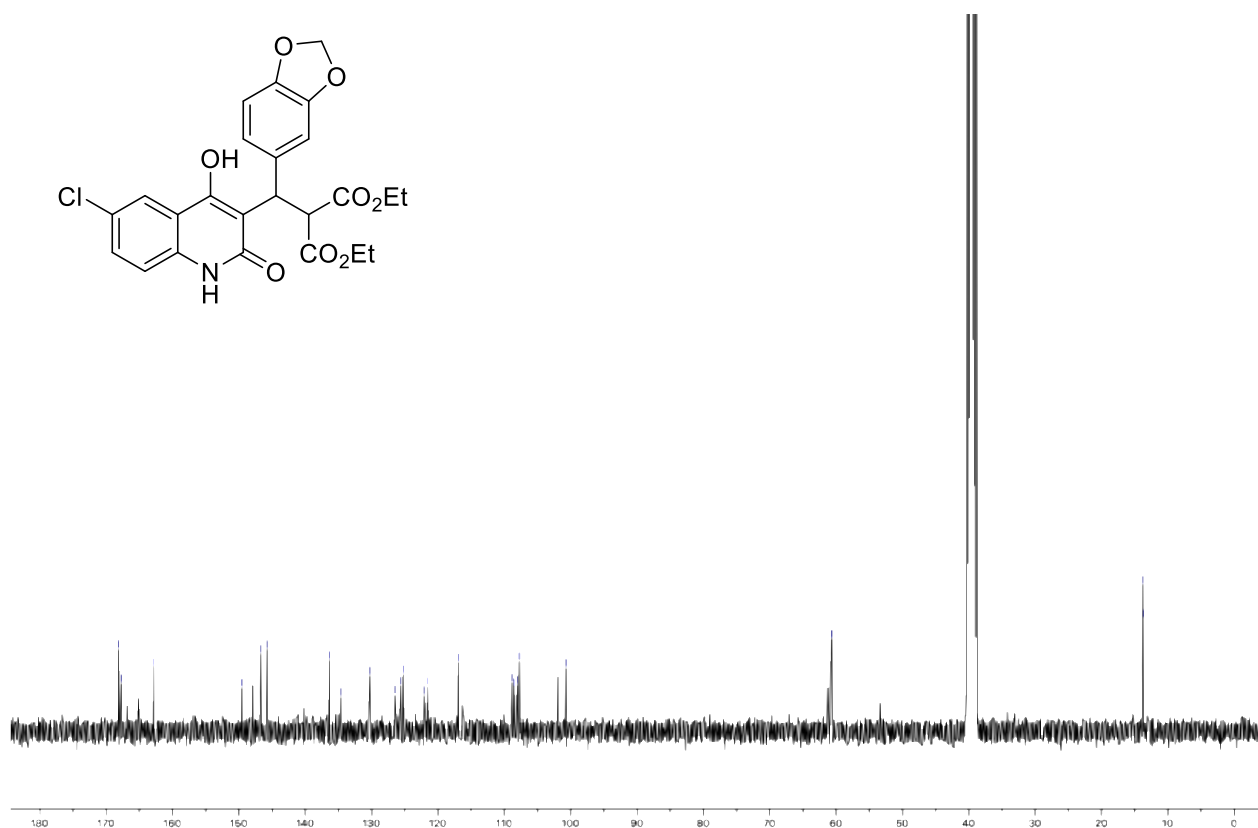
^{13}C NMR spectrum of compound 5a (100 MHz, $\text{DMSO}-d_6$)



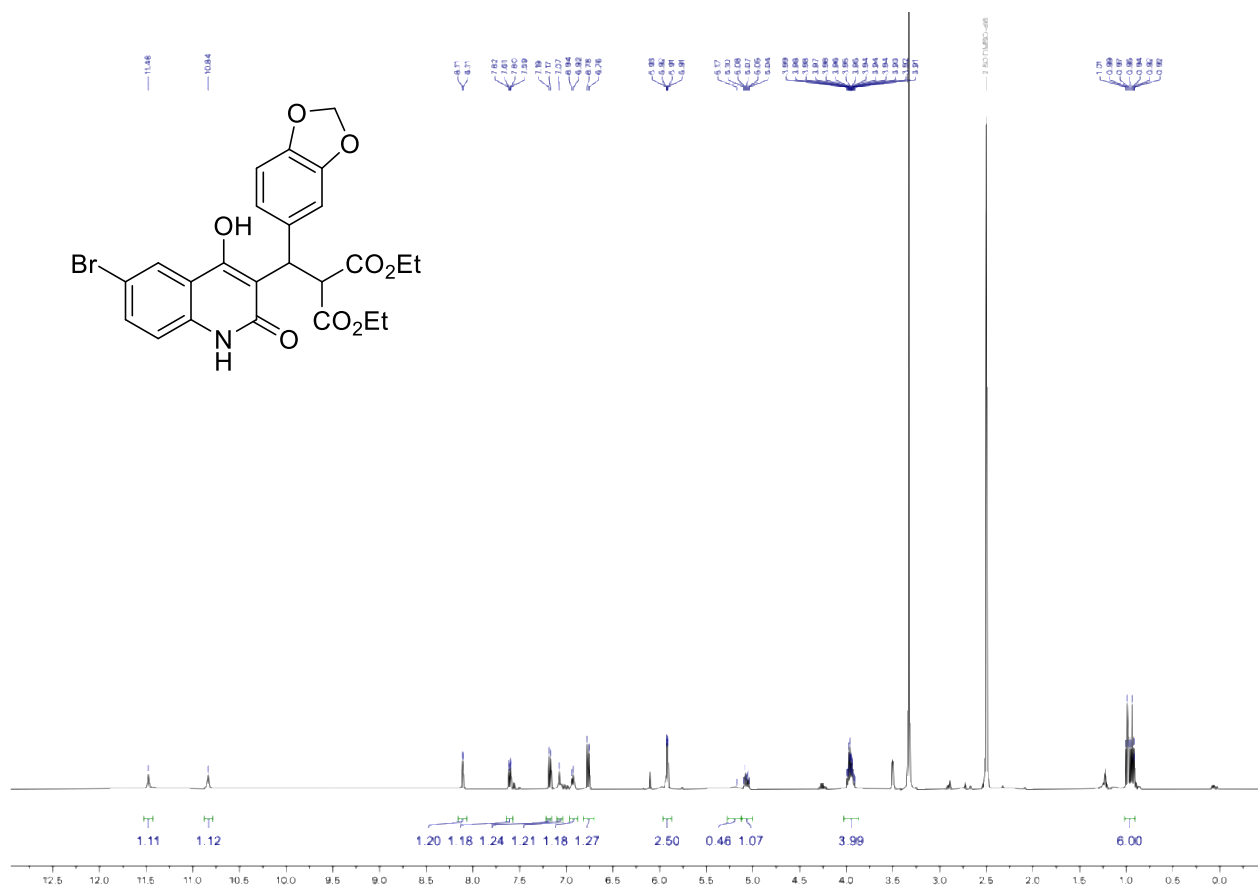
^1H NMR spectrum of compound 5b (400 MHz, $\text{DMSO}-d_6$)



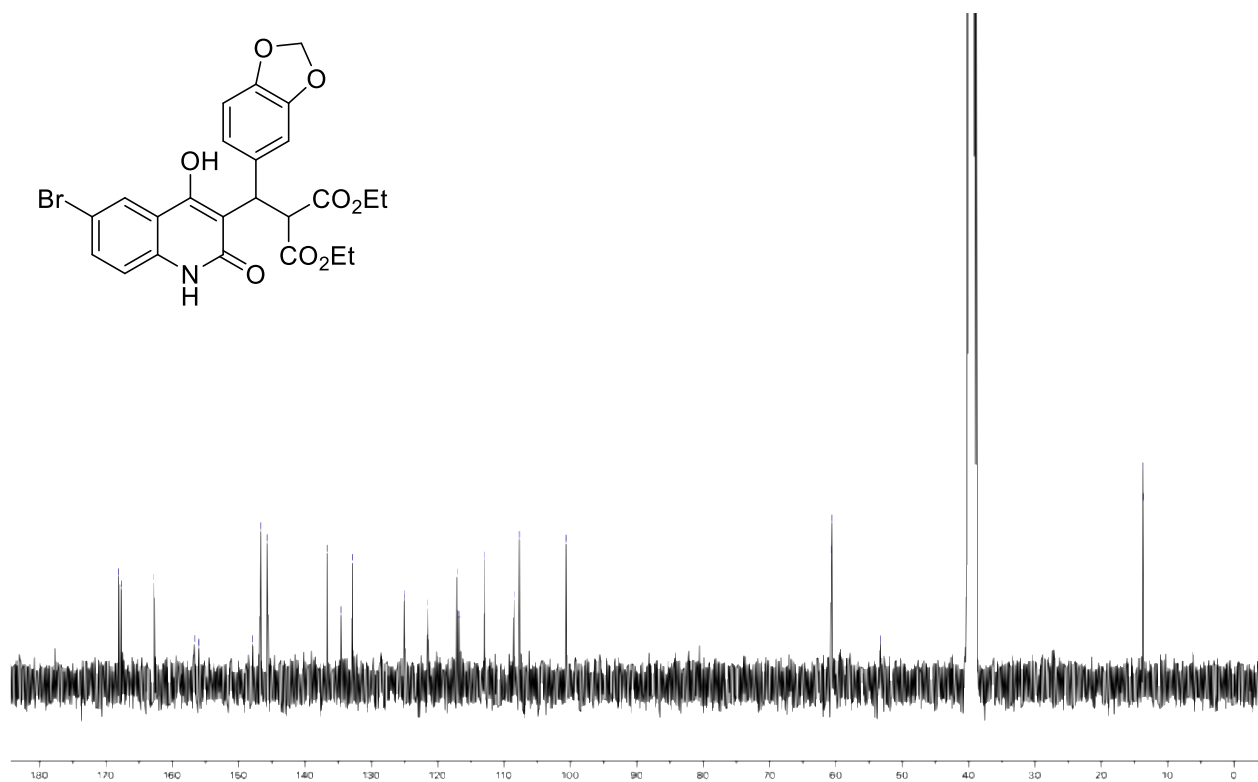
^{13}C NMR spectrum of compound 5b (100 MHz, $\text{DMSO}-d_6$)



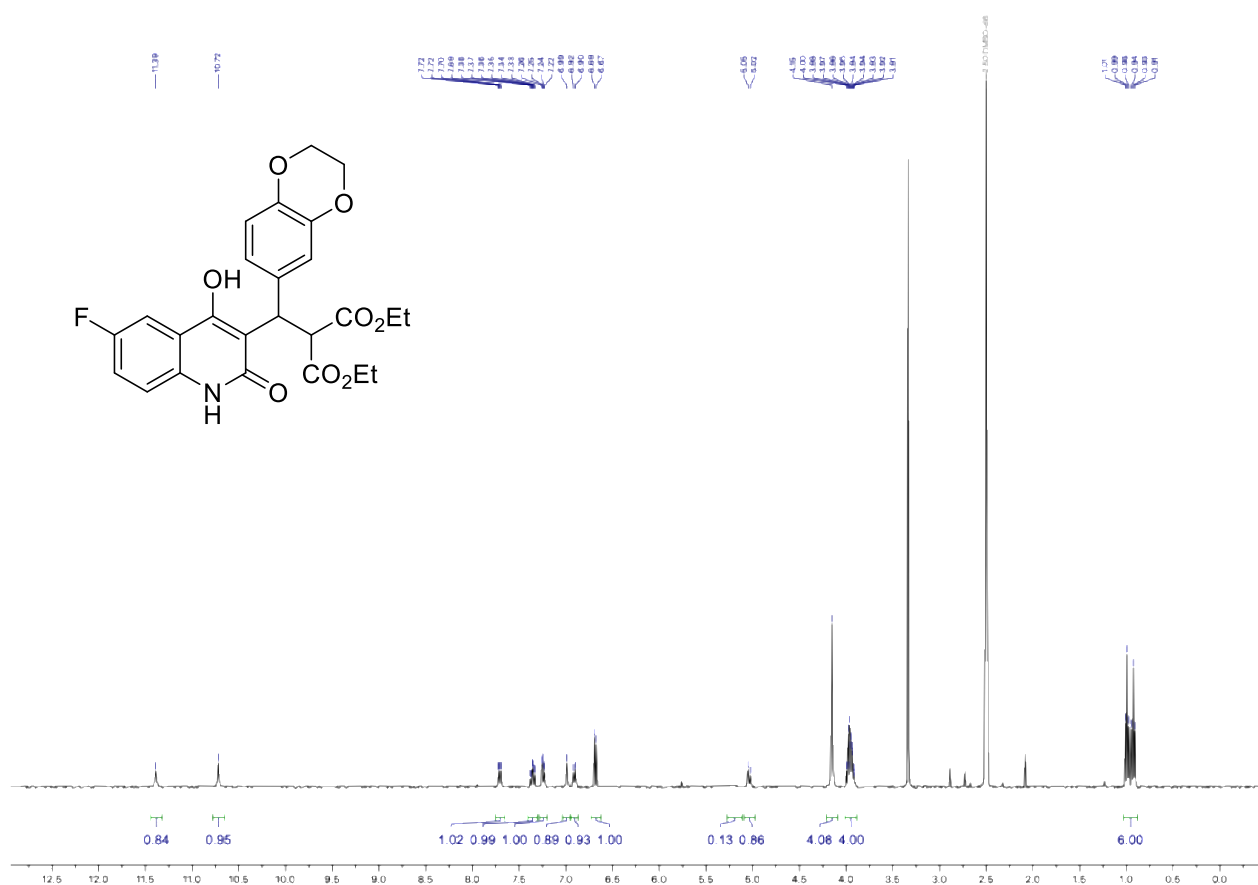
^1H NMR spectrum of compound 5c (400 MHz, $\text{DMSO}-d_6$)



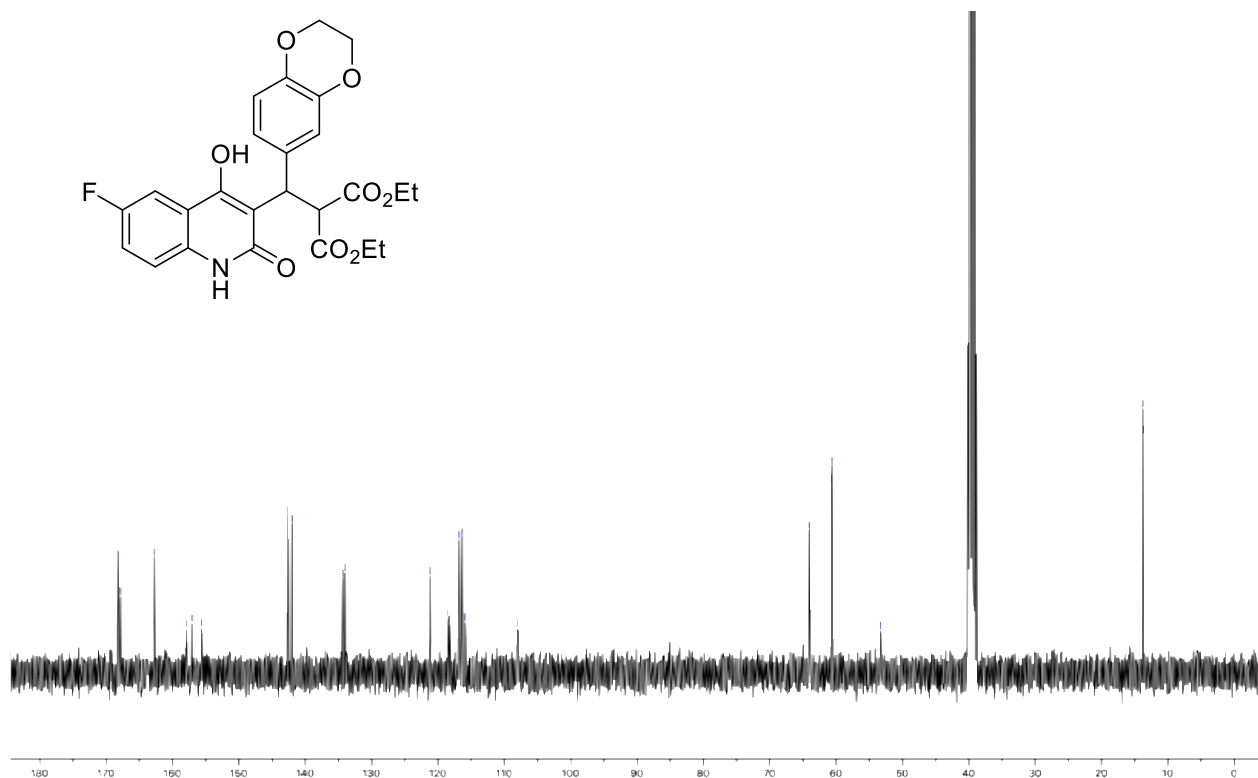
^{13}C NMR spectrum of compound 5c (100 MHz, $\text{DMSO}-d_6$)



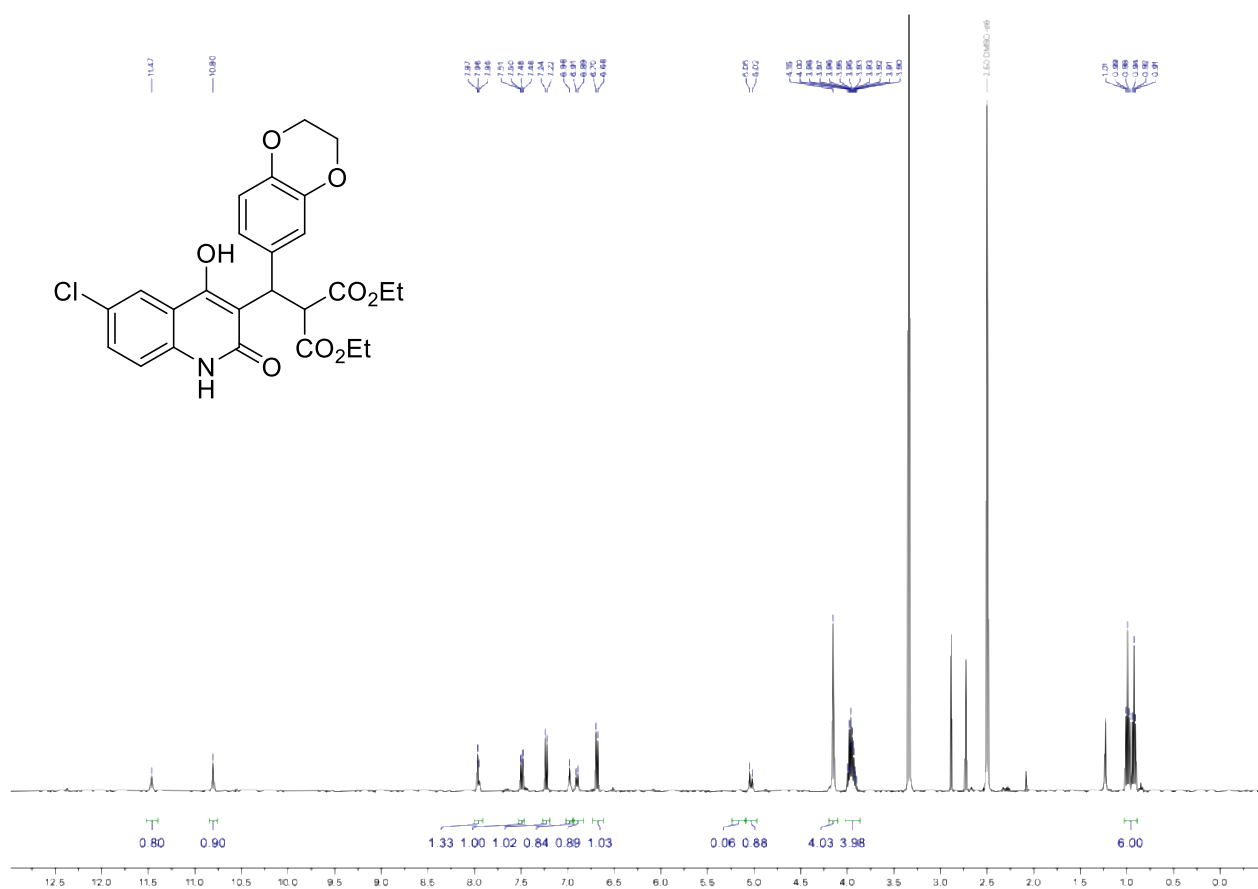
^1H NMR spectrum of compound 5d (400 MHz, $\text{DMSO}-d_6$)



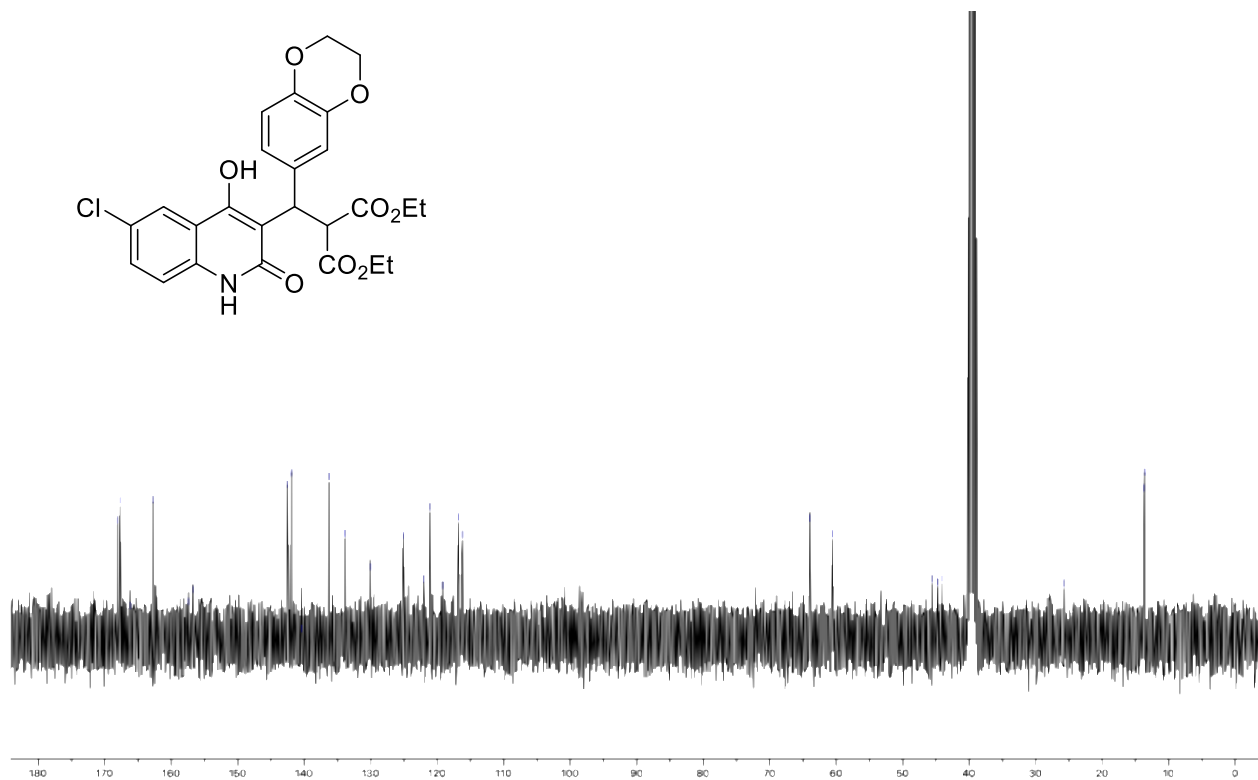
^{13}C NMR spectrum of compound 5d (100 MHz, $\text{DMSO}-d_6$)



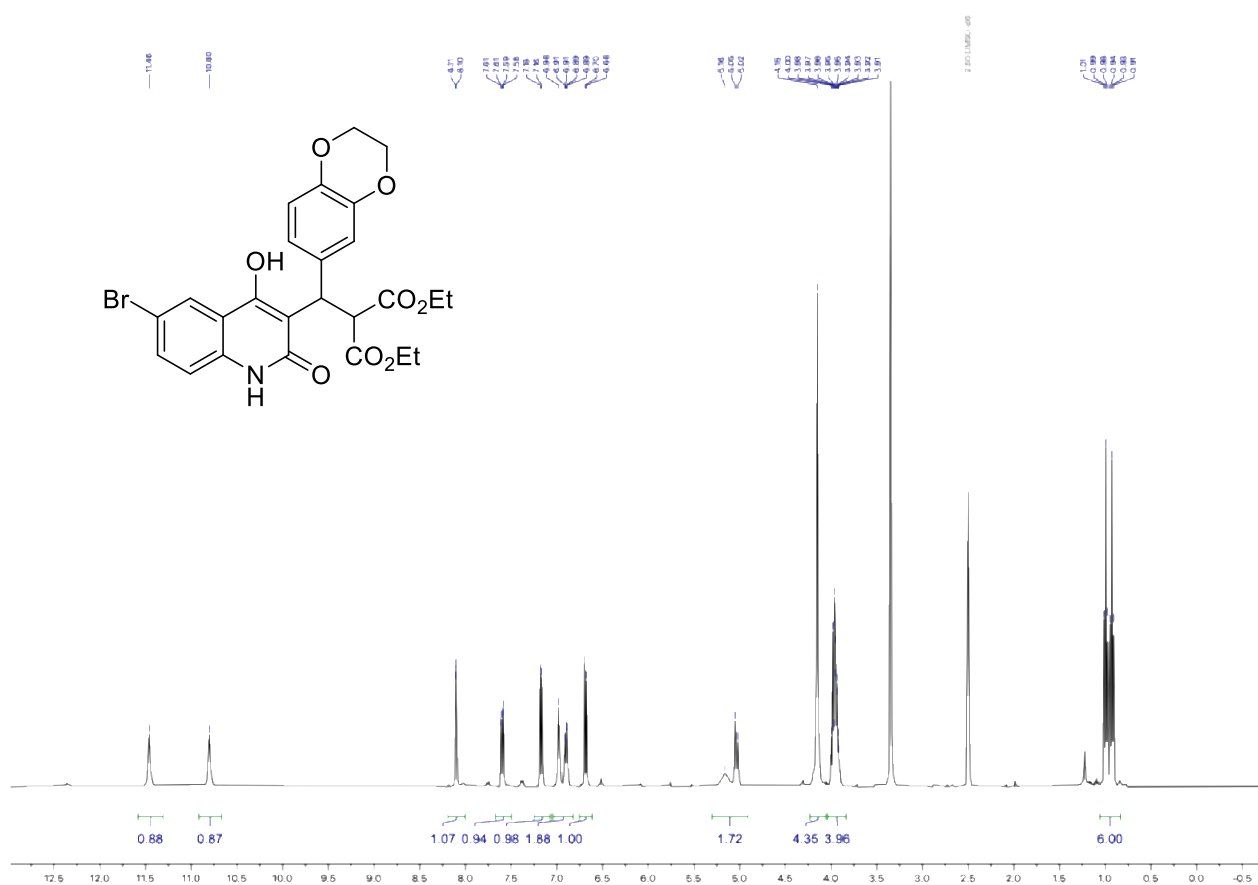
^1H NMR spectrum of compound 5e (400 MHz, $\text{DMSO}-d_6$)



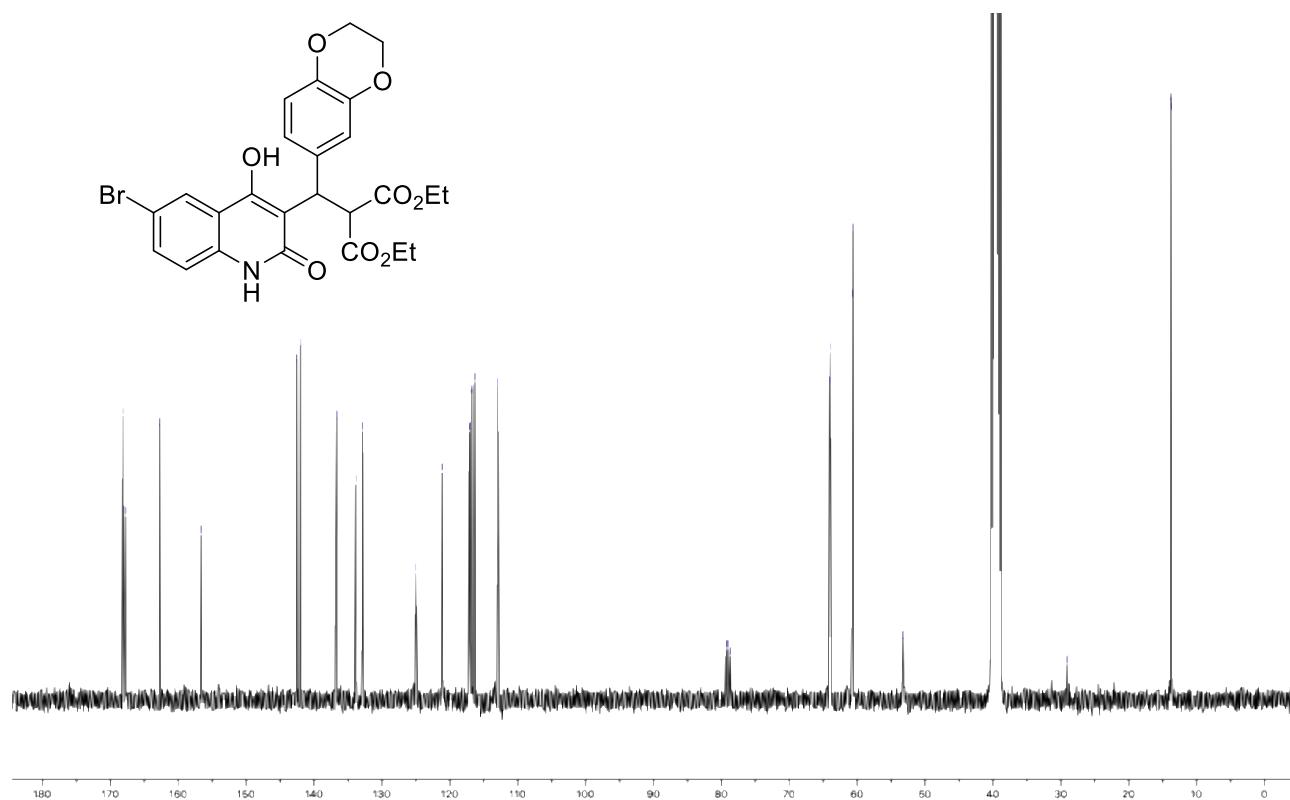
^{13}C NMR spectrum of compound 5e (100 MHz, $\text{DMSO}-d_6$)



^1H NMR spectrum of compound 5f (400 MHz, $\text{DMSO}-d_6$)



^{13}C NMR spectrum of compound 5f (100 MHz, DMSO- d_6)



Variable-Temperature and 2D NMR Studies

^1H and ^{13}C NMR spectra were recorded on Agilent NMR 400 (400 MHz for ^1H ; 100.6 MHz for ^{13}C) and Q.One Quantum 600 (599.5 MHz for ^1H ; 150.7 MHz for ^{13}C) spectrometers. Spectra were obtained in $\text{DMSO}-d_6$ and CDCl_3 at 298, 328 and 363 K. Chemical shifts (δ) are reported in ppm and referenced to residual solvent signals (2.50 and 7.26 ppm for ^1H ; 39.5 and 77.0 ppm for ^{13}C in $\text{DMSO}-d_6$ and CDCl_3 , respectively). Coupling constants (J) are given in Hz. Two-dimensional NMR experiments (HSQC and HMBC) were performed on Agilent NMR 400 and Q.One Quantum 600 spectrometers.

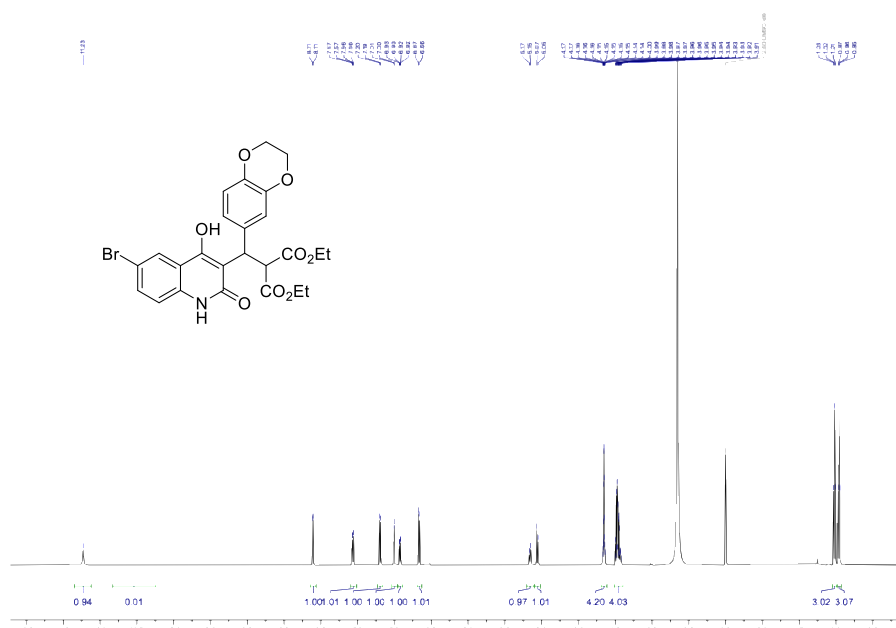


Figure S2 ^1H NMR spectrum of compound **5f** (600 MHz, 55 °C, $\text{DMSO}-d_6$)

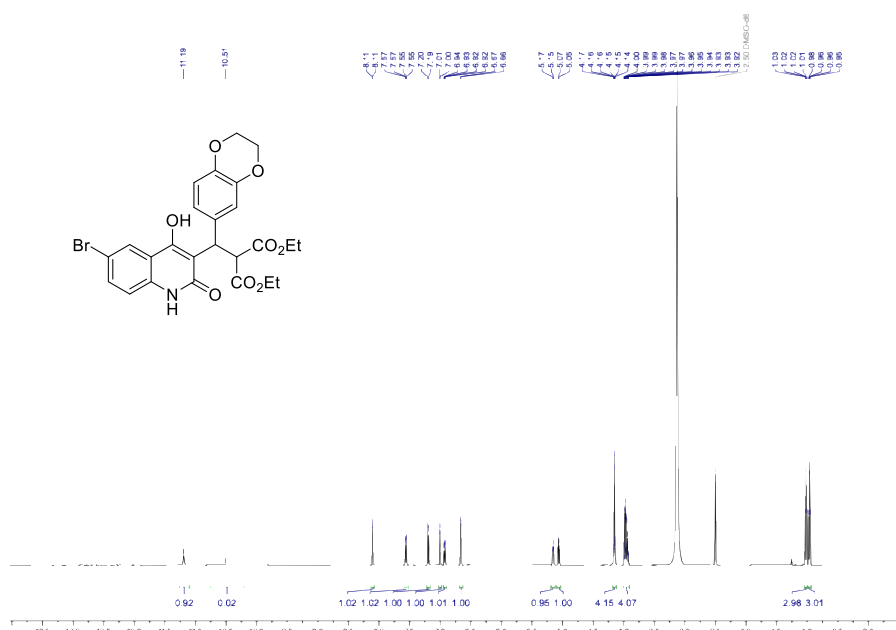


Figure S3 ^1H NMR spectrum of compound **5f** (600 MHz, 90 °C, $\text{DMSO}-d_6$)

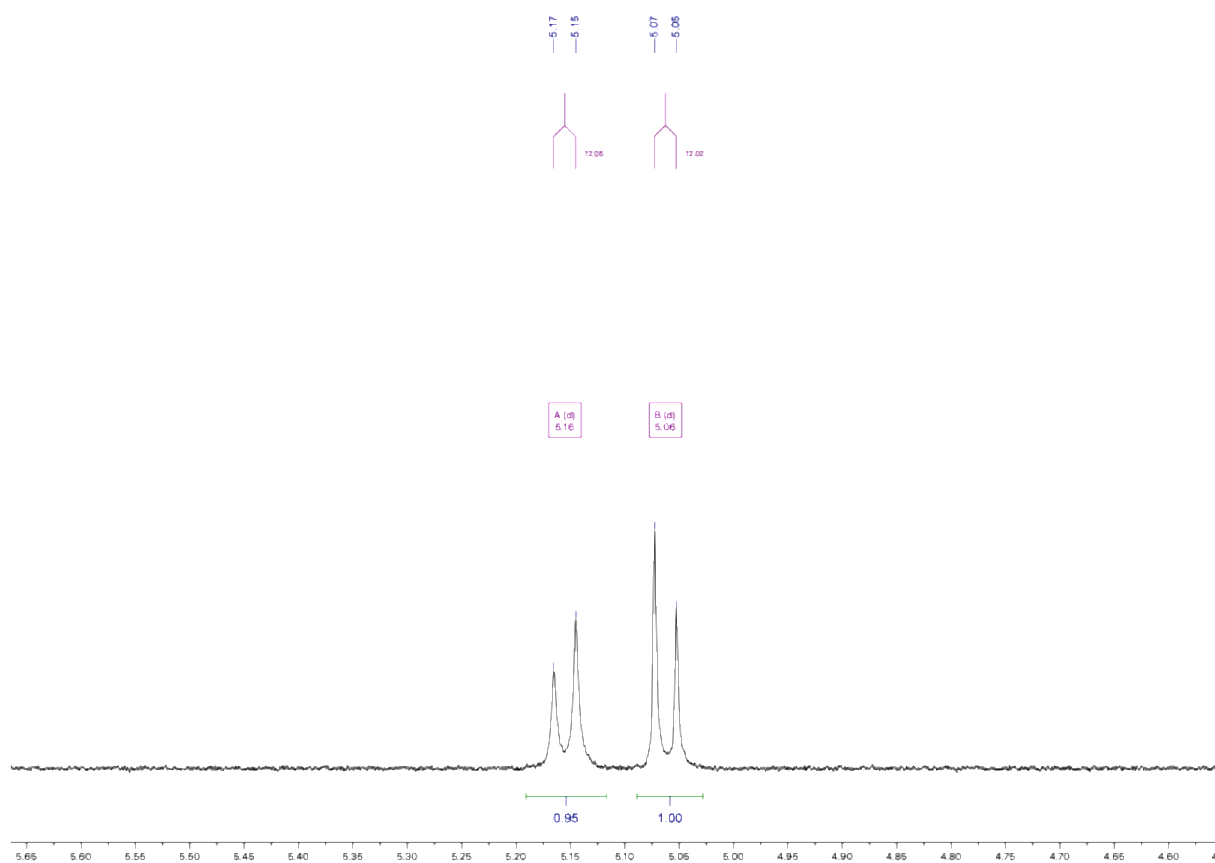


Figure S4 Expanded ^1H NMR region of compound **5f** at 90 °C showing two doublets with identical J values, confirming mutual coupling of the methine protons

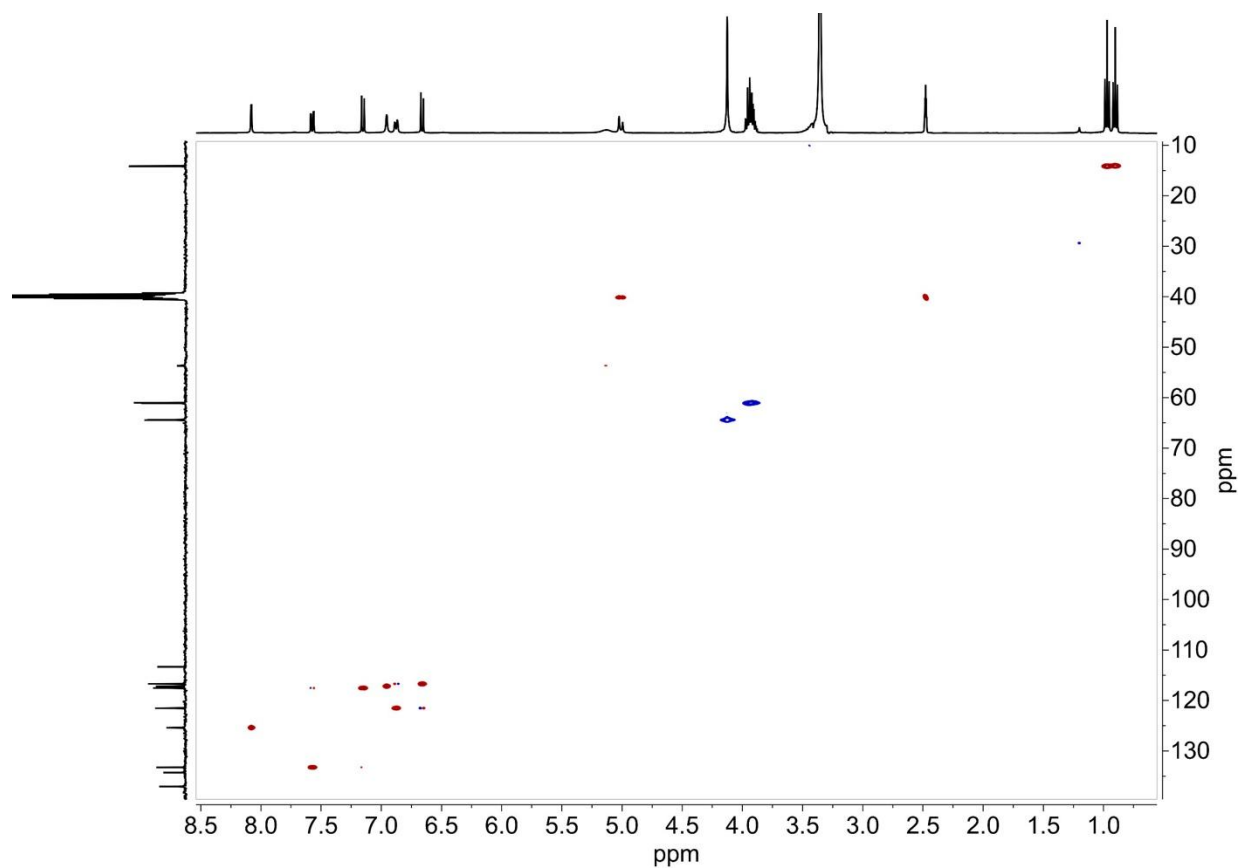


Figure S5 HSQC spectrum of compound **5f** at ambient temperature

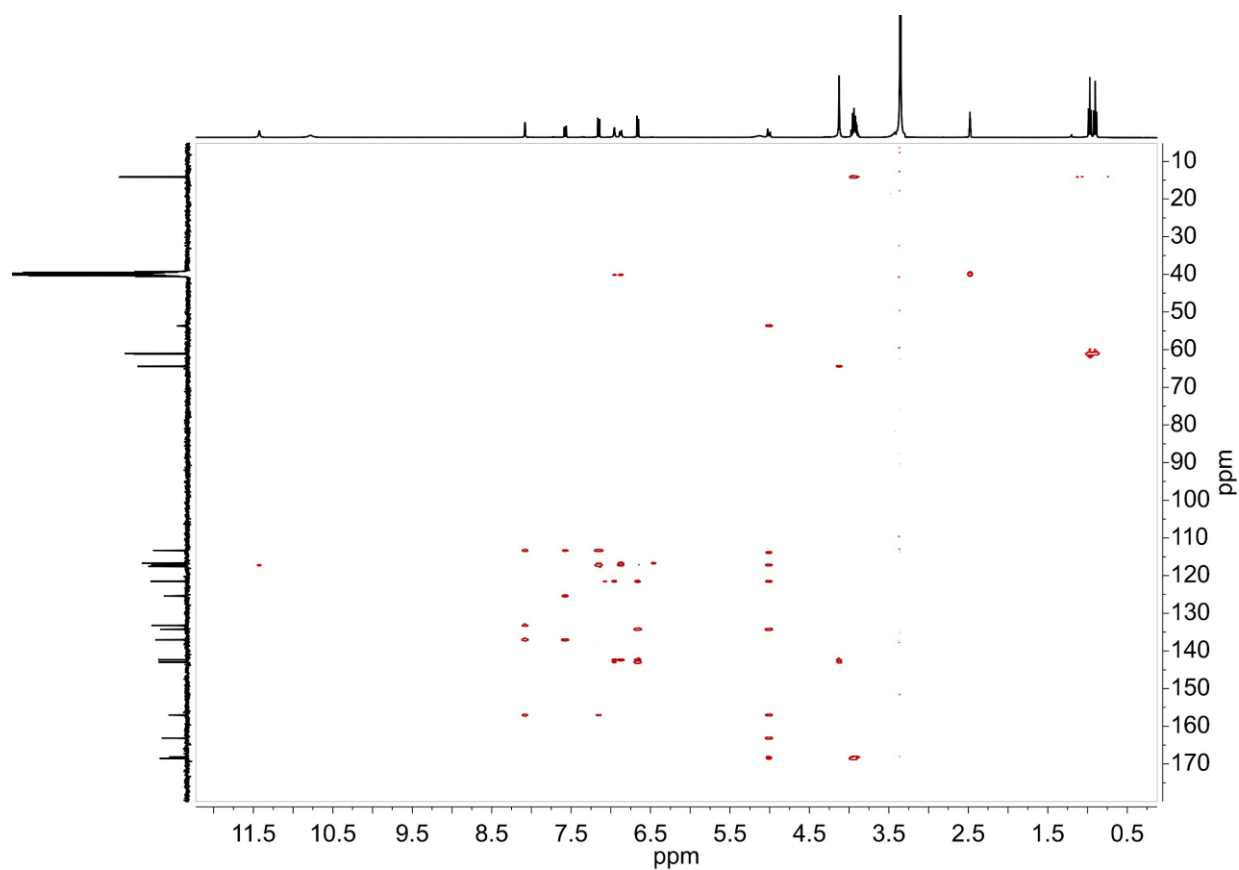


Figure S6 HMBC spectrum of compound **5f** at ambient temperature

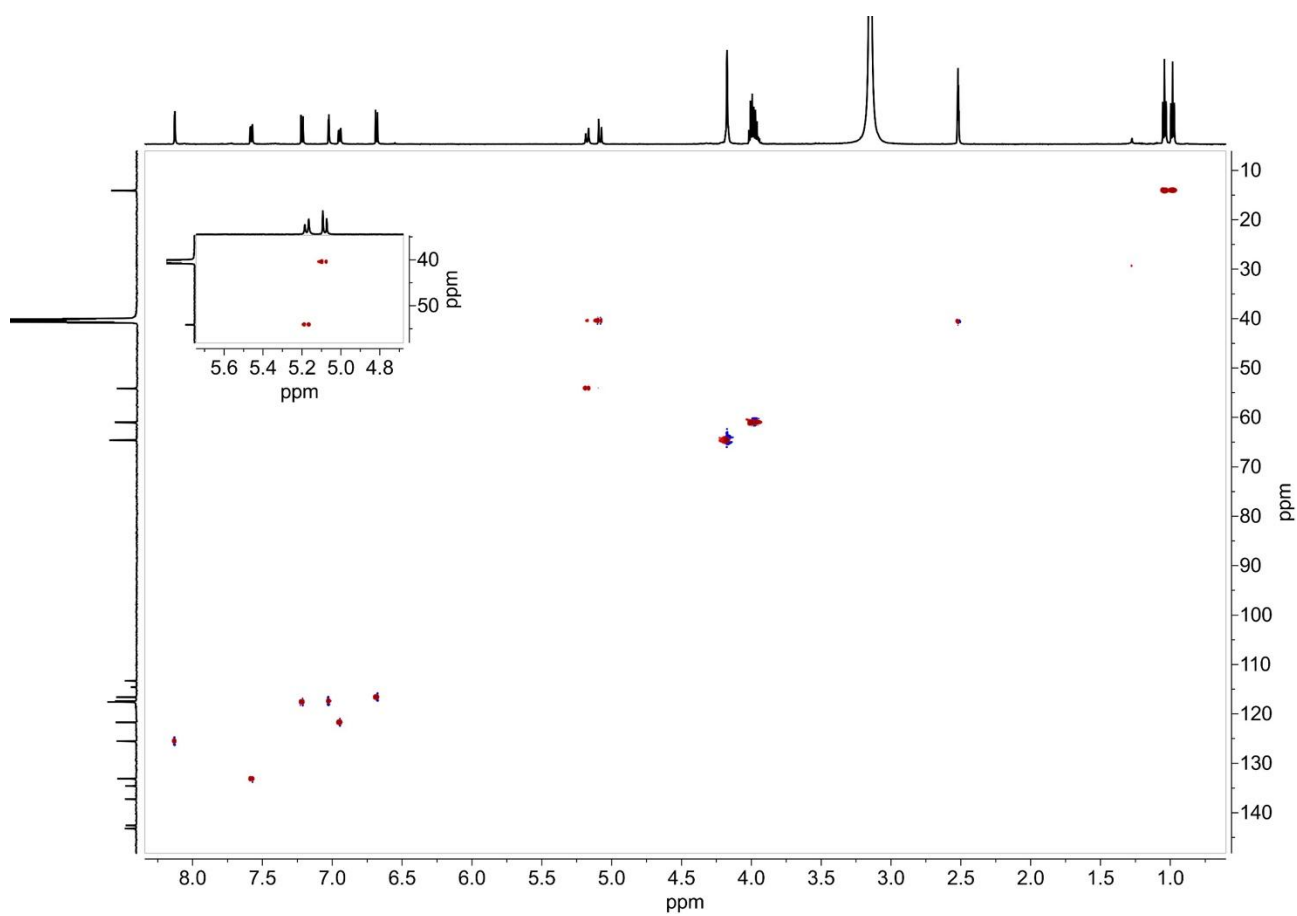


Figure S7 HSQC spectrum of compound **5f** at 90 °C

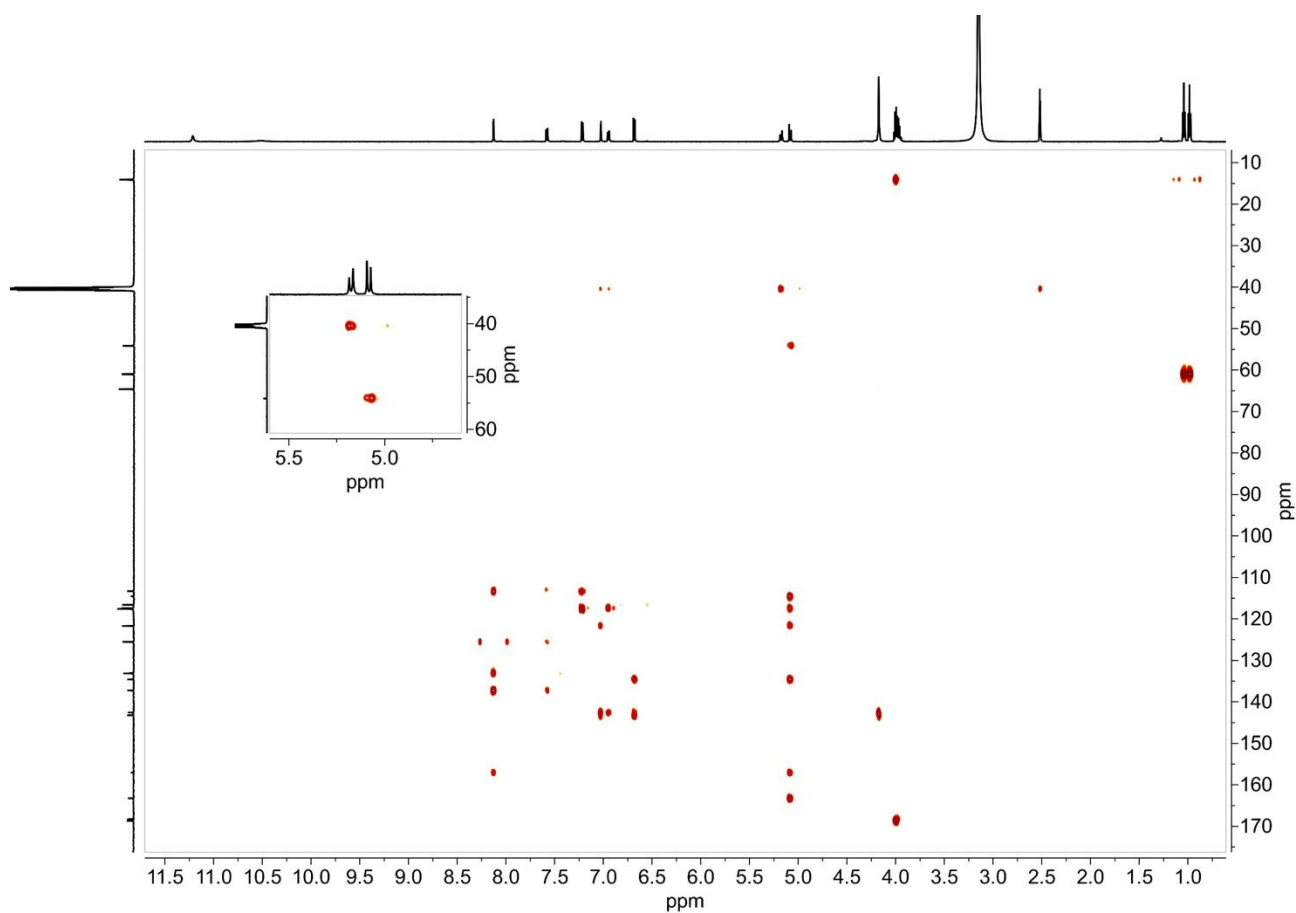


Figure S8 HMBC spectrum of compound **5f** at 90 °C

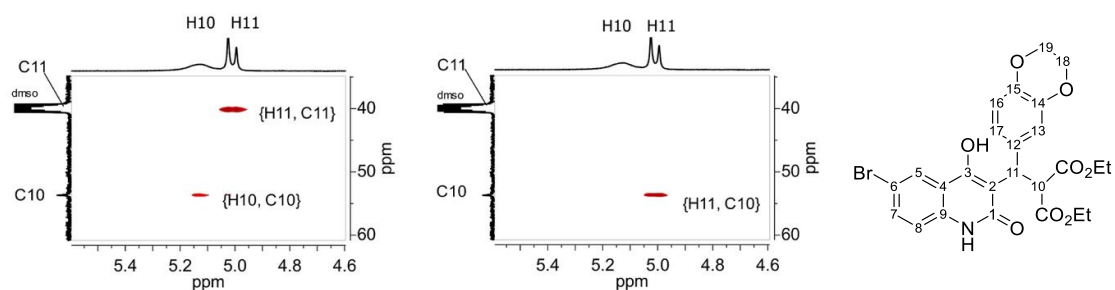


Figure S9 HSQC and HMBC spectra of compound **5f** at ambient temperature

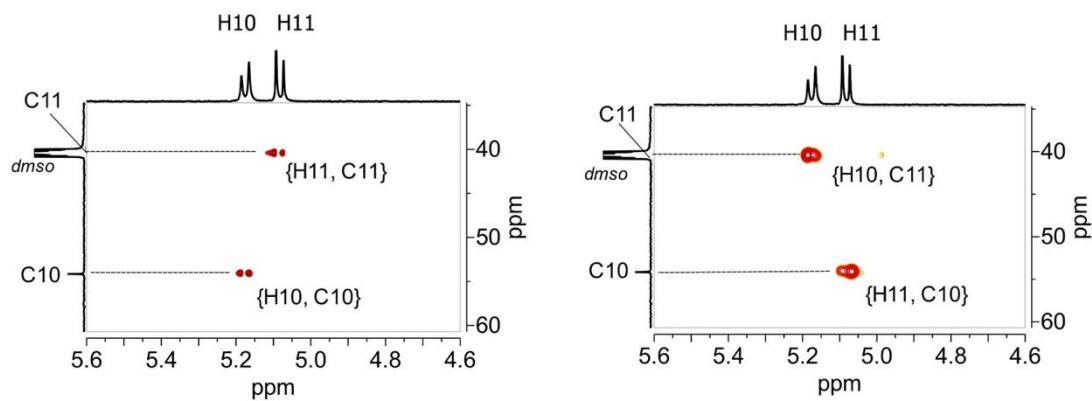


Figure S10 HSQC and HMBC spectra of compound **5f** at 90 °C

Biology

MTT cytotoxicity assay

MTT cytotoxicity assay [S5]. Four cell lines, including two cancerous (A549, MCF7) and two non-cancerous (HEK293T, VA13) were raised on DMEM/F-12 medium with 10% FBS and 1% anti-bacterial/anti-fungal solution (penicillin 50 U/ml, streptomycin 50 µg/ml) under 37 °C and 5% CO₂. In the first day of the test, cells were transferred to 96-well TC-treated plates in respective ratios (2500 per plate for HEK293T and A549, 5000 for MCF7, 6000 for VA13). Three identical plates were used for each cell line in order to detect the possible outliers. After 24 hours of cell incubation and attachment, compounds were added in serial three-fold dilutions. Stock concentration of the compound equaled 10 mg/ml, tested concentrations were in the range from 0.023 to 50 µg/ml. Compounds were dissolved in DMSO, and its concentration in cells was kept 0.5% and below to evade its possible cytotoxicity. DMSO in the same concentration was used as the negative control.

After three days of incubation under 37 °C and 5% CO₂, MTT reagent was added to the cells, its final concentration amounted to 0.5 g/l. Two hours afterwards, the supernatant was discarded and replaced with DMSO in order to dissolve the formed formazan crystals. After 10 minutes on a shaker, optical density of the solution in plates was measured at 555 nm wavelength by microplate reader VICTOR X5 (PerkinElmer, Waltham, MA, USA). Data processing and evaluation of cytotoxicity parameters were conducted in GrahPad Prism version 8.0.2 (GraphPad Software, Inc., San Diego, CA, USA).

Cytotoxicity evaluation

To evaluate the cytotoxicity, five synthesized compounds were tested via MTT assay. Sixth derivative couldn't be assessed due to the presence of residual DMSO, which prevented preparation of stock solution with precise concentration. The assay was conducted on four human cell lines: HEK293T (non-cancerous embryonal kidney), MCF7 (breast cancer), A549 (lung cancer), and VA13 (immortalized fibroblasts). All compounds, except 5d, expressed cytotoxicity only at the highest concentrations (10-50 mg/l). Four remaining substances lacked cytotoxicity towards VA13 cell line.

Table 1. The results of MTT cytotoxicity test for malonates **5b-5f**

Compound	IC50 (HEK293T, mg/ml)	IC50 (MCF7, mg/ml)	IC50 (A549 atcc, mg/ml)	IC50 (VA13, mg/ml)
5b	32±2	low tox	low tox	no tox
5c	34±2	low tox	≈50	no tox
5d	low tox	low tox	low tox	no tox
5e	14.5±0.7	45±2	24±2	low tox
5f	24±2	43±3	20±1	no tox

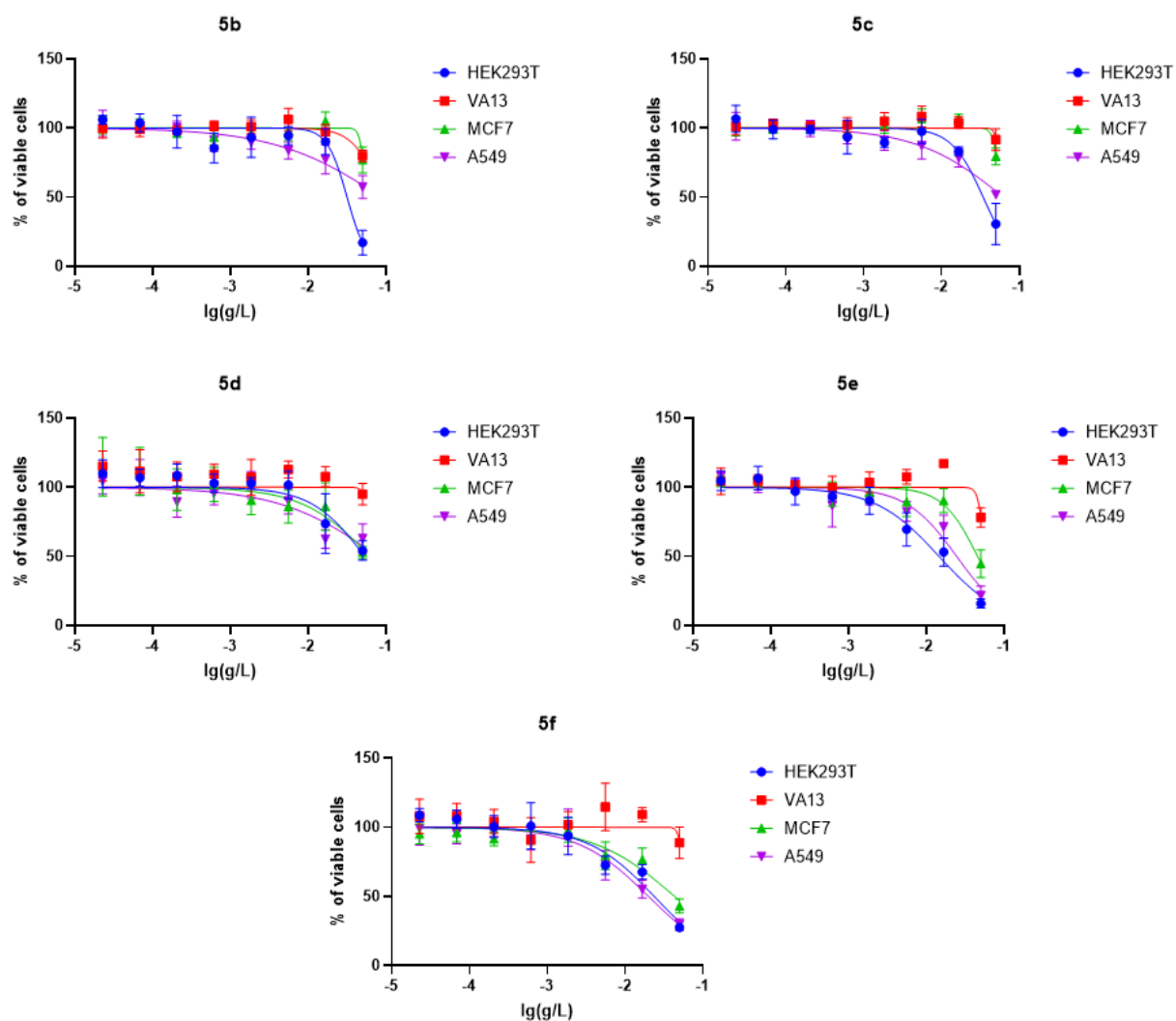


Figure S11 MTT assay results for malonates. The results are exhibited in form of dose-response curve, with error bars representing standard deviation (SD). SD was counted automatically in the process of curve fitting

Antibacterial properties

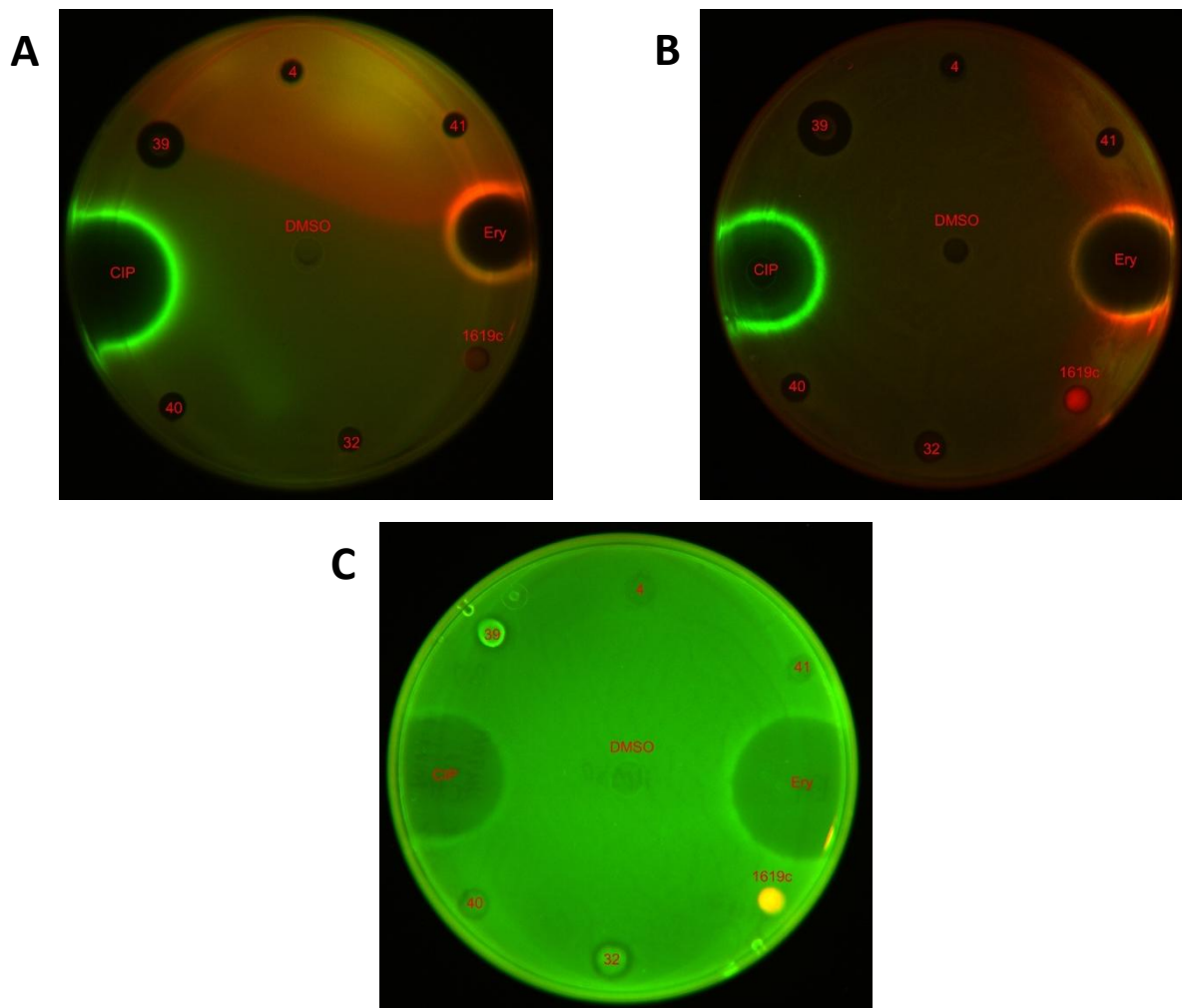


Figure S12 *In vitro* test of the molecules on reporter *E. coli* strains dTolC (A) and lptD (B), as well as *B. subtilis* 168 strain (C). The labels shown in the figure correspond to the compound numbering used in the main text (39 = **5a**, 41 = **5b**, 40 = **5c**, 4 = **5d**, 32 = **5e**, 1619c = **5f**). Only one molecule **5a** has demonstrated strong antibacterial activity against *E. coli* strains, but no conclusions can be drawn about the mechanism of action of this compound. In case of *B. subtilis* strain molecules **5a** and **5e** have also shown zones of inhibition but much smaller in diameter.

Reporter System Description

The reporter system used in this study is based on the pDualrep2 plasmid, as described previously [S6]. This system encodes two fluorescent proteins: turboRFP (red fluorescent protein) and Katushka2S (far-red fluorescent protein).

The expression of Katushka2S increases in the presence of translation-inhibiting antibiotics. This is achieved by placing the fluorescent gene downstream of a regulatory element derived from the tryptophan operon leader peptide. In nature, the translation rate of this leader peptide is

determined by tryptophan availability; when tryptophan is abundant, downstream biosynthetic genes are not expressed. In the pDualrep2 system, the tryptophan codons were replaced with alanine codons, which are always efficiently translated. Consequently, Katushka2S is expressed only when translation is inhibited by antibiotics, allowing detection of sublethal effects on protein synthesis.

The second reporter gene, RFP, is under the control of the *SulA* promoter, whose expression is upregulated during the SOS response triggered by DNA-damaging agents. Thus, RFP expression increases specifically in response to DNA damage.

Both fluorescent signals are observed in zones of sublethal antibiotic concentration, i.e., regions where the antibiotic affects the target without killing the cell. Since the lethal concentration for the tested compounds is initially unknown, assays are performed by applying a small droplet of the maximal feasible concentration to an agar plate seeded with the reporter strain. A concentration gradient is generated via diffusion, allowing visualization of sublethal zones around the application site.

The reporter system is optimized for *E. coli*, a representative Gram-negative bacterium. Many compounds that inhibit Gram-positive bacteria are ineffective against wild-type *E. coli* due to permeability and efflux barriers. To circumvent this limitation, two mutant strains were employed: Δ tolC, deficient in the TolC efflux pump, and LptD mutant, with altered outer membrane composition, which increases susceptibility to larger, primarily peptide antibiotics.

Screening of the compounds' activity was performed using an agar plate assay with a fluorescence reporter system based on *Escherichia coli* JW5503 Δ tolC [S7] and BW25113 lptD^{mut} [S8] strains harbouring the pDualrep2 [S6] and pDualrep2.1 [S9] plasmids respectively, which enables simultaneous determination of the antibiotic mechanism of action and inhibition efficiency. In addition, *B. subtilis* 168 without a reporter construct was used as a Gram-positive bacterial model. Fluorescence was visualized using a ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA) in the Cy3 and Cy5 channels for RFP and Katushka2S, respectively.

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