Supporting Information for "Combining de novo molecular design with semiempirical protein–ligand binding free energy calculations"

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S1 Chemical Synthesis

S1.1 Reagent and purification information

Reactions were set up and conducted in glassware. All chemicals were purchased from Sigma Aldrich (St. Louis, US), Thermo Scientific (Waltham, US) or obtained from the HCI-Shop at the ETH campus Hoenggerberg. All solids were dosed using a XS205 dual range balance from Mettler Toledo (Greifensee, CH). Liquids were dosed using syringes from B.Braun (Melsungen, DE). Anhydrous reactions were performed in oven-dried glassware (110°C), in absolute solvents, and under inert atmosphere (nitrogen or argon atmosphere). Na_2SO_4 or $MgSO_4$ were used as drying agents. Room temperature (RT) refers to 25°C. Reaction reflux conditions were obtained using DrySyn® heating blocks (Radnor, PA, USA) equipped with a standard thermometer. Solvent evaporations were performed under reduced pressure on a Büchi rotary evaporator. All reactions were monitored by thin layer chromatography (TLC) using precoated silica gel aluminum plates (Macherey-Nagel, Oensingen, Switzerland) and visualized by UV light at 254 and 366 nm. Flash column chromatographies (FCC) purifications were performed using a Biotage Isolera instrument.

S1.2 Analytical information

All compounds were characterized by nuclear magnetic resonance (NMR) spectroscopy and final compound, by analytical high-performance liquid chromatography (HPLC). The analytical and spectroscopic data of each compound are in good agreement with their structures. **NMR spectra** were recorded on a Bruker Avance Ultrashield, 400 MHz spectrometer equipped with a 5 mm Z-gradient iProbe. NMR data are reported as follows: chemical shift in reference to the residual solvent peak (δ ppm), multiplicity (s = singlet, d = doublet, br d = broad doublet, dd = doublet of doublet, br dd = broad doublet of doublet, t = triplet, br t = broad triplet, m = multiplet), coupling constant (Hz), and integration. ^{13}C NMR was selectively acquired for compounds where there was a possibility of side reactions leading to by-products. **High-resolution mass spectrum (HRMS)** of compound 2 was determined by using the services of Molecular and Biomolecular Analysis Service (MoBiAS) at ETH Zurich, on a Bruker maXis – ElectroSpray Ionisation - Quadrupole-Time-Of-Flight (ESI-Qq-TOF-MS; Bruker Corporation, Billerica, MA, USA). **HPLC-MS**: the purity of compound 2 was determined by reversed phase HPLC-MS with UV and ESI-MS detection on a Shimadzu LCMS 2020 system (Kyoto, Japan) with a Nucleodur C18 HTec column (150 × 3 mm, 5 μm, 110 Å; Macherey-Nagel, Düren, Germany) and a linear 30–95% acetonitrile in water (MilliQ) gradient containing 0.1% formic acid over 17 minutes with a flow rate of 0.5 mL min^{-1}C at 30°C. Reactions were additionally monitored by liquid chromatography electrospray ionization mass spectrometry (**LC-ESI-MS**) by using the services of Molecular and Biomolecular Analysis Service (MoBiAS) at ETH Zurich, on a Waters Acquity UPLC LCA 574 instrument (Waters, Milford, USA) connected to an Waters Acquity UPLC diode array detector with flow cell 10mm (5 μl) and a SQD2 LC/MSD ESI-Q-MS (Waters, Milford, USA) with a Acquity UPLC BEH C18 column (RHHD 1.7 μm particle size, 2.1 × 50 mm, Waters) and a linear 2-98% acetonitrile in water (HPLC grade) gradient containing 0.1% formic acid with a flow rate of 0.5 mL min^{-1} within 5 min, then isocratic for 1.5 min; UV spectra recorded from 190-500 nm at 4 nm resolution and 20 points s^{-1}.

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S1.3 Experimental procedures

Ethyl 3-(piperidin-4-yl)propanoate (**4**):

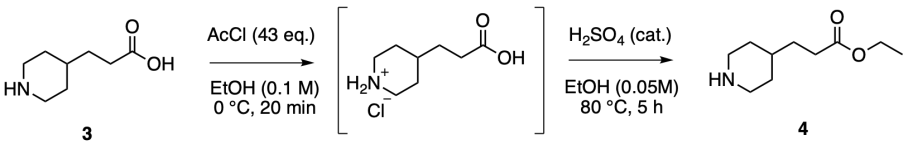


Fig. S1 Protection of carboxylic acid group by esterification of 3-(piperidin-4-yl)propanoic acid (3)

Compound **4** was synthesized as described in the patent WO 99/00367 by Novo Nordisk[92].
EtOH (0.1 M, 50 mL) was added to a flame dried three-necked round bottom flask. The flask was sealed with septa and purged with three vacuum/argon cycles. The solution was then cooled to 0 °C using a water-ice bath. After 10 minutes, acetyl-chloride (43 eq., 10 mL) was added using a syringe and the mixture stirred at 0 °C for 10 minutes. 3-(piperidin-4-yl)propanoic acid (**3**, 1.0 eq., 3.17 mmol) was added to the mixture and the septum was replaced with a glass stopper. The resulting mixture was stirred for 20 minutes provides a white precipitate. The HCl gas formed during the reaction was quenched by applying a vacuum driven suction force via a valve and directing it into a large round bottom flask containing saturated sodium hydrocarbonate solution. The reaction mixture was then concentrated *in vacuo* (50 °C, 60 mBar) under addition of toluene for co-evaporation to afford the intermediate ammonium chloride salt as a white powder. The ammonium chloride salt was transferred to a round bottom flask with EtOH (0.05 M, 100 mL). The solution was stirred until the salt was dissolved and sulfuric acid (cat., 5 drops) were added. The mixture was heated to 80 °C and stirred for 5 hours. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* (50 °C , 60 mBar) with toluene added for co-evaporation to afford ethyl 3-(piperidin-4-yl)propanoate (**4**, 500 mg, 83 % yield) as a light yellow oil. The obtained residue was used without further purifications.

^{1}**H NMR (400 MHz, MeOD)**: δ 4.04 (q, J = 7.1 Hz, 2H), 3.61 (q, J = 7.1 Hz, 1H), 3.43 – 3.34 (m, 2H), 3.05 – 2.91 (m, 2H), 2.39 – 2.33 (m, 2H), 2.00 – 1.87 (m, 2H), 1.67 – 1.56 (m, 3H), 1.42 (dddd, J = 17.4, 14.3, 11.3, 4.2 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H).

R$_f$ **=** 0.4 (50% EtOAc in *n*-pentanes, visualized with UV)

1-(5-(hydroxymethyl)thiophen-2-yl)ethan-1-one (**6**):

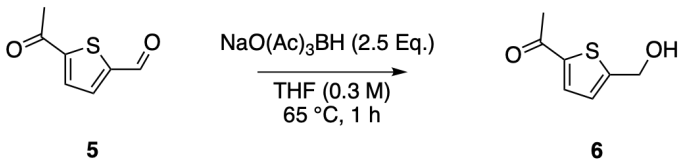


Fig. S2 Selective reduction of aldehyde in presence of keton of 5-acetylthiophene-2-carbaldehyde (5)

Compound 6 was synthesized as described in the patent: WO 2015134701 A1 by Bristol-Myers Squibb[93].
5-acetylthiophene-2-carbaldehyde (**5**, 1.0 eq., 6.49 mmol) was added to a flame dried three-necked round bottom flask. The flask was sealed with septums and purged with three vacuum/argon cycles. THF (0.3 M, 21.63 mL) was added using a syringe through a septum under argon atmosphere. The system was stirred until it became an homogeneous solution and, then, was cooled to 0 °C using a water-ice bath. After 20 minutes, sodium triacetoxyhydroborate (2.5 eq., 16.22 mmol) was added portionwise under argon atmosphere. The mixture was stirred at 65 °C for 1h. The reaction was cooled to 0 °C and quenched by addition of H_2O (13 mL) via syringe. The mixture was extracted with EtOAc and washed with H_2O (30 mL x 3). The organic phase was dried over $MgSO_4$, filtered using a funnel filled with cotton and concentrated *in vacuo* (50 °C, 240 mBar). Concentration of the dried extracts provided a yellow crude oil. The crude material was purified by flash column chromatography using EtOAc in *n*-pentanes to afford 1-(5-(hydroxymethyl)thiophen-2-yl)ethan-1-one

(**6**, 570 mg, 56%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$).: δ 7.57 (d, J = 3.8 Hz, 1H), 7.01 (dt, J = 3.8, 0.9 Hz, 1H), 4.85 (s, 2H), 2.53 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$). δ 191.01, 143.72, 132.80, 125.63, 60.38, 26.76, 24.93.

$\mathbf{R}_f$ = 0.25 (30% EtOAc in *n*-pentanes, visualized with UV)

1-(5-(iodomethyl)thiophen-2-yl)ethan-1-one (**7**):

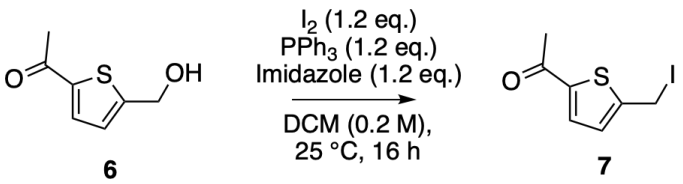


Fig. S3 Mitsunobu reaction of 1-(5-(hydroxymethyl)thiophen-2-yl)ethan-1-one (6)

PPh_3 (1.2 eq., 4.41 mmol) and imidazole (1.2 eq. 4.41 mmol) were added to a flame dried three-necked round bottom flask. The flask was sealed with septa and purged with three vacuum/argon cycles. Dry DCM (0.2 M, 18.38 mL) was added using a syringe and the resulting mixture stirred until it was homogeneous. The solution was cooled to 0 °C using a water-ice bath. After 15 minutes, I_2 was added portionwise under argon atmosphere. After 10 minutes 1-(5-(hydroxymethyl)thiophen-2-yl)ethan-1-one (**6**, 1.0 eq. 570 mg) was dissolved in DCM and added via syringe through the septum. The reaction mixture was stirred overnight (16 h) evolving to room temperature. The resulting mixture was extracted with DCM and filtered using funnel filled with cotton and concentrated *in vacuo* (50 °C, 800 mbar). Concentration of the mixture resulted in a dark brown oil which was purified by flash column chromatography (60% DCM in *n*-pentanes) to afford 1-(5-(iodomethyl)thiophen-2-yl)ethan-1-one (**7**, 514 mg, 52.4% yield) as a dark brown oil.

^{1}H NMR (400 MHz, $CDCl_3$).: δ 7.49 (d, J = 3.8 Hz, 1H), 7.13 (dd, J = 3.9, 0.8 Hz, 1H), 4.66 (s, 2H), 2.52 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$).: δ 190.70, 151.09, 144.28, 132.74, 128.40, 26.74, -4.39.

$\mathbf{R}_f$ = 0.42 (60% EtOAc in *n*-pentanes, visualized with UV)

Ethyl 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoate (**8**):

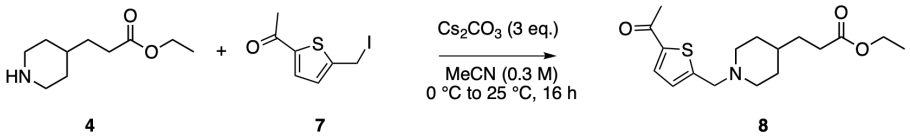


Fig. S4 N-alkylation via nucleophilic substitution SN_2 of Ethyl 3-(piperidin-4-yl)propanoate (7)

1-(5-(iodomethyl)thiophen-2-yl)ethan-1-one (**6**, 1.5 eq., 7.94 mmol) and ethyl 3-(piperidin-4-yl)propanoate (**4**, 1.0 eq., 5.40 mmol) were dissolved in MeCN and transferred to a flame-dried three-necked round bottom flask. The flask was then sealed and purged with three vacuum/argon cycles. Dry MeCN (0.3 M, 4.50 mL) was added using a syringe and the resulting solution stirred until it was homogeneous. The solution was cooled to 0 °C using a water-ice bath. After 10 minutes, Cs_2CO_3 (3.0 eq., 4.05 mmol) was slowly added by briefly lifting one of the septa and performing vacuum/argon cycles between additions. The reaction evolved from 0 °C to room temperature overnight, extracted with EtOAc and washed with H_2O (3 x 30 mL). Brine and sodium hydroxyde solution (1M, 50 mL) were added to basify the aqueous phase. Subsequently, the aqueous phase was washed with EtOAc (3 x 50 mL) and DCM (2 x 50 mL). The

resulting organic layer extracted, dried over $MgSO_4$ and concentrated *in vacuo* (145 mbar, 50 °C) resulting in a crude brown oil. The crude was purified by flash column chromatography (75 % EtOAc, 1% Et_3N 24 % pentan) to afford ethyl 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoate (**8**, 1141 mg, 65% yield) as a light brown oil.

^{1}H NMR (400 MHz, MeOD): δ 7.73 (d, J = 3.8 Hz, 1H), 7.13 (d, J = 3.8 Hz, 1H), 3.96 (s, 2H), 3.57 (q, J = 7.0 Hz, 2H), 3.05 (d, J = 11.1 Hz, 2H), 2.50 (s, 3H), 2.31 (t, J = 11.9 Hz, 2H), 2.16 (t, J = 7.7 Hz, 2H), 1.75 (d, J = 10.5 Hz, 2H), 1.52 (dt, J = 9.0, 6.0 Hz, 2H), 1.32 (dd, J = 7.6, 3.6 Hz, 3H), 1.14 (t, J = 7.0 Hz, 3H).

$\mathbf{R}_f$ = 0.42 (60% EtOAc in *n*-pentanes, visualized with UV)

3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoic acid (**9**):

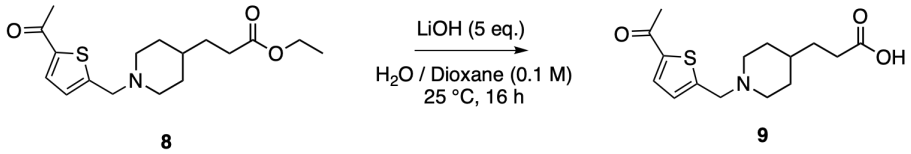


Fig. S5 Hydrolysis of the ethyl ester of 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoate (8)

In a flame-dried round bottom flask ethyl 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoate (**7**, 1.0 eq., 0.49 mmol). The flask was then sealed with a septum and dioxane (0.1 M, 5 mL) was added using a syringe. The resulting solution was stirred until being homogeneous. LiOH (5 eq., 2.45 mmol) was dissolved in H_2O (4.9 M, 0.5 mL) and added to the round bottom flask. The reaction was then stirred at 25 °C for 16 h. After that time, pentane was added, and a precipitate was formed. The crude was concentrated *in vacuo* (800 mbar, 50 °C). The precipitate was re-dissolved in EtOH, filtered through a cotton funnel, concentrated *in vacuo* to afford a light brown oil. Residual solvent in the oil was removed by addition of MeCN (100 mL), filtration through cotton funnel and subsequent concentration *in vacuo* (145 mbar, 50°C) to afford 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoic acid (**9**, 144 mg, quant. yield) as a light-brown solid.

^{1}H NMR (400 MHz, MeOD): δ 7.74 (d, J = 3.8 Hz, 1H), 7.07 (dd, J = 3.8, 0.8 Hz, 1H), 3.79 (s, 2H), 2.95 (d, J = 11.1 Hz, 2H), 2.53 (s, 3H), 2.20 (t, J = 7.7 Hz, 2H), 2.12 (t, J = 10.7 Hz, 2H), 1.80 – 1.72 (m, 2H), 1.60 – 1.52 (m, 2H), 1.38 – 1.22 (m, 3H).

$\mathbf{R}_f$ = 0.17 (50% MeOH in EtOAc, visualized with UV)

3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)-1-(4-methylpiperazin-1-yl)propan-1-one (**2**)

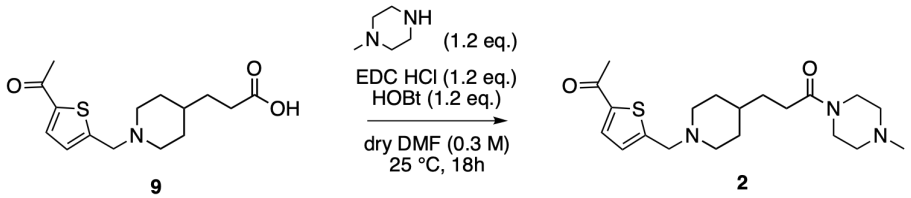


Fig. S6 Amide coupling of 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoic acid (8) with 1-methylpiperazine

3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)propanoic acid (**8**, 1 eq., 1.02 mmol), HOBt (1.2 eq., 1.22 mmol) and EDC HCl (1.2 eq., 1.22 mmol) and a stirring bar are placed in a round-bottomed flask. The flask is then sealed with a septum and purged with three vacuum/nitrogen cycles. Dry DMF (0.3 M, 4 mL) is added and the resulting mixture is stirred for 10 min at room temperature under a nitrogen atmosphere. After that time, commercially available

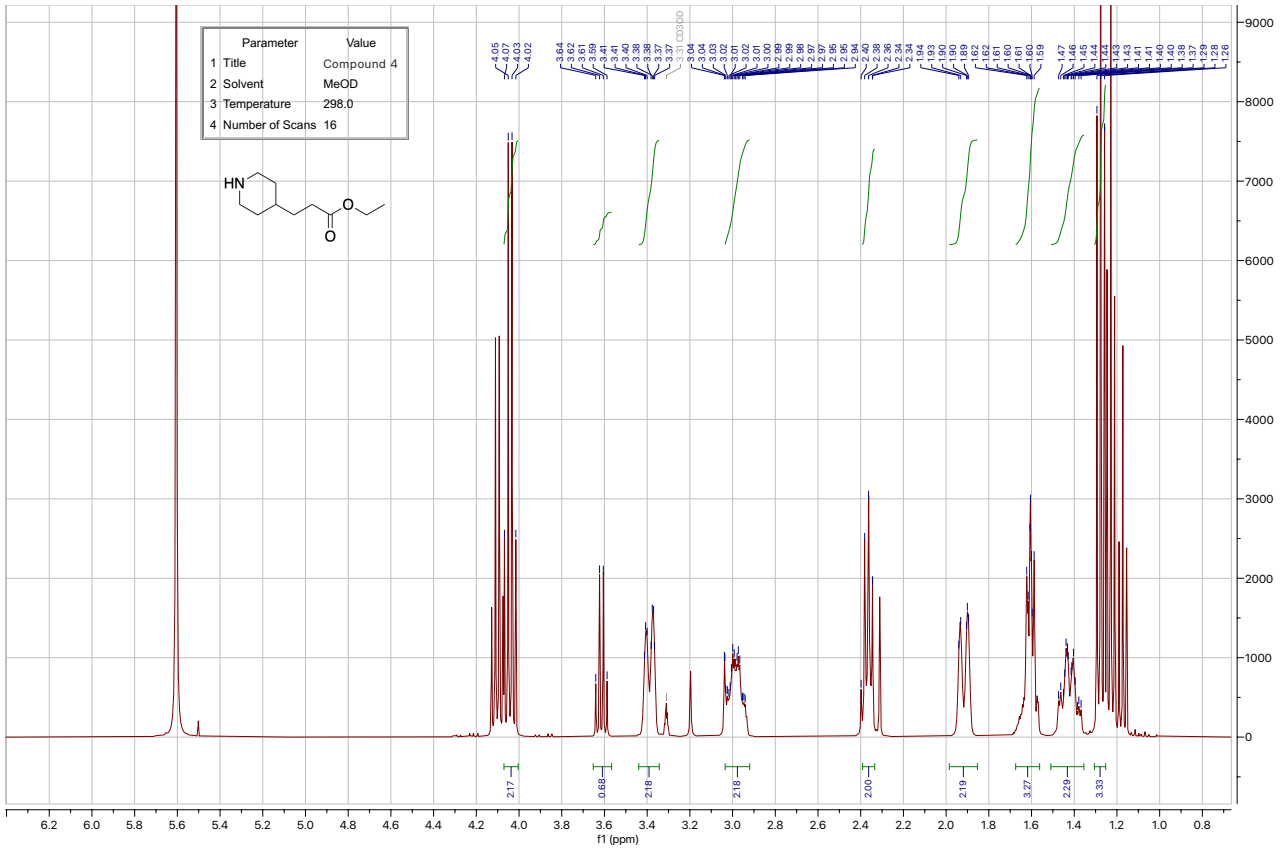
1-methylpiperazine (1.2 eq., 1.22 mmol) is added dropwise. The resulting mixture is stirred at room temperature for 18h. The crude reaction is then reduced under pressure conditions to finally be purified by reversed phase flash column chromatography using 5% MeCN in H_2O as the eluent system. 3-(1-((5-acetylthiophen-2-yl)methyl)piperidin-4-yl)-1-(4-methylpiperazin-1-yl)propan-1-one is eluted the second as a pale yellow oil that solidified after 72h under high vacuum (**2**, 72.20 mg, 0.19 mmol, 16% yield) .

^{1}H NMR (400 MHz, MeOD): δ 7.73 (d, J = 3.8 Hz, 1H), 7.05 (dt, J = 3.7, 0.8 Hz, 1H), 3.75 (d, J = 0.9 Hz, 2H), 3.57 (dt, J = 13.2, 5.4 Hz, 4H), 2.94 (ddd, J = 12.7, 3.9, 2.0 Hz, 2H), 2.53 (s, 3H), 2.48 – 2.38 (m, 6H), 2.31 (s, 3H), 2.08 (td, J = 11.2, 2.5 Hz, 2H), 1.78 – 1.69 (m, 2H), 1.58 – 1.49 (m, 2H), 1.28 (dq, J = 11.4, 3.9 Hz, 3H).

^{13}C NMR (101 MHz, MeOD): δ 193.08, 174.11, 152.59, 144.73, 134.77, 128.96, 58.23, 56.04, 55.54, 54.55, 46.36, 45.96, 42.31, 36.39, 32.98, 32.89, 31.34, 26.48.

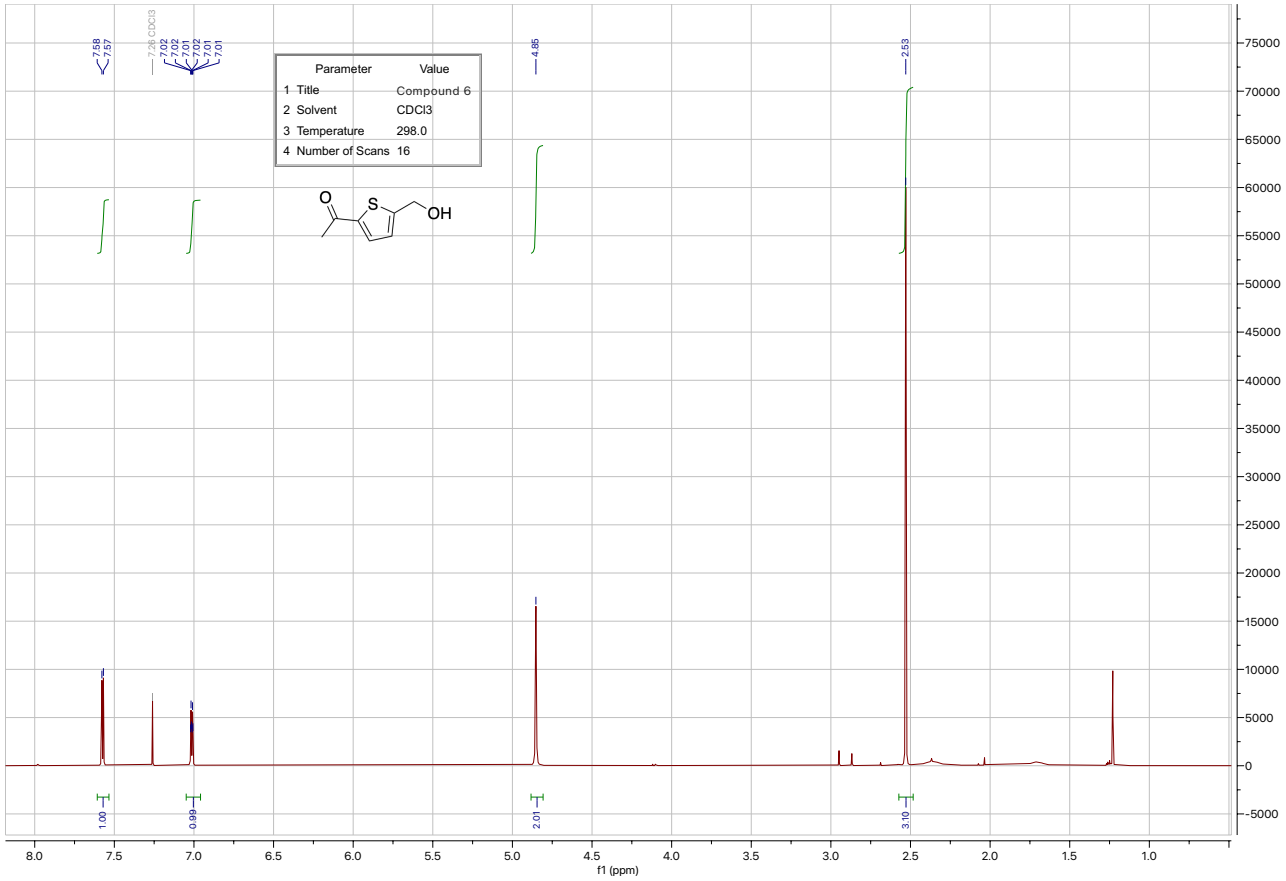
HRMS (ESI): $[M+H]^+$ m/z calcd. for $C_{20}H_{32}N_3O_2S$: 378.2210, found: 378.2205.

S1.4 NMR spectra



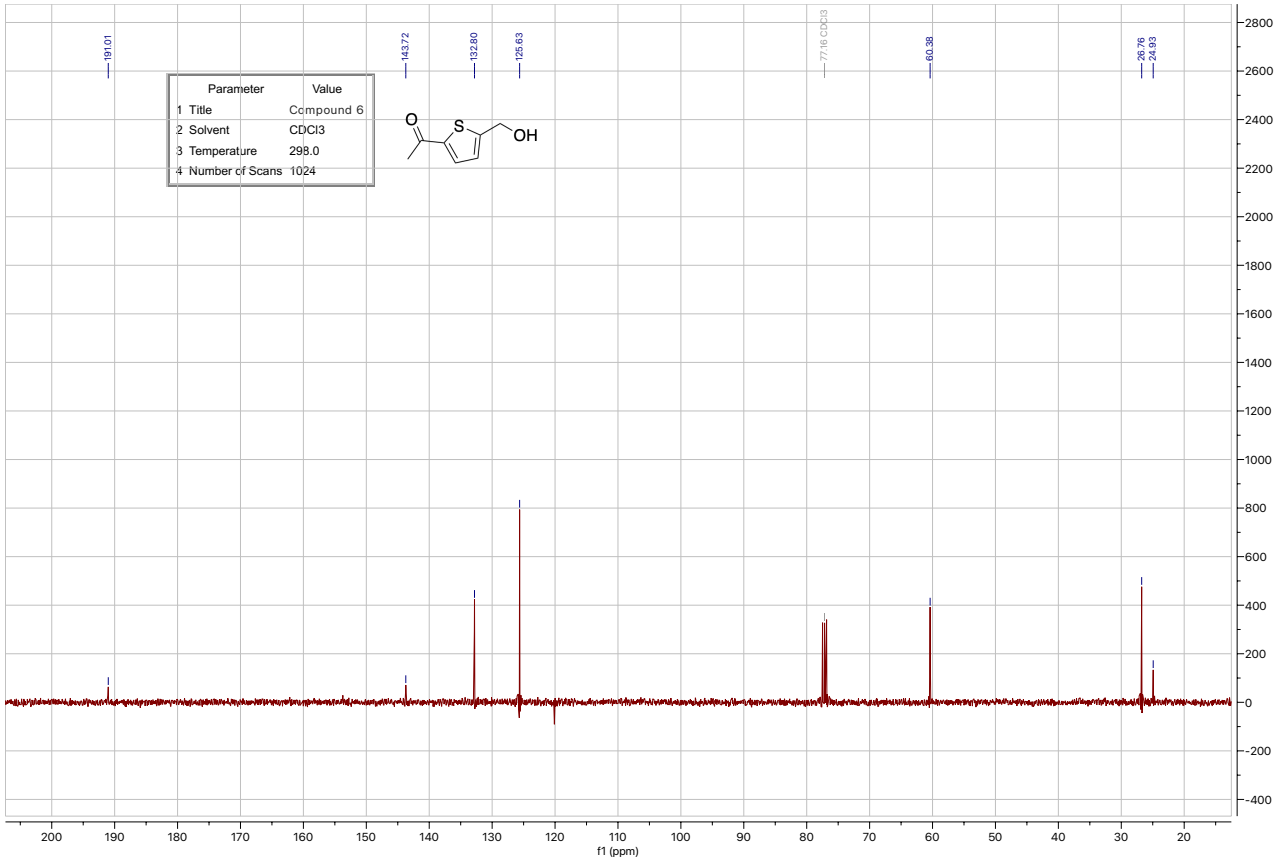
^{1}H NMR (400 MHz, MeOD) δ 4.04 (q, J = 7.1 Hz, 2H), 3.61 (q, J = 7.1 Hz, 1H), 3.43 – 3.34 (m, 2H), 3.05 – 2.91 (m, 2H), 2.39 – 2.33 (m, 2H), 2.00 – 1.87 (m, 2H), 1.67 – 1.56 (m, 3H), 1.42 (dddd, J = 17.4, 14.3, 11.3, 4.2 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H).

Fig. S7 Compound 4, ^{1}H-NMR spectra (400 MHz, MeOD).



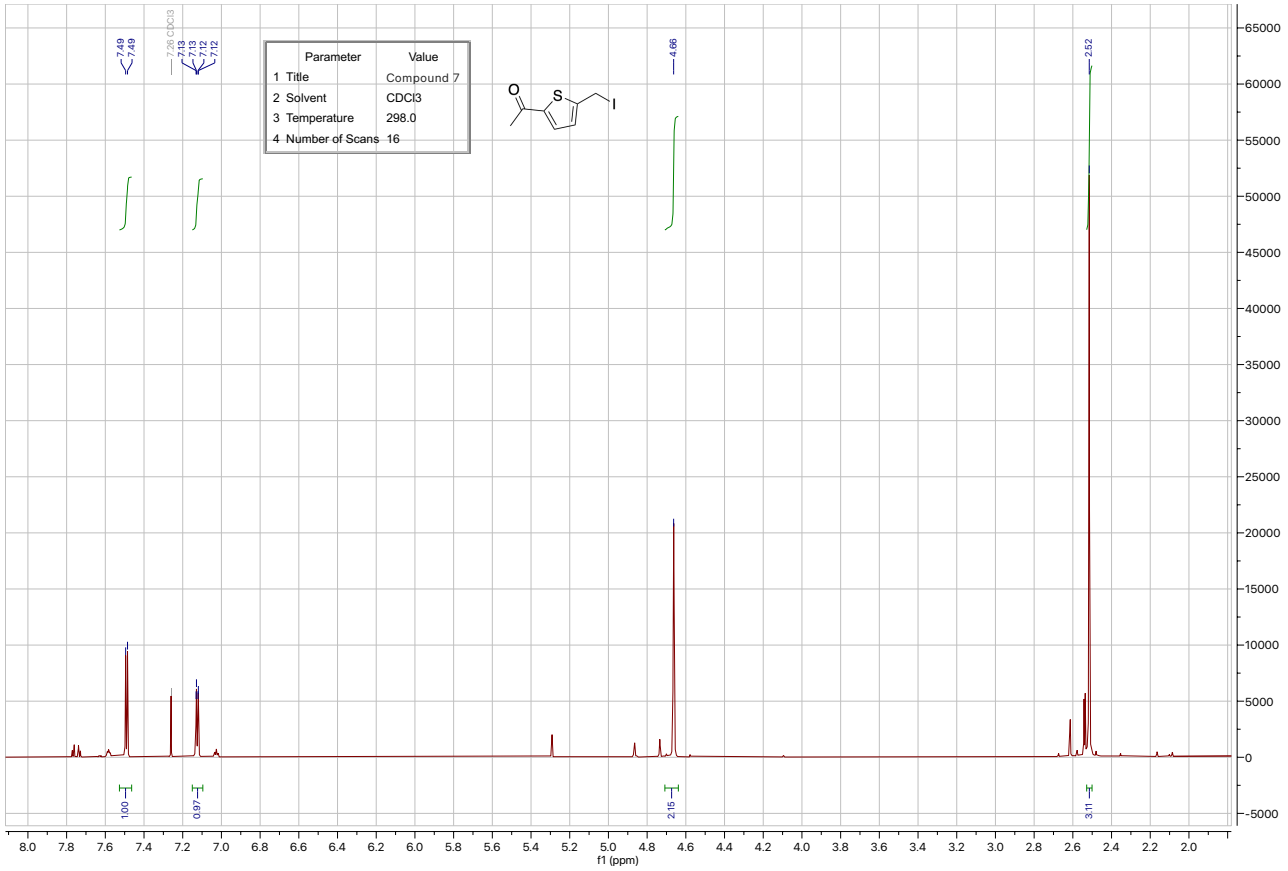
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 3.8 Hz, 1H), 7.01 (dt, J = 3.8, 0.9 Hz, 1H), 4.85 (s, 2H), 2.53 (s, 3H).

Fig. S8 Compound 6, ^{1}H-NMR spectra (400 MHz, $CDCl_3$).



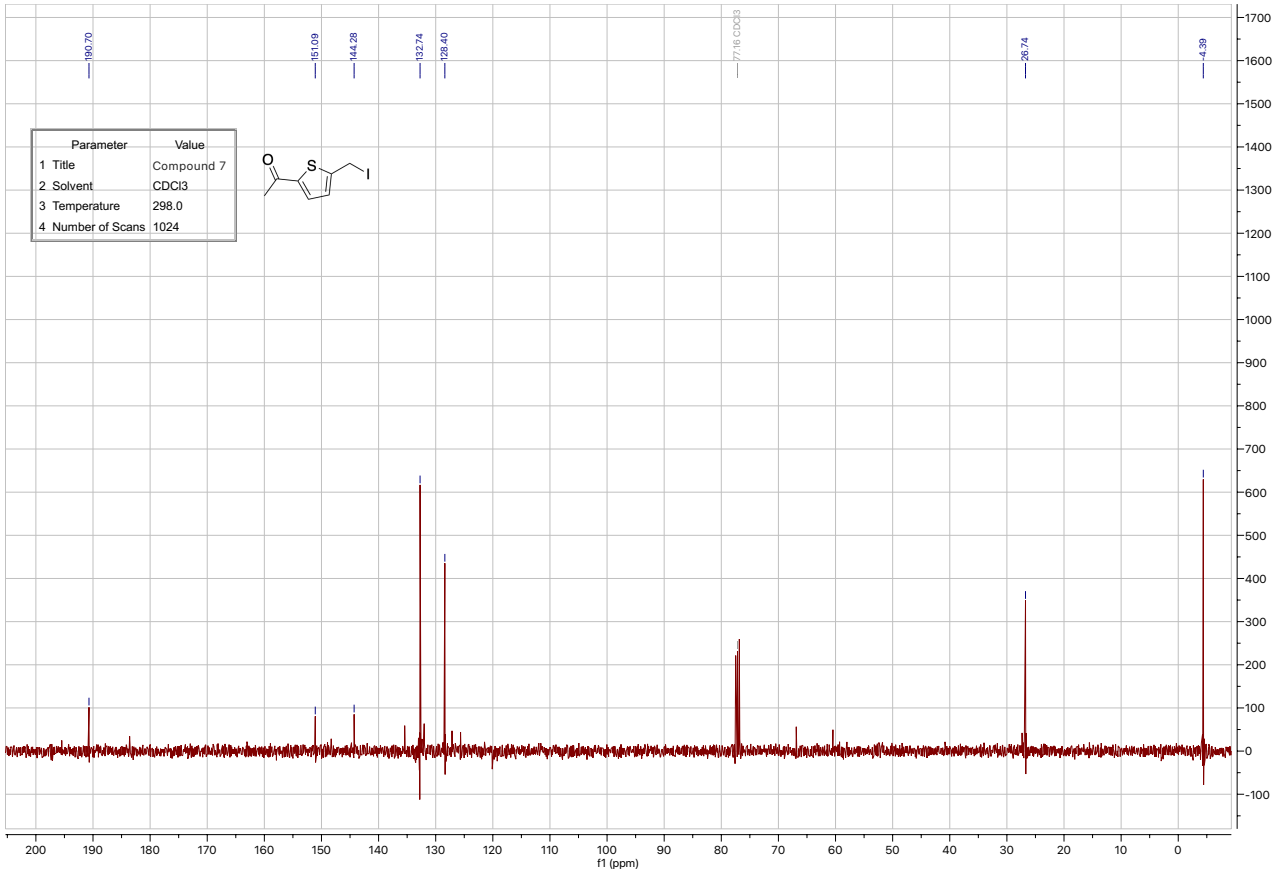
^{13}C NMR (101 MHz, $CDCl_3$) δ 191.01, 143.72, 132.80, 125.63, 60.38, 26.76, 24.93.

Fig. S9 Compound 6, ^{13}C-NMR spectra (101 MHz, $CDCl_3$).



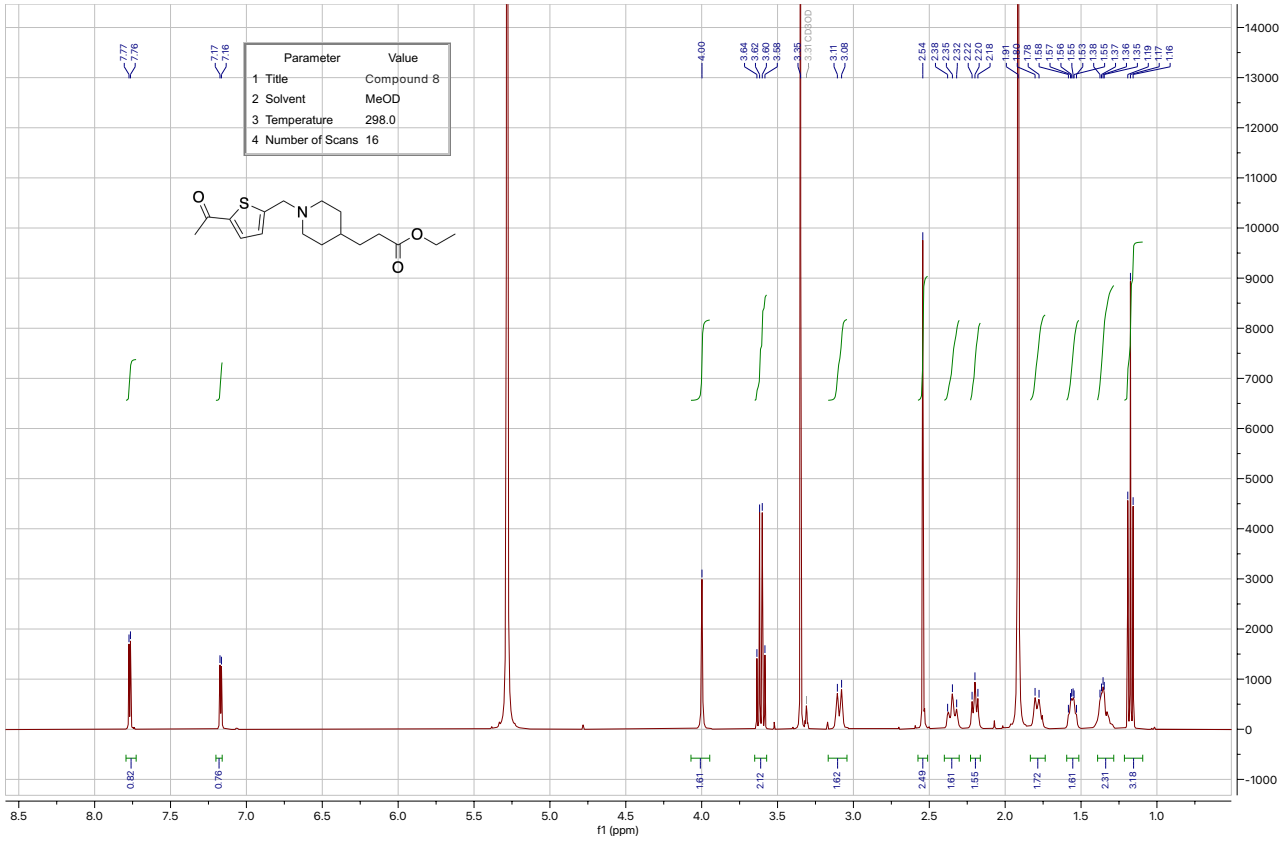
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 3.8 Hz, 1H), 7.13 (dd, J = 3.9, 0.8 Hz, 1H), 4.66 (s, 2H), 2.52 (s, 3H).

Fig. S10 Compound 7, ^{1}H-NMR spectra (400 MHz, $CDCl_3$).



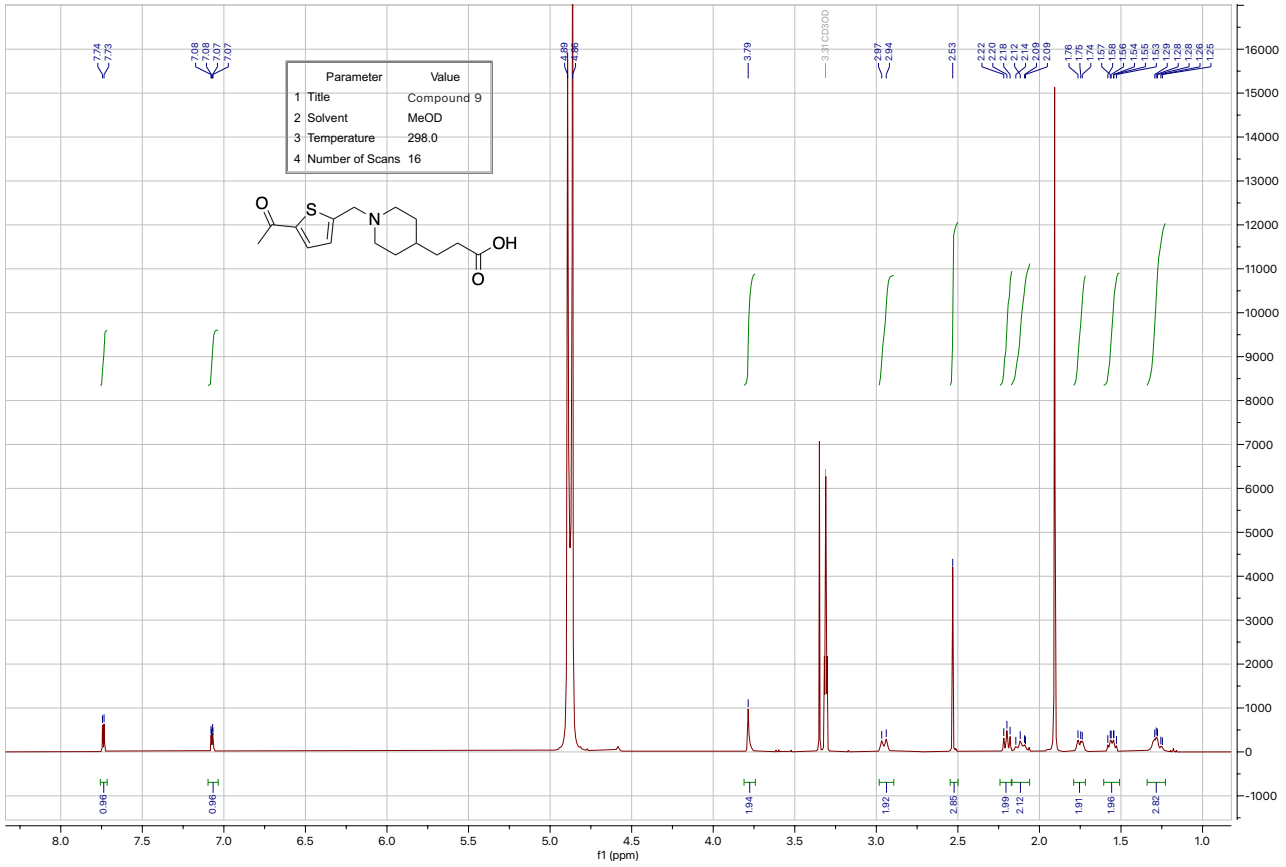
^{13}C NMR (101 MHz, $CDCl_3$) δ 190.70, 151.09, 144.28, 132.74, 128.40, 26.74, -4.39.

Fig. S11 Compound 7, ^{13}C-NMR spectra (101 MHz, $CDCl_3$).



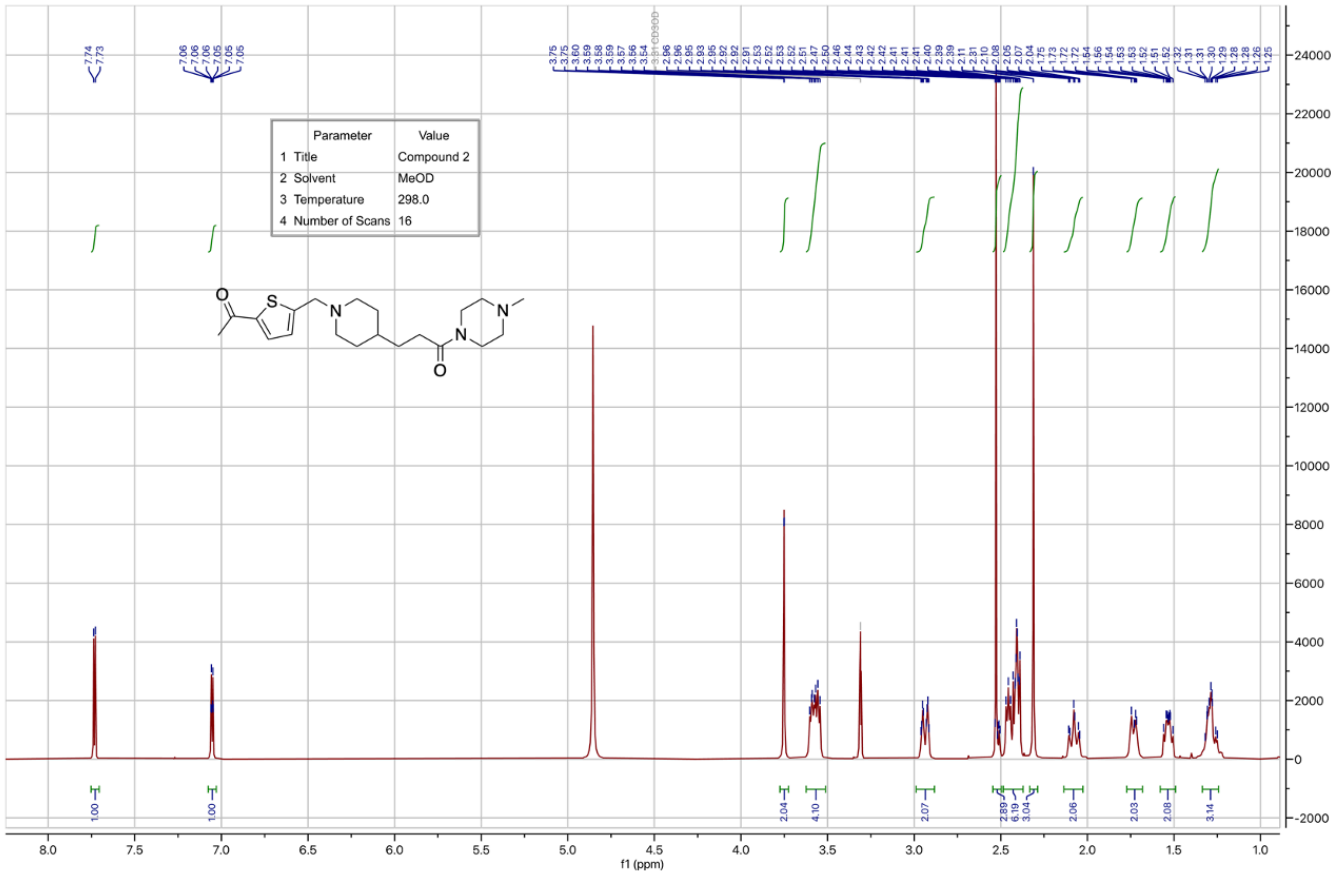
^{1}H NMR (400 MHz, MeOD) δ 7.73 (d, J = 3.8 Hz, 1H), 7.13 (d, J = 3.8 Hz, 1H), 3.96 (s, 2H), 3.57 (q, J = 7.0 Hz, 2H), 3.05 (d, J = 11.1 Hz, 2H), 2.50 (s, 3H), 2.31 (t, J = 11.9 Hz, 2H), 2.16 (t, J = 7.7 Hz, 2H), 1.75 (d, J = 10.5 Hz, 2H), 1.52 (dt, J = 9.0, 6.0 Hz, 2H), 1.32 (dd, J = 7.6, 3.6 Hz, 3H), 1.14 (t, J = 7.0 Hz, 3H).

Fig. S12 Compound 8, ^{1}H-NMR spectra (400 MHz, MeOD).



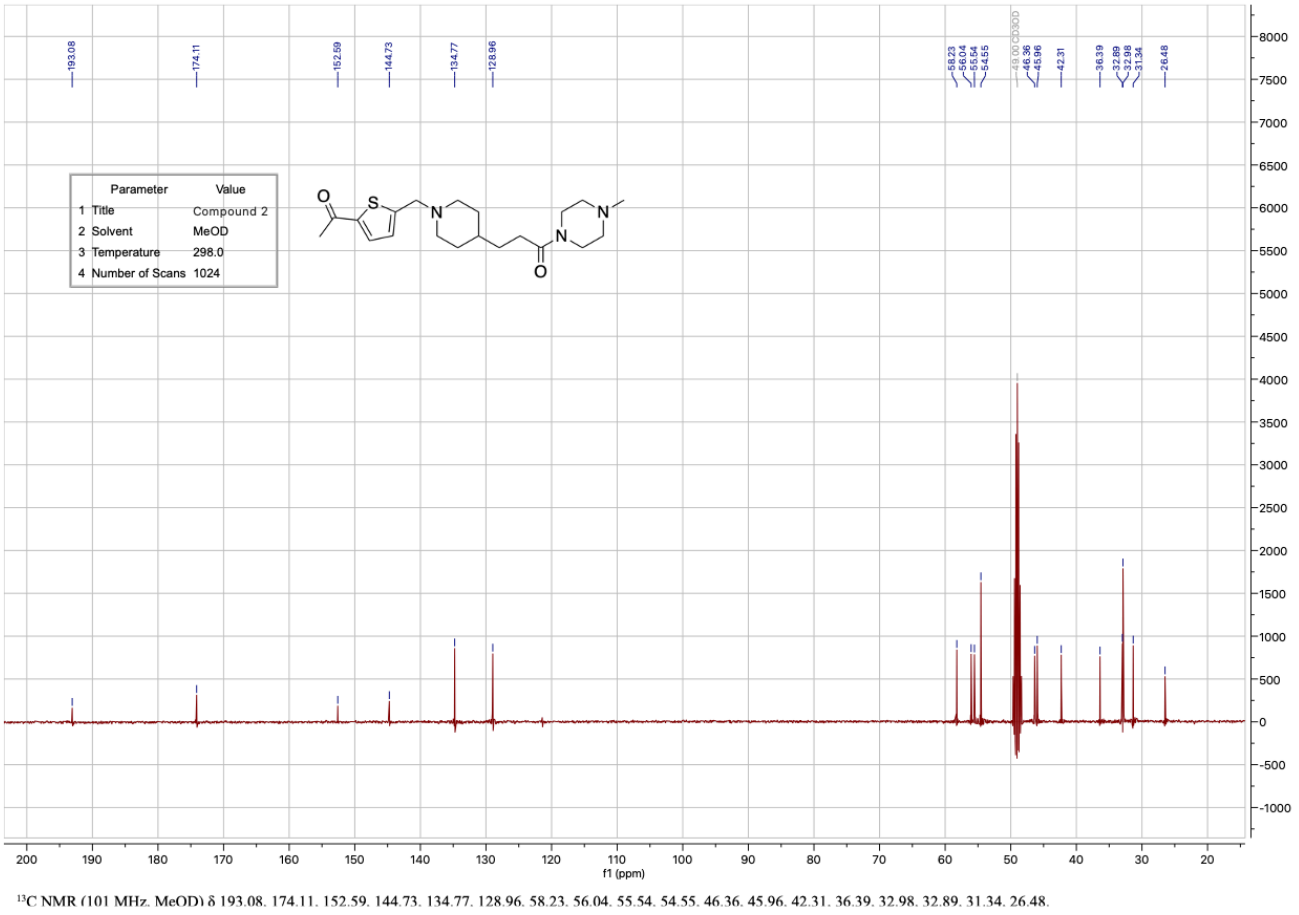
^{1}H NMR (400 MHz, MeOD) δ 7.74 (d, J = 3.8 Hz, 1H), 7.07 (dd, J = 3.8, 0.8 Hz, 1H), 3.79 (s, 2H), 2.95 (d, J = 11.1 Hz, 2H), 2.53 (s, 3H), 2.20 (t, J = 7.7 Hz, 2H), 2.12 (t, J = 10.7 Hz, 2H), 1.80 – 1.72 (m, 2H), 1.60 – 1.52 (m, 2H), 1.38 – 1.22 (m, 3H).

Fig. S13 Compound 9, ^{1}H-NMR spectra (400 MHz, MeOD).



^{1}H NMR (400 MHz, MeOD) δ 7.73 (d, J = 3.8 Hz, 1H), 7.05 (dt, J = 3.7, 0.8 Hz, 1H), 3.75 (d, J = 0.9 Hz, 2H), 3.57 (dt, J = 13.2, 5.4 Hz, 4H), 2.94 (ddd, J = 12.7, 3.9, 2.0 Hz, 2H), 2.53 (s, 3H), 2.48 – 2.38 (m, 6H), 2.31 (s, 3H), 2.08 (td, J = 11.2, 2.5 Hz, 2H), 1.78 – 1.69 (m, 2H), 1.58 – 1.49 (m, 2H), 1.28 (dq, J = 11.4, 3.9 Hz, 3H).

Fig. S14 Compound 2, ^{1}H-NMR spectra (400 MHz, MeOD).



^{13}C NMR (101 MHz, MeOD) δ 193.08, 174.11, 152.59, 144.73, 134.77, 128.96, 58.23, 56.04, 55.54, 54.55, 46.36, 45.96, 42.31, 36.39, 32.98, 32.89, 31.34, 26.48.

Fig. S15 Compound 2, ^{13}C-NMR spectra (101 MHz, $CDCl_3$).