Supporting Information

Discovery of Irreversible Inhibitors Targeting Histone Methyltransferase, SMYD3

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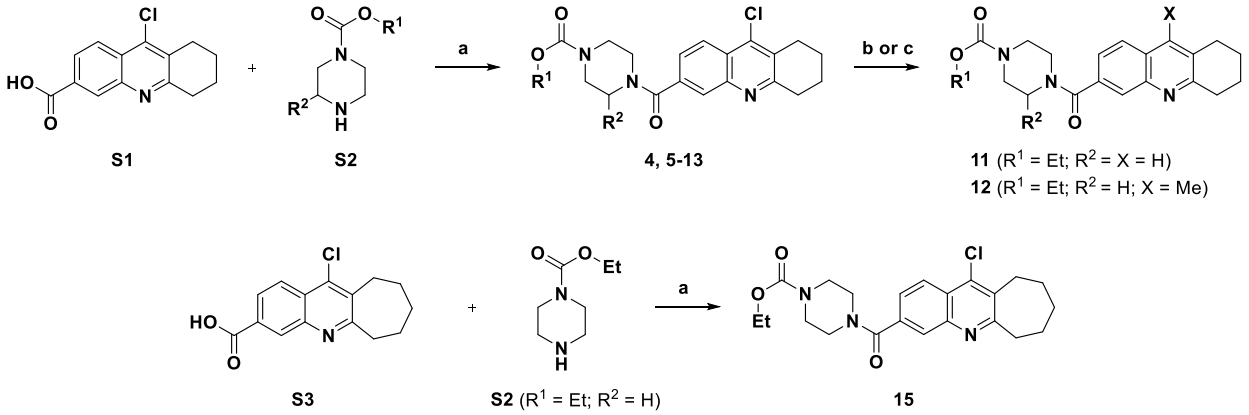
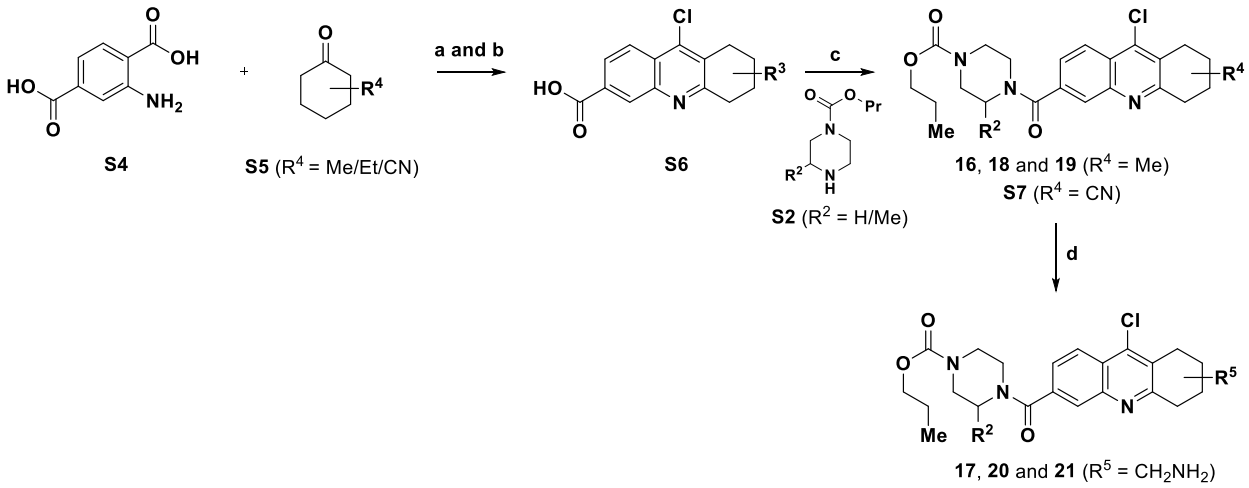
Scheme 1. Synthesis of compounds **4**, **5**–**13** and **15**.[a]



[a]Reagents and conditions: (a) HATU (1.2–1.5 equiv), Et_3N (2 equiv), DMF, 0 ºC, 20 min; (b) $CH_3B(OH)_2$ (1.2 equiv), $Pd(PPh_3)_4$ (0.1 equiv), K_2CO_3 (2 equiv), 1,4-dioxane, 110 ºC, 18 h; (c) 10% Pd/C, H_2, DMF/EtOH (1:1), 15 min.

The tetrahydroacridine compounds **4**, **5**–**13** were synthesized in a single step using amide coupling between commercially available 9-substituted-5,6,7,8-tetrahydroacridine-3-carboxylic acid (**S1**) and piperazine **S2** bearing N1-carbamates with different chain lengths (Scheme 1). In a similar way compound **15** with a 7-membered aliphatic ring was synthesized using commercially available acid **S3**. Compounds **11** and **12** were both synthesized from compound **4** (R^1 = Et and R^2 = H) using Pd-catalyzed hydrogenation and Suzuki cross-coupling with methyl boronic acid respectively.

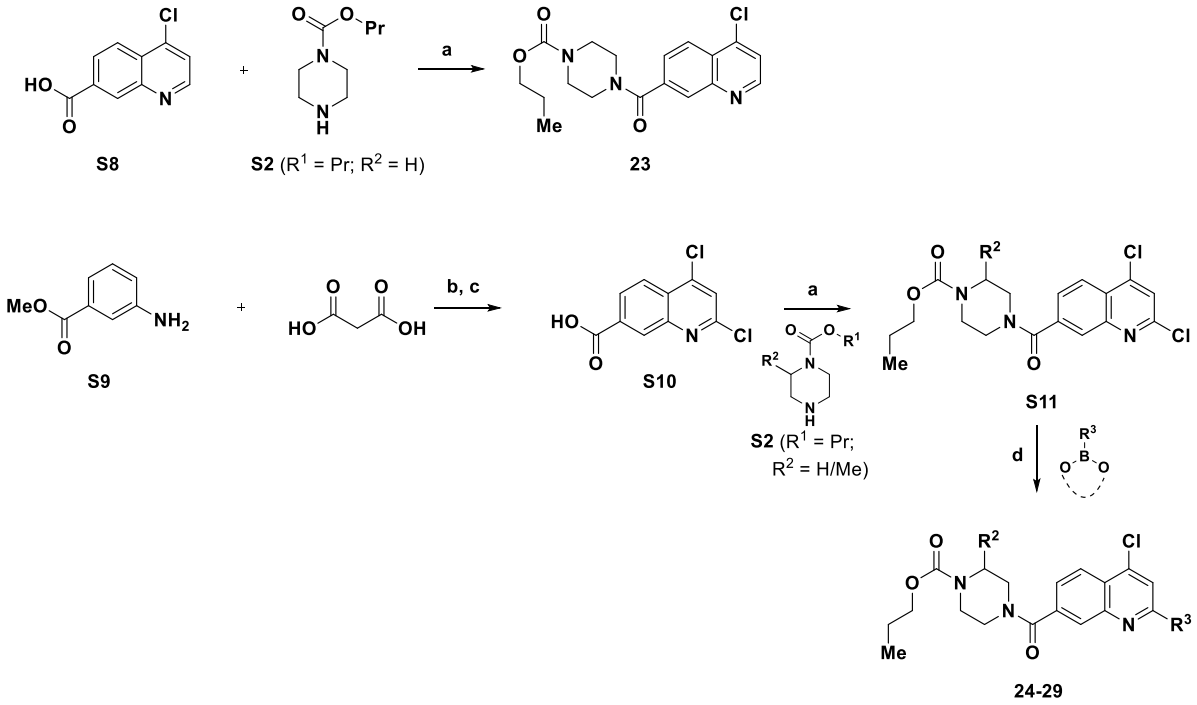
Scheme 2. Synthesis of compounds **16**–**21** bearing R^4/R^5 substituent.[a]



[a]Reagents and conditions: (a) Ph_2O (10 mL/g), 300 ºC, 1–3 h in a sealed tube; (b) $POCl_3$ (10 mL/g), 100 ºC, 4 h; (c) T3P® (2–3 equiv, 50% in ethyl acetate), Et_3N (10 equiv), THF, 0–25 ºC; (d) KBH_4 (4 equiv), Raney nickel (until complete), EtOH/THF, rt.

Compounds substituted at the C-6, -7 or -8 (see Scheme 2) position of the tetrahydroacridine system such as **16**–**21** were synthesized using a Friedländer-type condensation between **S4** and the respective cyclic ketones **S5** (Scheme 2). 4-Substituted ketones **S5** gave exclusively C7-substituted compounds **S6** upon heating with **S4** followed by treatment with $POCl_3$. The resulting acid **S6** was reacted with the respective piperazine **S2** using an amide coupling reaction with propylphosphonic anhydride (T3P®). C6- or 8-substituted compounds were obtained in the same pot upon reaction between **S4** and 3-substituted ketone **S5** as regioisomers. These isomers were separated using chromatography before being taken on further. Amines **17**, **20** and **21** were synthesized from their corresponding nitrile intermediates (collectively labeled as **S7**) via a Raney nickel reduction in the presence of KBH_4 (Wu, B.; Zhang, J.; Yang, M.; Yue, Y.; Ma, L.-J.; Yu, X.-Q. Raney Ni/KBH_4: an efficient and mild system for the reduction of nitriles to amines. *ARKIVOC* **2008**, *7*, 95–102). This was the only condition where the C9-chlorine atom remained intact and the desired product could be isolated.

Scheme 3. Synthesis of quinoline compounds **23** and **24**–**29**.[a]



[a]Reagents and conditions: (a) HATU (1.2–1.5 equiv), Et_3N (2 equiv), DMF, 0 ºC, 20 min; (b) $POCl_3$ (4–10 equiv), 100–120 ºC, 4–12 h; (c) LiOH (10 equiv), MeOH/THF/H_2O, 25–50 ºC, 30 min; (d) R^3-boronic acid (or pinacol ester) (1.2 equiv), $Pd(dppf)_2Cl_2$.DCM (0.1 equiv), K_3PO_4 (3 equiv), 1,4-dioxane/H_2O, 110 ºC, 20 min.

Quinoline compound **23** was synthesized from commercially available 4-chloroquinoline-7-carboxylic acid (**S8**) and piperazine **S2** via amide coupling (Scheme 3). Compounds **24**–**29** were synthesized via Suzuki cross-coupling from the dichloro-intermediate **S11**. The Suzuki cross-coupling proceeded with excellent regioselectivity at the C2-position; the C4-isomer was never observed as a reaction product. Occasionally cross-couplings at both C2 and C4 were observed when excess boronic acid was used or if the reaction was allowed to proceed for long durations. Intermediate **S11** was in turn synthesized using amide coupling between acid **S10** and piperazine **S2**. Acid **S10** was prepared in two-steps, first a Knorr-quinoline synthesis between **S9** and malonic acid, followed by hydrolysis of the ester using LiOH.

General. All reagents were purchased at the highest commercial quality and used as received, unless otherwise stated. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merk silica gel plates (60F-254) using ultraviolet light as visualizing agent and potassium permanganate and heat as developing agents. LC-MS analyses were carried out using an Agilent 1290 Infinity system with a Zorbax Eclipse Plus C18 column (1.8 µm, 50 × 2.1 mm). Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained using a Bruker 400 spectrometer and were calibrated using residual undeuterated solvent as internal reference (DMSO-d_6: ^{1}H NMR = 2.50, ^{13}C NMR = 39.52 ppm). The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, br = broad. The compounds' purities were ≥95% determined by a Varian ProStar HPLC instrument.

General procedure 1A. Amide coupling with piperazine derivatives using HATU. To a mixture of the carboxylic acid (1 equiv), piperazine (1.1–1.2 equiv) was added DMF (0.2 M) and Et_3N (2 equiv). The mixture was cooled to 0 ºC before the addition of HATU (1.5 equiv). After 20 min, the

reaction was quenched by addition of water and then extracted 5 times with ethyl acetate. The combined organic layer was washed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography or preparative HPLC (eluent ACN, water, formic acid 0.1%) to afford the purified product.

General procedure 1B. Amide coupling with piperazine derivatives using T3P®. To a suspension of carboxylic acid (1 equiv) and piperazine (0.5–1.1 equiv) in THF (0.2 M) at 0 °C were added T3P® (50% solution in ethyl acetate) (2–3 equiv) and Et_3N (10 equiv). The resulting mixture was stirred at room temperature for 2 h. The reaction mass was diluted with ethyl acetate and the organic layer was washed with water, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography or preparative HPLC (eluent ACN, water, formic acid 0.1%) to afford the purified product.

Ethyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (4). Compound **4** was prepared according to the general procedure 1A using commercially available 9-chloro-5,6,7,8-tetrahydroacridine-3-carboxylic acid (**S1**) and ethyl piperazine-1-carboxylate to give a pale yellow oil (yield, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.6 Hz, 1 H), 7.94 (d, J = 1.3 Hz, 1 H), 7.65 (dd, J = 8.6, 1.6 Hz, 1 H), 4.06 (q, J = 7.1 Hz, 2 H), 3.74–3.33 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 1.90 (t, J = 2.9 Hz, 4 H), 1.19 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.6, 154.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.5, 124.8, 123.9, 60.9, 46.9, 43.3, 41.4, 33.6, 27.0, 21.9, 21.9, 14.5; MS (ESI) *m/z* 402.2 $[C_{21}H_{24}ClN_3O_3 + H]^+$. Melting point = 98.5–100.0 ºC.

Methyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (5). Compound **5** was prepared according to general procedure 1A using commercially available **S1** and methyl piperazine-1-carboxylate to give a white solid (yield, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.6 Hz, 1 H), 7.94 (d, J = 1.2 Hz, 1 H), 7.65 (dd, J = 8.6, 1.5 Hz, 1 H), 3.62 (s, 3 H), 3.75–

3.34 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 1.90 (t, *J* = 2.8 Hz, 4 H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.3, 160.6, 155.0, 145.4, 140.0, 136.6, 129.9, 126.7, 125.5, 124.9, 123.9, 52.5, 46.9, 43.3, 41.4, 33.6, 27.1, 21.9, 21.9; MS (ESI) *m/z* 388.1 $[C_{20}H_{22}ClN_3O_3 + H]^+$. Melting point = 181.8–182.8 ºC.

Propyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (6). Compound **6** was prepared according to general procedure 1A using commercially available **S1** and propyl piperazine-1-carboxylate to give a yellow solid (yield, 72%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.94 (d, *J* = 1.3 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.6 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz, 2 H), 3.73–3.35 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 1.90 (t, *J* = 2.8 Hz, 4 H), 1.65–1.51 (m, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.3, 160.6, 154.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.5, 124.8, 123.9, 66.4, 46.7, 43.3, 41.4, 33.6, 27.0, 21.9, 21.9, 10.2; MS (ESI) *m/z* 416.2 $[C_{22}H_{26}ClN_3O_3 + H]^+$. Melting point = 135.5–136.9 ºC.

Butyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (7). *Tert*-butyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate was synthesized according to general procedure 1. The crude material was dissolved in 50% TFA/CH_2Cl_2 (10 equiv) and evaporated to dryness after 10 min. Ethyl acetate was added and the organic mixture was basified by saturated sodium bicarbonate. The aqueous layer was further extracted by ethyl acetate and the combined extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography to give (9-chloro-5,6,7,8-tetrahydroacridin-3-yl)(piperazin-1-yl)methanone as a yellow foam (yield, 70% over 2 steps). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.18 (d, *J* = 8.6 Hz, 1 H), 7.87 (d, *J* = 1.3 Hz, 1 H), 7.62 (dd, *J* = 8.6, 1.6 Hz, 1 H), 3.60 (s, 2 H), 3.05 (s, 2 H), 2.98 (s, 2 H), 2.84–2.57 (m, 6 H), 1.89 (t, *J* = 3.0 Hz, 4 H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 167.9, 160.5, 145.5, 139.9, 137.1, 129.7, 126.4, 125.5, 124.7, 123.8, 48.5, 45.8, 45.4, 42.8, 33.5, 27.0, 21.9, 21.9; MS (ESI) *m/z* 330.1 $[C_{18}H_{20}ClN_3O + H]^+$.

To a solution of (9-chloro-5,6,7,8-tetrahydroacridin-3-yl)(piperazin-1-yl)methanone (48.8 mg, 0.148 mmol) in CH_2Cl_2 (1.1 mL) and Et_3N (30 µL, 0.215 mmol, 1.5 equiv) was added n-butyl chloroformate (20 µL, 0.154 mmol, 1.04 equiv). After 30 min, saturated ammonium chloride was added and the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate and dried under reduced pressure. The crude material was purified by column chromatography (0–75% ethyl acetate/hexanes) to give compound **7** as a pale yellow oil (49 mg, 78%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.94 (d, *J* = 1.3 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.6 Hz, 1 H), 4.02 (t, *J* = 6.5 Hz, 2 H), 3.74–3.34 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 1.90 (t, *J* = 3.0 Hz, 4 H), 1.62–1.48 (m, 2 H), 1.33 (dd, *J* = 14.9, 7.5 Hz, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 168.3, 160.5, 154.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.5, 124.8, 123.9, 64.7, 46.8, 43.1, 41.4, 33.5, 30.6, 27.0, 21.9, 21.9, 18.6, 13.6; MS (ESI) *m/z* 430.2 $[C_{23}H_{28}ClN_3O_3 + H]^+$.

1-(4-(9-Chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazin-1-yl)pentan-1-one (8). To a solution of (9-chloro-5,6,7,8-tetrahydroacridin-3-yl)(piperazin-1-yl)methanone (51.8 mg, 0.157 mmol) in CH_2Cl_2 (1.2 mL) and Et_3N (40 µL, 0.287 mmol, 1.8 equiv) was added valeroyl chloride (20 µL, 0.165 mmol, 1.06 equiv). After 30 min, saturated ammonium chloride was added and the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate and dried under reduced pressure. The crude material was purified by column chromatography (0–75% ethyl acetate/hexanes) to give compound **8** as a pale yellow oil (43.5 mg, 67%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.20 (d, *J* = 8.6 Hz, 1 H), 7.95 (d, *J* = 1.3 Hz, 1 H), 7.66 (dd, *J* = 8.6, 1.5 Hz, 1 H), 3.76–3.36 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 2.33 (br s, 2 H), 1.90 (t, *J* = 2.9 Hz, 4 H), 1.53–1.41 (m, 2 H), 1.36–1.18 (m, 2 H), 0.87 (t, *J* = 8.0 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.9, 168.2, 160.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.6, 124.8, 123.9, 47.0, 44.8, 41.9, 41.0, 33.6, 32.0, 27.0, 26.9, 21.9, 21.9, 13.8; MS (ESI) *m/z* 414.2 $[C_{23}H_{28}ClN_3O_2 + H]^+$.

2-Aminoethyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (9). *Tert*-butyl *N*-(2-hydroxyethyl)carbamate (161 mg, 1 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and Et_3N (0.28 mL. 2.0 mmol, 2 equiv). 4-Nitrophenyl chloroformate (202 mg, 1 mmol, 1 equiv) was added and the mixture was stirred at room temperature for 15 min. The mixture was concentrated under reduced pressure to give crude *tert*-butyl (2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)carbamate which was used without further purification.

To a solution of (9-chloro-5,6,7,8-tetrahydroacridin-3-yl)(piperazin-1-yl)methanone (164.9 mg, 0.50 mmol) in CH_2Cl_2 (5.0 mL) was added crude *tert*-butyl (2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)carbamate (163.2 mg, 0.50 mmol, 1 equiv). After 30 min of stirring at room temperature, another portion of carbamate (163.2 mg, 0.50 mmol, 1 equiv) was added. After 2 h, CH_2Cl_2 (100 mL) was added and the organic layer was washed with saturated sodium bicarbonate, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography (0–3% MeOH/CH_2Cl_2) to give Boc-**9** as a yellow gum (100 mg, 39%). MS (ESI) *m/z* 517.2 $[C_{40}H_{51}ClN_6O_{12} + H]^+$.

To a solution of Boc-**9** (100 mg, 0.193 mmol) in CH_2Cl_2 (5.0 mL) was added TFA (0.5 mL, 6.53 mmol, 33 equiv) and stirred at room temperature for 2 h. CH_2Cl_2 (50 mL) was added and the organic layer was washed with saturated sodium bicarbonate, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by preparative HPLC (30% ACN/H_2O; 0.1% formic acid) to afford **9** as a yellow oil (50 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.94 (d, *J* = 1.3 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.6 Hz, 1 H), 3.97 (t, *J* = 5.8 Hz, 2 H), 3.78–3.13 (m, 10 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 2.75 (s, 2 H), 1.90 (t, *J* = 2.8 Hz, 4 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.6, 154.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.5, 124.8, 123.9, 67.2, 46.8, 43.3, 43.1, 41.3, 40.5, 33.6, 27.0, 21.9, 21.9; MS (ESI) *m/z* 417.1 $[C_{21}H_{25}ClN_4O_3 + H]^+$.

4-Amino-1-(4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazin-1-yl)butan-1-one (10). Fmoc-**10** was synthesized via general procedure 1A using (9-chloro-5,6,7,8-tetrahydroacridin-3-yl)(piperazin-1-yl)methanone and commercially available *N*-Fmoc-$_{DL}$-4-amino-butyric acid. The crude material (0.1379 mmol, assume quantitative) was dissolved in 50% piperidine (0.15 mL, 1.52 mmol, 11 equiv) in CH_2Cl_2 (0.15 mL). After 1 h, the mixture was acidified with formic acid and purified directly via preparative HPLC (30% ACN/H_2O; 0.1% formic acid) to afford compound **10** as a colorless oil (37.4 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 8.6 Hz, 1 H), 7.95 (d, J = 1.0 Hz, 1 H), 7.66 (dd, J = 8.6, 1.3 Hz, 1 H), 3.76–3.38 (m, 8 H), 3.06 (s, 2 H), 2.99 (s, 2 H), 2.53 (s, 2 H), 2.35 (br s, 2 H), 1.90 (t, J = 2.7 Hz, 4 H), 1.56 (dt, J = 13.3, 6.6 Hz, 2 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.0, 168.2, 160.6, 145.4, 139.9, 136.6, 129.8, 126.7, 125.6, 124.8, 123.9, 47.1, 44.7, 41.9, 41.1, 33.6, 29.9, 28.8, 27.0, 21.9, 21.9; MS (ESI) *m/z* 415.2 $[C_{22}H_{27}ClN_4O_2 + H]^+$.

Ethyl 4-(5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (11). Compound **4** (49.5 mg, 0.123 mmol) was dissolved in DMF (1 mL) and EtOH (1 mL). 10% Pd/C (50 mg) was added to the solution and H_2 was bubbled through for 15 min. The mixture was filtered and purified by preparative HPLC (50% ACN/H_2O; 0.1% formic acid) to give compound **11** as a colorless oil (26 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (s, 1 H), 7.91 (d, J = 8.4 Hz, 1 H), 7.85 (d, J = 0.6 Hz, 1 H), 7.48 (dd, J = 8.3, 1.4 Hz, 1 H), 4.06 (q, J = 7.1 Hz, 2 H), 3.74–3.32 (m, 8 H), 3.03 (t, J = 6.5 Hz, 2 H), 2.97 (t, J = 6.2 Hz, 2 H), 1.98–1.88 (m, 2 H), 1.82 (dd, J = 11.4, 6.1 Hz, 2 H), 1.19 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.9, 160.0, 154.6, 145.2, 135.5, 134.4, 132.0, 127.7, 127.1, 126.1, 124.1, 60.9, 46.8, 43.2, 41.5, 32.9, 28.5, 22.7, 22.3, 14.5; MS (ESI) *m/z* 368.2 $[C_{21}H_{25}N_3O_3 + H]^+$.

Propyl (*RS*)-4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)-3-methylpiperazine-1-carboxylate (13). *Tert*-butyl 4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)-3-methylpiperazine-1-carboxylate was synthesized using general procedure 1A with compound **S1** and commercially available *tert*-butyl 3-methylpiperazine-1-carboxylate (yield, 90%). To a solution of this compound (220 mg, 0.496 mmol) in CH_2Cl_2 (10 mL) was added TFA (1.0 mL, 13.0 mmol, 26 equiv). The mixture was stirred for 24

h before diluting with CH_2Cl_2 (50 mL). The organic layer was washed with saturated sodium bicarbonate, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material (120 mg, 0.349 mmol) was dissolved in CH_2Cl_2 (10 mL) before Et_3N (80 µL, 0.574 mmol, 1.6 equiv) and propyl chloroformate (50 µL, 0.428 mmol, 1.2 equiv) were added. After 30 min, the mixture was quenched by saturated ammonium chloride and extract with CH_2Cl_2. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography (0–2% MeOH/CH_2Cl_2) to give **13** as a white solid (60 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.91 (d, *J* = 1.3 Hz, 1 H), 7.63 (dd, *J* = 8.6, 1.5 Hz, 1 H), 4.06–3.68 (m, 5 H), 3.26–2.87 (m, 7 H), 1.90 (t, *J* = 2.9 Hz, 4 H), 1.57 (dt, *J* = 14.1, 7.0 Hz, 2 H), 1.16 (d, *J* = 5.3 Hz, 3 H), 0.89 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.6, 155.1, 145.5, 139.9, 137.0, 132.2, 129.8, 126.2, 125.2, 124.7, 124.0, 66.4, 47.4, 43.2, 33.6, 27.0, 21.9, 21.9, 15.2, 10.2; MS (ESI) *m/z* 430.2 $[C_{23}H_{28}ClN_3O_3 + H]^+$. Melting point = 92.6–93.8 ºC.

Propyl (*R*)-4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)-3-methylpiperazine-1-carboxylate (13). Compound (*R*)-**13** was synthesized as above. $[\alpha]_D^{25}$ = –29.4 (*c* 1.0, $CHCl_3$).

Propyl (*S*)-4-(9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)-3-methylpiperazine-1-carboxylate (13). Compound (*S*)-**13** was synthesized as above. $[\alpha]_D^{25}$ = +32.1 (*c* 1.0, $CHCl_3$).

Ethyl 4-(9-methyl-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (12). Compound **4** (50 mg, 0.124 mmol), methyl boronic acid (8.9 mg, 0.149 mmol, 1.2 equiv), potassium carbonate (34.3 mg, 0.248 mmol, 2 equiv) and $Pd(PPh_3)_4$ (17.2 mg, 0.015 mmol, 0.1 equiv) were charged with previously degassed 1,4-dioxane (1.5 mL) and heated at 110 ºC for 18 h. The crude mixture was purified by preparative HPLC (50% ACN/H_2O; 0.1% formic acid) to give compound **12** as a white solid (12 mg, 25%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.6 Hz, 1 H), 7.83 (d, *J* = 1.4 Hz, 1 H), 7.50 (dd, *J* = 8.6, 1.6 Hz, 1 H), 4.06 (q, *J* = 7.1 Hz, 2 H), 3.77–3.34 (m, 8 H), 3.01 (s, 2 H), 2.89 (s, 2 H), 2.55 (s, 3 H), 1.92–1.82 (m, 4 H), 1.19 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8, 159.2,

154.6, 144.7, 141.1, 135.1, 129.7, 126.8, 124.4, 123.8, 60.9, 43.3, 33.9, 26.4, 22.6, 22.2, 14.5, 13.2; MS (ESI) *m/z* 382.5 $[C_{22}H_{27}N_3O_3 + H]^+$. Melting point = 100.2–102.3 ºC.

Ethyl 4-(11-chloro-7,8,9,10-tetrahydro-6H-cyclohepta[b]quinoline-3-carbonyl)piperazine-1-carboxylate (15). Compound **15** was synthesized according to general procedure 1A using commercially available acid **S3** and ethyl piperazine-1-carboxylate to give a tan solid (yield, 19%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.95 (d, *J* = 1.3 Hz, 1 H), 7.67 (dd, *J* = 8.6, 1.6 Hz, 1 H), 4.06 (q, *J* = 7.1 Hz, 2 H), 3.74–3.34 (m, 8 H), 3.22 (d, *J* = 7.2 Hz, 4 H), 1.91–1.81 (m, 2 H), 1.71 (br s, 4 H), 1.19 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.2, 165.7, 154.6, 145.3, 138.2, 136.6, 134.8, 126.9, 125.7, 124.9, 124.6, 60.9, 46.8, 43.2, 41.4, 31.0, 29.7, 27.0, 26.3, 14.5; MS (ESI) *m/z* 416.2 $[C_{22}H_{26}ClN_3O_3 + H]^+$. Melting point = 50.8–52.5 ºC.

General procedure 2. Synthesis of compound S6. A solution of 2-amino-terephthalic acid (**S4**) (1 equiv) and ketone **S5** (1.2 equiv) were mixed in Ph_2O (10 mL/g) and heated to 300 ºC for 1–3 h in a sealed tube. The reaction was cooled to room temperature and diluted with hexanes. The resulting solid was collected by filtration and washed with hexanes to afford the crude cyclized product. The cyclized product (1 equiv) was heated at 100 ºC in the presence of $POCl_3$ (10 mL/g) for 4 h. The reaction was cooled and concentrated under reduced pressure. Cold water was added and stirred until a free solid was formed. The solid was collected by filtration, washed with hexanes and dried to afford compound **S6** which was used without further purification.

Propyl 4-(9-chloro-7-methyl-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (16). Compound **16** was synthesized according to general procedure 2 using **S4** and 4-methylcyclohexanone, followed by general procedure 1B with propyl piperazine-1-carboxylate as a tan solid (yield, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.94 (d, *J* = 1.0 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.5 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz, 2 H), 3.80–3.36 (m, 9 H), 3.23–3.02 (m, 3 H), 1.99 (br d, *J* = 10.2 Hz, 2 H), 1.56 (dt, *J* = 15.4, 6.9 Hz, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H), 0.89 (t, *J* = 7.3 Hz, 3 H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.3, 154.7, 145.5, 139.8, 136.6, 129.5, 126.7, 125.5, 124.8, 123.9, 66.4, 46.8, 43.3, 41.4, 35.3, 33.2, 30.0, 28.2, 21.9, 21.5, 10.2; MS (ESI) *m/z* 430.2 [$C_{23}H_{28}ClN_3O_3$ + H]$^+$. Melting point = 48.0–49.5 ºC.

Propyl 4-(9-chloro-8-methyl-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (18). Compound **18** was synthesized according to general procedure 2 using **S4** and 3-methylcyclohexanone, followed by general procedure 1B with propyl piperazine-1-carboxylate and was isolated as the minor product in the form of a yellow solid (yield, 5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, *J* = 8.6 Hz, 1 H), 7.93 (d, *J* = 1.1 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.5 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz, 2 H), 3.75–3.43 (m, 8 H), 3.21–2.92 (m, 3 H), 2.13–1.97 (m, 1 H), 1.97–1.80 (m, 3 H), 1.58 (dd, *J* = 14.1, 6.8 Hz, 2 H), 1.28 (d, *J* = 7.0 Hz, 3 H), 0.89 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.1, 154.7, 145.5, 140.0, 136.8, 134.4, 126.6, 125.6, 125.1, 124.2, 66.5, 46.9, 43.4, 41.3, 33.2, 30.2, 28.8, 21.9, 19.9, 17.1, 10.3; MS (ESI) *m/z* 430.9 [$C_{23}H_{28}ClN_3O_3$ + H]$^+$. Melting point = 36.8–38.0 ºC.

Propyl 4-(9-chloro-6-methyl-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (19). Compound **19** was synthesized according to general procedure 2 using **S4** and 3-methylcyclohexanone, followed by general procedure 1B with propyl piperazine-1-carboxylate and was isolated as the major product in the form of a yellow solid (yield, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.6 Hz, 1 H), 7.94 (d, *J* = 1.3 Hz, 1 H), 7.65 (dd, *J* = 8.6, 1.6 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz, 2 H), 3.75–3.36 (m, 8 H), 3.20–3.07 (m, 2 H), 2.98–2.83 (m, 1 H), 2.69 (dd, *J* = 17.3, 10.8 Hz, 1 H), 2.00 (br s, 2 H), 1.66–1.42 (m, 3 H), 1.10 (d, *J* = 6.4 Hz, 3 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3, 160.3, 154.6, 145.5, 139.9, 136.6, 129.2, 126.7, 125.6, 124.8, 123.8, 66.4, 46.8, 43.2, 41.7, 41.5, 29.8, 28.1, 26.6, 21.9, 21.1, 10.2; MS (ESI) *m/z* 430.9 [$C_{23}H_{28}ClN_3O_3$ + H]$^+$. Melting point = 39.2–39.8 ºC.

General procedure 3. Reduction of nitrile 25 to give amines 26–28. To a mixture of KBH_4 (4 equiv) in dry ethanol (20 mL) was added Raney Ni (moist weight, approximately 1 equiv). Nitrile **25** (1

equiv) was added and stirred at room temperature. Upon full consumption of **25** as monitored by LC-MS, the mixture was decanted and filtered and directly purified via preparative HPLC (ACN, water and 0.1% formic acid) to afford amines **26–28**.

Propyl 4-(7-(aminomethyl)-9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (17). Propyl 4-(9-chloro-7-cyano-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate was synthesized according to general procedure 2 using **S4** and 4-oxocyclohexanonecarbonitrile, followed by general procedure 1B with propyl piperazine-1-carboxylate and was isolated as the major product in the form of an yellow solid (yield, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.4 Hz, 1 H), 7.98 (s, 1 H), 7.69 (dd, J =1.6, 1.6 Hz, 1 H), 3.96 (t, J = 6.4 Hz, 2 H), 3.66–3.31 (m, 8 H), 3.21–3.16 (m, 4 H), 2.32–2.16 (m, 2 H), 1.60–1.55 (m, 3 H), 0.89 (t, J = 7.6 Hz, 3 H); MS (ESI) *m/z* 441.2 $[C_{23}H_{25}ClN_4O_3 + H]^+$.

Compound **17** was synthesized according to general procedure 3 using the above intermediate. It was isolated as an off-white solid (yield, 20%). ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) δ 8.20 (d, J = 8.6 Hz, 1 H), 7.94 (d, J = 1.0 Hz, 1 H), 7.64 (dd, J = 8.6, 1.2 Hz, 1 H), 4.00 (t, J = 6.6 Hz, 2 H), 3.59–3.42 (m, 8 H), 3.30–3.12 (m, 4 H), 2.73–2.56 (m, 3 H), 2.16–2.04 (m, 1 H), 1.86 (s, 1 H), 1.67–1.49 (m, 4 H), 0.91 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, 80 ºC, DMSO-d_6) δ 168.1, 160.4, 154.4, 145.3, 139.6, 136.3, 129.3, 126.4, 125.0, 124.6, 123.5, 66.1, 46.6, 43.0, 36.3, 32.6, 30.9, 25.3, 21.5, 9.7; MS (ESI) *m/z* 445.2 $[C_{23}H_{29}ClN_4O_3 + H]^+$. Melting point = 62.0–64.0 ºC.

Propyl 4-(6-(aminomethyl)-9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate (20). Propyl 4-(9-chloro-6-cyano-5,6,7,8-tetrahydroacridine-3-carbonyl)piperazine-1-carboxylate was synthesized according to general procedure 2 using **S4** and 3-oxocyclohexanonecarbonitrile, followed by general procedure 1B with propyl piperazine-1-carboxylate and was isolated as the major product in the form of an yellow solid (yield, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 8.0 Hz, 1 H), 7.99 (d, J = 0.8 Hz, 1 H), 7.70 (dd, J = 1.2, 8.4 Hz, 1 H), 3.97 (t, J

= 6.8 Hz, 2 H), 3.75–3.28 (m, 11 H), 3.09 (t, *J* = 6.6 Hz, 2 H), 2.30–2.15 (m, 2 H), 1.65–1.50 (m, 2 H), 0.89 (t, *J* = 7.4 Hz, 3 H); MS (ESI) *m/z* 441.2 $[C_{23}H_{25}ClN_4O_3 + H]^+$.

Compound **20** was synthesized according to general procedure 3 using the above intermediate. It was isolated as an off-white solid (yield, 14%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.4 Hz, 1 H), 7.94 (s, 1 H), 7.65 (dd, *J* = 1.6 Hz, 1 H), 3.98 (t, *J* = 6.4 Hz, 3 H), 3.71–3.20 (m, 8 H), 3.21–3.11 (m, 2 H), 2.91–2.82 (m, 1 H), 2.74–2.66 (m, 1 H), 2.62–2.61 (m, 2 H), 2.40–1.84 (m, 4 H), 1.61–1.47 (m, 3 H), 0.89 (t, *J* = 7.2 Hz, 3 H); MS (ESI) *m/z* 445.3 $[C_{23}H_{25}ClN_4O_3 + H]^+$.

Propyl (3*S*)-4-(6-(aminomethyl)-9-chloro-5,6,7,8-tetrahydroacridine-3-carbonyl)-3-methylpiperazine-1-carboxylate (21). Compound **21** was synthesized the same way as compound **20** but propyl (*S*)-3-methylpiperazine-1-carboxylate was used in general procedure 1B. Compound **21** was isolated as an off-white solid (yield, 20%). ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) δ 8.20 (d, *J* = 8.1 Hz, 1 H), 7.90 (s, 1 H), 7.62 (d, *J* = 8.6 Hz, 1 H), 4.44–3.66 (m, 7 H), 3.28–3.15 (m, 5 H), 3.03–2.65 (m, 5 H), 2.09 (br s, 1 H), 1.91 (br s, 1 H), 1.67–1.47 (m, 3 H), 1.19 (d, *J* = 6.6 Hz, 3 H), 0.90 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, 80 ºC, DMSO-d_6) δ 168.2, 160.1, 155.0, 145.5, 139.4, 136.9, 129.3, 126.0, 124.8, 124.6, 123.6, 66.2, 47.2, 45.9, 43.0, 37.4, 35.6, 25.9, 25.2, 21.6, 14.9, 9.7; MS (ESI) *m/z* 459.2 $[C_{24}H_{31}ClN_4O_3 + H]^+$. Melting point = 76.0–79.0 ºC.

Propyl 4-(4-chloroquinoline-7-carbonyl)piperazine-1-carboxylate (23). Compound **23** was synthesized according to general procedure 1A using commercially available 4-chloroquinoline-7-carboxylate (**S8**) and propyl piperazine-1-carboxylate as a colorless oil (yield, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (d, *J* = 4.7 Hz, 1 H), 8.29 (d, *J* = 8.6 Hz, 1 H), 8.12 (d, *J* = 1.3 Hz, 1 H), 7.86 (d, *J* = 4.7 Hz, 1 H), 7.79 (dd, *J* = 8.6, 1.6 Hz, 1 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.77–3.35 (m, 8 H), 1.58 (dd, *J* = 13.9, 6.9 Hz, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0, 154.7, 151.5, 148.0, 141.2, 137.8, 127.7, 126.6, 125.9, 124.4, 122.5, 66.5, 46.8, 43.3, 43.1, 41.4, 21.9, 10.2; MS (ESI) *m/z* 362.1 $[C_{18}H_{20}ClN_3O_3 + H]^+$.

2,4-Dichloroquinoline-7-carboxylic acid (S10). $POCl_3$ (10 mL) was added to a mixture of methyl 3-aminobenzoate (**S9**) (2 g, 13.23 mmol) and malonic acid (1.52 g, 14.61 mmol, 1.1 equiv). The mixture was heated under reflux for 8 h before allowing it to cool to room temperature. The black mixture was diluted with CH_2Cl_2 and basified to pH > 8 using cold 4N aqueous NaOH. The organic layer was separated and the aqueous layer was extracted thrice more with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography (0–10% ethyl acetate/hexanes) to afford methyl 2,4-dichloroquinoline-7-carboxylate as a white solid (238.1 mg, 7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, *J* = 0.8 Hz, 1 H), 8.36 (d, *J* = 8.8 Hz, 1 H), 8.25 (dd, *J* = 8.6, 1.3 Hz, 1 H), 8.14 (s, 1 H), 3.96 (s, 3 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 165.2, 150.6, 146.8, 143.7, 132.6, 130.0, 127.4, 127.1, 125.0, 124.1, 52.8; MS (ESI) *m/z* 256.0, 258.0 $[C_{11}H_7Cl_2NO_2 + H]^+$. Melting point = 121.7–123.4 ºC.

The above intermediate (429.4 mg, 1.68 mmol) was dissolved in methanol (5 mL) and THF (2.5 mL) followed by LiOH (410 mg, 17.12 mmol, 10.2 equiv) in H_2O (5 mL). The mixture was heated to 50 ºC for 30 min and then allowed to cool. Concentrated HCl was added until pH < 3 and white solid was collected via filtration using copious amounts of water for washing. Compound **S10** was obtained as a white solid (389.2 mg, 98%) after heating under vacuum (50 ºC) for 2 h. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (s, 1 H), 8.47 (d, *J* = 1.2 Hz, 1 H), 8.28 (d, *J* = 8.7 Hz, 1 H), 8.21 (dd, *J* = 8.7, 1.6 Hz, 1 H), 8.06 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 150.3, 146.9, 143.6, 133.9, 130.0, 127.8, 126.9, 124.7, 123.8; MS (ESI) *m/z* 241.9, 243.9 $[C_{10}H_5Cl_2NO_2 - H]^-$. Melting point = 250.0–255.0 ºC.

Propyl 4-(2,4-dichloroquinoline-7-carbonyl)piperazine-1-carboxylate (S11a) or propyl (*R*)-4-(2,4-dichloroquinoline-7-carbonyl)-2-methylpiperazine-1-carboxylate (S11b). Compound **S11** was synthesized according to general procedure 1A using compound **S10** and either propyl piperazine-1-carboxylate or propyl 2-(*R*)-methylpiperazine-1-carboxylate respectively. **S11a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, *J* = 8.6 Hz, 1 H), 8.05 (s, 2 H), 7.81 (dd, *J* = 8.6, 1.5 Hz, 1 H), 3.97 (t, *J* = 6.6 Hz, 2 H), 3.75–3.28 (m, 8 H), 1.58 (dd, *J* = 13.9, 6.9 Hz, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz,

DMSO-d_6) δ 167.5, 154.6, 150.1, 146.9, 143.6, 139.0, 127.1, 126.5, 124.9, 124.7, 122.9, 66.4, 46.7, 43.3, 43.0, 41.4, 21.8, 10.2; MS (ESI) *m/z* 396.1, 398.1 $[C_{18}H_{19}Cl_2N_3O_3 + H]^+$. Melting point = 120–124 ºC. **S11b**: $[\alpha]_D^{20}$ = –2.26 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) δ 8.30 (d, *J* = 8.6 Hz, 1 H), 8.02 (s, 1 H), 7.95 (s, 1 H), 7.80 (d, *J* = 8.5 Hz, 1 H), 4.37–3.57 (m, 6 H), 3.37–3.12 (m, 3 H), 1.68–1.52 (m, 2 H), 1.13 (d, *J* = 6.0 Hz, 3 H), 0.90 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, 80 ºC, DMSO-d_6) δ 168.0, 154.2, 149.8, 146.7, 143.3, 138.8, 126.6, 126.2, 124.6, 124.3, 122.5, 66.1, 46.6, 37.9, 21.5, 14.8, 9.7; MS (ESI) *m/z* 410.1, 412.1 $[C_{19}H_{21}Cl_2N_3O_3 + H]^+$. Melting point = 90.0–93.0 ºC.

General procedure 4. Suzuki-coupling of compound S11 and boronic acid derivatives. To a pre-evacuated reaction vessel was added compound **S11** (1 equiv), boronic acid derivative (1.1–1.3 equiv), K_3PO_4 (3 equiv) and a 4:1 mixture of 1,4-dioxane/H_2O (0.08 M). The mixture was degassed by bubbling N_2, and Pd(dppf)Cl_2.DCM (0.1 equiv) was added. The mixture was heated at 110 ºC for 10–20 min before cooling it to room temperature. The mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was purified by column chromatography or preparative HPLC (ACN, H_2O and 0.1% formic acid) to afford compounds **24**–**29**.

Propyl 4-(4-chloro-2-phenylquinoline-7-carbonyl)piperazine-1-carboxylate (24). Compound **24** was synthesized according to general procedure 4 using phenyl boronic acid and was isolated as a white solid (yield, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1 H), 8.33 (dd, *J* = 7.8, 1.7 Hz, 2 H), 8.28 (d, *J* = 8.5 Hz, 1 H), 8.14 (d, *J* = 1.3 Hz, 1 H), 7.75 (dd, *J* = 8.5, 1.5 Hz, 1 H), 7.61–7.51 (m, 3 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.77–3.35 (m, 8 H), 1.58 (dd, *J* = 13.9, 6.9 Hz, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0, 157.2, 154.6, 147.7, 142.3, 138.2, 137.2, 130.4, 128.9, 127.7, 127.4, 126.3, 124.9, 124.3, 119.7, 66.4, 46.8, 43.3, 43.1, 41.4, 21.9, 10.2; MS (ESI) *m/z* 438.1 $[C_{24}H_{24}ClN_3O_3 + H]^+$. Melting point = 79.0–82.0 ºC.

Propyl (*R*)-4-(4-chloro-2-phenylquinoline-7-carbonyl)-2-methylpiperazine-1-carboxylate (25). Compound **25** was synthesized according to general procedure 4 using phenyl boronic acid and was isolated as a white solid (yield, 50%). $[\alpha]_D^{25}$ = +13.42 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1 H), 8.36–8.31 (m, 2 H), 8.29 (d, *J* = 8.5 Hz, 1 H), 8.12 (br s, 1 H), 7.75 (br s, 1 H), 7.63–7.50 (m, 3 H), 4.52–3.35 (m, 7 H), 3.23–2.91 (m, 3 H), 1.67–1.51 (m, 2 H), 1.12 (br d, *J* = 65.0 Hz, 3 H), 0.89 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.7, 157.2, 154.5, 147.8, 142.3, 138.2, 137.2, 130.4, 128.9, 127.6, 127.5, 126.3, 124.9, 124.4, 119.7, 66.4, 50.7, 46.7, 45.4, 41.5, 21.9, 15.1, 10.3; MS (ESI) *m/z* 452.2 $[C_{25}H_{26}ClN_3O_3 + H]^+$. Enantiomeric excess, *ee* = 99.4%; melting point = 57.8–59.3 ºC.

Propyl 4-(4-chloro-2-(p-tolyl)quinoline-7-carbonyl)piperazine-1-carboxylate (26). Compound **26** was synthesized according to general procedure 4 using 4-methylphenylboronic acid and was isolated as a colorless oil (yield, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1 H), 8.29–8.21 (m, 3 H), 8.12 (d, *J* = 1.0 Hz, 1 H), 7.73 (dd, *J* = 8.5, 1.6 Hz, 1 H), 7.39 (d, *J* = 8.1 Hz, 2 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.78–3.34 (m, 8 H), 2.41 (s, 3 H), 1.59 (d, *J* = 7.1 Hz, 2 H), 0.89 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0, 157.2, 154.6, 147.7, 142.2, 140.2, 138.1, 134.4, 129.6, 127.6, 127.3, 126.1, 124.8, 124.3, 119.4, 66.4, 46.9, 43.3, 43.1, 41.4, 21.9, 20.9, 10.2; MS (ESI) *m/z* 452.2 $[C_{25}H_{26}ClN_3O_3 + H]^+$.

Propyl 4-(4-chloro-2-(4-methoxyphenyl)quinoline-7-carbonyl)piperazine-1-carboxylate (27). Compound **27** was synthesized according to general procedure 4 using 4-methoxyphenylboronic acid and was isolated as a white solid (yield, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1 H), 8.31 (d, *J* = 8.9 Hz, 2 H), 8.24 (d, *J* = 8.5 Hz, 1 H), 8.09 (d, *J* = 1.2 Hz, 1 H), 7.70 (dd, *J* = 8.5, 1.5 Hz, 1 H), 7.12 (d, *J* = 8.9 Hz, 2 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.86 (s, 3 H), 3.75–3.34 (m, 8 H), 1.59 (dd, *J* = 13.4, 6.8 Hz, 2 H), 0.89 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.1, 161.2, 156.9, 154.7, 147.8, 142.1, 138.1, 129.6, 129.0, 127.5, 125.8, 124.5, 124.2, 119.2, 114.3, 66.4, 55.4, 46.7, 43.4, 43.1, 41.4, 21.9, 10.2; MS (ESI) *m/z* 468.1 $[C_{25}H_{26}ClN_3O_4 + H]^+$. Melting point = 53.5–56.5 ºC.

Propyl 4-(4-chloro-2-(4-(methylcarbamoyl)phenyl)quinoline-7-carbonyl)piperazine-1-carboxylate (28). Compound **28** was synthesized according to general procedure 4 using 4-(methylcarbamoyl)phenylboronic acid and was isolated as a white solid (yield, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62–8.57 (m, 1 H), 8.56 (s, 1 H), 8.42 (d, *J* = 8.5 Hz, 2 H), 8.30 (d, *J* = 8.6 Hz, 1 H), 8.18 (d, *J* = 1.2 Hz, 1 H), 8.02 (d, *J* = 8.5 Hz, 2 H), 7.77 (dd, *J* = 8.5, 1.6 Hz, 1 H), 3.98 (t, *J* = 6.6 Hz, 2 H), 3.77–3.35 (m, 8 H), 2.83 (d, *J* = 4.5 Hz, 3 H), 1.59 (dd, *J* = 13.8, 6.8 Hz, 2 H), 0.89 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0, 166.0, 156.3, 154.7, 147.7, 142.5, 139.4, 138.3, 135.9, 127.8, 127.7, 127.3, 126.6, 125.0, 124.4, 119.9, 66.4, 46.8, 43.3, 41.5, 26.3, 21.9, 10.2; MS (ESI) *m/z* 495.2 $[C_{26}H_{27}ClN_4O_4 + H]^+$. Melting point = 79.5–83.5 ºC.

Propyl (*R*)-4-(2-(4-(1-aminocyclopropyl)phenyl)-4-chloroquinoline-7-carbonyl)-2-methylpiperazine-1-carboxylate (29). Compound **29** was synthesized according to general procedure 4 using 4-(1-aminocyclopropyl)phenylboronic acid hydrochloride and was isolated as a white solid (yield, 59%). $[\alpha]_D^{20}$ = –4.0 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) δ 8.37 (s, 1 H), 8.27 (d, *J* = 8.5 Hz, 1 H), 8.22 (d, *J* = 8.4 Hz, 2 H), 8.10 (s, 1 H), 7.71 (dd, *J* = 8.6, 1.3 Hz, 1 H), 7.50 (d, *J* = 8.4 Hz, 2 H), 4.34–3.61 (m, 6 H), 3.39–3.13 (m, 3 H), 2.37 (s, 2 H), 1.66–1.53 (m, 2 H), 1.15 (d, *J* = 5.4 Hz, 3 H), 1.09–0.99 (m, 4 H), 0.91 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, 80 ºC, DMSO-d_6) δ 168.5, 157.0, 154.2, 150.0, 147.6, 141.8, 137.9, 133.9, 127.2, 126.7, 125.5, 124.9, 124.4, 123.9, 119.1, 66.1, 46.6, 38.0, 35.7, 21.5, 18.9, 14.8, 9.7, –0.4; MS (ESI) *m/z* 507.2 $[C_{28}H_{31}ClN_4O_3 + H]^+$. Enantiomeric excess, *ee* >99.9%; melting point = 75.8–76.8 ºC.

Propyl (*R*)-2-methyl-4-(2-phenylquinoline-7-carbonyl)piperazine-1-carboxylate (30). To a solution of compound **25** (30 mg, 0.066 mmol) in ethanol (3 mL) and THF (0.9 mL) was added KBH_4 (14.2 mg, 0.263 mmol, 4 equiv) and ~30 drops of Raney nickel ®2800 slurry. After the reaction is complete (~30 min) the mixture was decanted with ethyl acetate. The mixture was filtered and concentrated under reduced pressure. The crude material was purified by preparative HPLC (20–95% MeCN/H_2O; 0.1% formic acid) to afford compound **30** as a white solid upon lyophilization (16.8 mg,

60%). $[\alpha]_D^{20}$ = –36.0 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) δ 8.53 (d, *J* = 8.7 Hz, 1 H), 8.29 (d, *J* = 7.0 Hz, 2 H), 8.23 (d, *J* = 8.7 Hz, 1 H), 8.10 (d, *J* = 8.3 Hz, 1 H), 8.05 (br s, 1 H), 7.69–7.47 (m, 4 H), 4.49–3.57 (m, 7 H), 3.23–2.95 (m, 2 H), 1.65–1.50 (m, 2 H), 1.27–0.98 (m, 3 H), 0.89 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.3, 157.0, 154.5, 146.9, 138.3, 137.2, 137.0, 129.8, 128.9, 128.5, 127.3, 127.2, 127.1, 124.9, 116.7, 66.4, 50.7, 46.7, 41.5, 21.9, 15.1, 10.3, 0.1; MS (ESI) *m/z* 418.2 $[C_{25}H_{27}N_3O_3 + H]^+$. Melting point = 63.9–65.1 ºC.

Pseudo 1st-order kinetics of 22, 13 and 21 with GSH. All reactions were conducted at room temperature and the stock solutions or solvents used were degassed by bubbling with N_2 30 min prior to use. All reactions were run under an inert atmosphere of N_2. 250 µL of compound stock (5.0 mM in DMSO), 250 µL of indoprofen stock (internal standard, 1 mM in DMSO) and 500 µL of DMSO were added to a reaction vessel. Addition of 4.0 mL of GSH stock (5.55 mM in pH 7.4 phosphate buffer (100 mM)) marked the start of the reaction. [Final concentration in vessel: 0.25 mM of compound, 0.05 mM of indoprofen and 5.0 mM of GSH in 5 mL of 20% DMSO in phosphate buffer]. An aliquot was drawn from the reaction in fixed time intervals and analyzed with the Agilent 1290 Infinity HPLC-MS. The reactant, product and indoprofen were separated by LC using the Eclipse Plus C18 RRHD column (1.8 (D) × 2.1 × 50 mm) and quantified by area under curve of the UV spectrum. The LC mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in ACN). LC was initiated with 95% solvent A, followed by a linear increase of solvent B to 95% in 1.2 min. Solvent B was held at 95% for 0.5 min and then rapidly decreased to 5%. At the end of the run, 95% of solvent A was held for 0.2 min. For plotting of calibration curves, compounds and indoprofen were prepared in solutions of 20% DMSO in phosphate buffer and ran at concentrations of 0.01563 mM, 0.03125 mM, 0.0625 mM, 0.125 mM, 0.25 mM, 0.5 mM and 1 mM. The linearity and $k_{pseudo,\ 1st}$ were determined by plotting the natural logarithm of the consumption of compound as a function of time. The rate information ($T_{1/2}$ and $k_{pseudo,\ 1st}$) were calculated according to the equations below:

$$ln[electrophile] = -k_{pseudo,1st}t + ln[electrophile]_0$$

$$t_{1/2} = \frac{0.693}{60 \times k_{pseudo,1st}}$$

See **Table S1–6** and **Figures S1–3** for results.

X-ray crystallization and data collection. SMYD3 was crystallized at 297 K by incubating 1:1 equivolume ratio of protein (11 mg/mL) and reservoir solution (0.2 M magnesium acetate and 17% PEG 3350) in a hanging drop vapor diffusion set up. The crystals obtained were soaked overnight in reservoir solution containing 10 mM final concentration of compound. Crystals were then cryoprotected using 25% glycerol and flash frozen in liquid nitrogen. 2.1 Å and 2.4 Å datasets were collected for SMYD3 crystals soaked overnight with compounds **21** and **29** respectively using a home source Rigaku MicroMax™ 007 HF. The datasets were indexed, integrated and scaled using HKL2000 (Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode, *Methods in Enzymology* **1997**, *276: Macromolecular Crystallography, part A*, 307–326, C.W. Carter, Jr. & R. M. Sweet, Eds., Academic Press (New York)). The structure of SMYD3 (PDB ID: 1MEK) after the removal of waters was used as the search model for molecular replacement and refined using the PHENIX suite of programs (PHENIX: a comprehensive Python-based system for macromolecular structure solution. Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. *Acta Cryst.* **2010**, *D66*, 213–221.) The SMYD3 co-crystal structures with compounds **21** and **29** were refined to a final R_{free} value of 22.9% and 27.3% respectively. See Tables **S7–8** for results.

SMYD3 biochemical assay. For IC_{50} determination, the compounds were incubated with 0.4 µM of SMYD3 enzyme for 0.5 h in low volume 384 well plates. A final concentration of 1.0 µM SAM and 10 µM MAP3K2 peptide were added and further incubated for 0.5 h at room temperature. MTase Glo and detection reagents were added. Reaction signals were detected using microplate reader on luminescent

mode (Safire Tecan). The IC_{50} was determined by non-linear regression, using GraphPad Prism (v, 5.03). See **Figure S4** for biochemical IC_{50} data for **29**.

Determination of k_{inact}/K_I for 24, 28 and 29. The k_{inact}/K_I measurement was performed at room temperature by adding SMYD3 (100.0 nM) at a 5 min time interval to the reaction buffer (100 mM Tris, 20 mM NaCl, 0.02% Triton-X, 2.0 mM DTT, pH = 8.5) consisting of 120.0 μM MAP3K2 peptide (Genescript), 10.0 μM of SAM, DMSO (2.5% v/v) and inhibitor (0–20.0 μM with 2-fold serial dilution). The reaction was carried out for a total time of 65 min and terminated by the addition of 0.1% TFA followed by 5 min incubation. Subsequently, the MTA-Glo™ reagent (1 ×) and MTA-Glo™ detection buffer (1 ×) (Promega) were added into the assay solution under dim light, followed by 30 min incubation respectively. The end-point signal was detected on a microplate reader (Tecan Safire II) at room temperature. The substrate concentration used in the assay ensured that the reaction progress curve in the absence of inhibitor was essentially linear for the first 10% of the reaction. The reaction progress curves were fit to the following equation to obtain k_{obs}.

$L_t = L_0 - v_i * [1 - \exp(-k_{obs}t)]/k_{obs}$ where L_t and L_0 are the luminescent signal at times t and 0, respectively, v_i is the initial velocity, and k_{obs} is the pseudo-first order rate constant for the approach to steady state. The k_{obs} obtained at different inhibitor concentrations are subsequently fit into the following equation to obtain k_{inact} and K_I.

$k_{obs} = \frac{k_{inact}[I]}{K_I + [I]}$ where k_{inact} is the maximum rate of inactivation at infinite inhibitor concentration, and K_I corresponds to the concentration of the inhibitor where the rate of inactivation reaches half of k_{inact}. Data was analyzed with Kaleidograph version 4.5. See **Figure S5** for k_{inact}/K_I data for **29**.

Generation of SMYD3 knockout HepG2 cells. The all-in-one expression plasmid system containing OFP (Orange Fluorescent Protein) reporter, Cas9 and sgRNA for SMYD3 was purchased from ThermoFisher Scientific (GeneArt). The target sequence for SMYD3 sgRNA is: CTTGCACACCGTGTACGCCA (PAM sequence is AGG). HepG2 cells were transfected with the

SMYD3 sgRNA plasmid using Lipofectamine 3000 (Invitrogen). At 48 h after transfection, OFP-negative (control cells) and OFP-positive cells were sorted and cultured at 0.5 cell/well in 96-well TC plates. Single clones of HepG2 cells were grown and expanded for SMYD3 protein levels testing by Western analysis. HepG2 clones which express significantly low to undetectable levels of SMYD3 protein were selected and expanded for subsequent studies. CRISPR-induced indels on SMYD3 gene were validated by performing Topo TA cloning on the SMYD3 knockout clones genomic DNA with the following primers: SMYD3_topo_Fed: 5'-ACTTTTCGTCTCCCAGCAAA-3' and SMYD3_topo_Rev: 5'-GCTGAAGGTGGAAAAGTTCG-3'.

Quantification of MAP3K2 methylation using NanoPro immunoassay. HepG2 cells treated with DMSO or compounds for 24 h were lyzed in Bicine-CHAPS lysis buffer containing protease inhibitor and phosphatase inhibitor (ProteinSimple). The lysate samples were then mixed with ProteinSimple's Premix G2, pH 3–10 separation gradient and pI standards before loading onto the NanoPro 100 system for analysis. Isoelectric focusing was performed in capillaries filled with the mixture of cell lysate and the proteins were separated based on their pI value. MAP3K2 methylation was detected in the NanoPro 100 system as a single peak with pI value of 5.64 by immune-probing with our customized rabbit anti-methylated MAP3K2 antibody (1:50 dilution) and goat-anti-rabbit secondary antibody (HRP conjugated, 1:100 dilution, ProteinSimple). The rabbit anti-β-2 microglobulin (B2M) antibody (1:100 ab75853, Abcam) recognized B2M as a single peak at pI value of 5.88 was used as internal standard. The signal was visualized by luminol (ProteinSimple) and was captured by embedded camera in NanoPro 100. The digital image was analyzed and the area of peaks was quantified with the Compass software (ProteinSimple). The Ratio ($\frac{Area\ (methylated\ MAP3K2)}{Area\ (B2M)}$) represents the protein level of methylated MAP3K2 relative to loading control and the % inhibition was calculated based on $\frac{Ratio\ (compound\ treatment)}{Ratio\ (DMSO)} \times 100\%$. See **Table S9** for details.

2D Cell proliferation assay. Cell proliferation assay was performed using CellTiter-Glo Luminescent Cell Viability Assay (Promega) following manufacturer's instructions. HepG2 cell line was treated with compound by preparing the dilution in cell culture media (replace with Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin). Plates were incubated for 3 d at 37 ºC in 5% CO_2. After 72 h, an equal volume of Cell Titer Glo reagent was added. Plates were rocked on a rotator for 2 h. 100 µL of each well was transferred to a 96-well opaque plate, and luminescence emitted was measured with the Tecan Safire II. The GI_{50} was determined by non-linear regression, using GraphPad Prism version, 5.03. See **Table S10** for results.

Soft agar colony formation assay for HepG2. Cells were cultured in EMEM media as mentioned above. 600 µL of 0.6% agar was added to a 24-well plate to form the base layer. This is followed by the addition of 500 µL of 0.36% agar middle layer (containing 10000 cells). Lastly, 500 µL of fresh growth medium (containing the serially diluted compound) was added above the middle layer. The plates were incubated at 37 ºC with 5% carbon dioxide in a humidified incubator for 1–2 weeks. 70 µL of thiazolyl blue tetrazolium bromide (5 mg/mL) was added to each well and the plates were incubated at 37 ºC for 2 h. Colonies were counted with GelCount® instrument (Oxford Optronix). The colony counts were plotted against compound concentrations using GraphPad Prism. In addition, the software was used for perform non-linear curve fitting and the calculation of IC_{50}. See **Table S11** for details.

Analysis of SMYD3 protein levels in HepG2 cells using Western blot. The target engagement assay was performed in HepG2 cells. Prior to treatment, 1.2×10^6 cells were seeded in 60 mm^2 dish. The cells were treated for 24 h with either 1% DMSO or compound (1 µM, 5 µM and 20 µM). The cells were harvested and lyzed with Ripa Buffer containing SDS and protease and phosphatase inhibitor cocktail (*Santa Cruz* Biotechnology, Inc). The total protein concentration of lysate was quantified using the standard Bradford assay. A Western blot was performed using anti-SMYD3 primary antibody rabbit (Ab

199361, Abcam, 1:2,000 dilution). Percentage inhibition was calculated by quantifying the pixel density of compounds against 1% DMSO using Alphaview Software ™ (Protein Simple, FluorChem R FR0108). See **Table S12** for results.

Bidirectional Caco-2 transport assay. Permeability of compounds were determined as reported earlier (*J. Med. Chem.* **2018**, *61*, 4348−4369).

Microsomal stability assay. Microsomal stability of compounds in mouse/human liver microsomes were determined as reported earlier (*J. Med. Chem.* **2018**, *61*, 4348−4369).

Histone methyltransferase selectivity assay. To determine the specificity of **29**, the biochemical assays were performed against several methyltransferases such as G9a, SMYD1, SMYD2, PRDM9 and PRMT5. Compound potency was evaluated in 4 µL reaction volume in a 384-well low volume assay plate (Greiner 784075). All assays were performed with Methyltransferase-Glo™ reagent (V7601). Reaction signals were detected using a Tecan Safire II microplate reader and IC_{50} values were determined through non-linear regression fit of the inhibition response (GraphPad Prism version 5.03).

(A) SMYD1 and SMYD2 methyltransferase inhibition assay. For the IC_{50} determination, **29** with 3-fold dilution ranging from (0 µM – 750 µM) was incubated with 0.1 µM SMYD1 enzyme (HMT-11-304) or 0.5 µM SMYD2 for 30 min in assay buffer. A final concentration of 2.0 µM of SAM and 0.4 µM of Histone H3 recombinant protein (NEB M2507S) were added to SMYD1 reaction (Sirinupong, N.; Brunzelle, J.; Ye, J.; Pirzada, A.; Nico, L.; Yang, Z. Crystal structure of cardiac-specific histone methyltransferase SmyD1 reveals unusual active site architecture, *J. Biol. Chem.* **2010**, *285*, 40635-40644) and further incubated for 90 min at room temperature with shaking at 28 rpm. For the SMYD2 reaction, 1.0 µM of SAM and 20.0 µM p53 peptide was added to the enzyme inhibitor mix and incubated for 30 min. The reaction was terminated by the addition of 1 µL of 0.4% trifluoroacetic acid (TFA). The SMYD2 protein was expressed and purified as previously described (Ferguson, A. D.; Larsen, N. A.; Howard, T.; Pollard, H.; Green, I.; Grande, C.; Cheung, T.; Garcia-Arenas, R.; Cowen, S.; Wu, J.; Godin, R.; Chen, H.; Keen, N. Structural basis of substrate methylation and inhibition of SMYD2, *Structure* **2011**, *19*, 1262-1273). The p53 peptide substrate (GSRAHSSHLK-SKKGQSTSRH) and H3 peptides were

synthesized at GenScript. Human SMYD1 (residues 2-490; GenBank Accession No. NM_198274) with a GST tag expressed in E.coli was purchased from Reaction Biology Corp. **(B) G9a Methyltransferase inhibition assay**. G9a assay was carried out similar to SMYD assays. The reaction contained a final concentration of 0.04 µM G9a enzyme, 6.0 µM SAM and 1.0 µM H3 (1-21) peptide substrate. It was incubated for 15 min at RT and the methyltransferase reaction was measured. Human G9a (residues 785-1210; GenBank Accession No. NM_006709) with N-terminal GST tag expressed in E.coli was purchased from BPS Bioscience (Catalog: 51000). The protein was supplied in 40 mM Tris-HCl, pH 8.0, 110 mM NaCl, 2.2 mM KCl, 16 mM glutathione, 3 mM DTT, and 20% Glycerol. **(C) PRDM9 and PRMT5 Methyltransferase inhibition assay**. **29** was diluted three-fold in 100% DMSO, followed by further dilution to form a working stock in 40% DMSO, such that final concentrations ranged between 5.6 nM and 1 mM. The final reaction buffer consisted of 50 mM Tris (pH 8.0) with 20 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 10% glycerol and 10% DMSO. 2.5 µL of the working stock of compound was incubated with 2.5 µL of enzyme and incubated at room temperature for 30 min prior to addition of 5 µL of SAM and substrate to initiate the methyltransferase reaction. The reaction was allowed to proceed for 60 min at 30 ^{o}C, followed by transfer of 4 µL to duplicate wells in a 384-well plate (Greiner 784075). Enzyme and substrate concentrations used for the PRDM9 inhibition assay were 50 nM PRDM9, 3 µM H3 peptide 1-21 and 8 µM SAM. Enzyme and substrate concentrations used for the PRMT5/MEP50 inhibition assay were 150 nM PRMT5/MEP50, 1.5 µM H2A peptide 1-21 and 4 µM SAM. Recombinant mouse PRDM9 was produced in-house as previously described (Koh-Stenta, X.; Joy, J.; Poulsen, A.; Li, R.; Tan, Y.; Shim, Y.; Min, J. H.; Wu, L.; Ngo, A.; Peng, J.; Seetoh, W. G.; Cao, J.; Wee, J. L.; Kwek, P. Z.; Hung, A.; Lakshmanan, U.; Flotow, H.; Guccione, E.; and Hill, J. Characterization of the histone methyltransferase PRDM9 using biochemical, biophysical and chemical biology techniques, *Biochem. J.* **2014**, *461*, 323-334). Full length human PRMT5 (GenBank Accession No. NM_006109), with *N*-terminal His tag and human MEP50 (GenBank Accession No. NM_024102), with *N* terminal His tag co-expressed in Sf9 cells was purchased from BPS Bioscience (Cat. 51048). See results in **Table S13**.

Pseudo 1st-Order Kinetics of 22, 13 and 21 with GSH

Table S1. Calibration curve of reference compound **22** in 20% DMSO/buffer (pH 7.4).

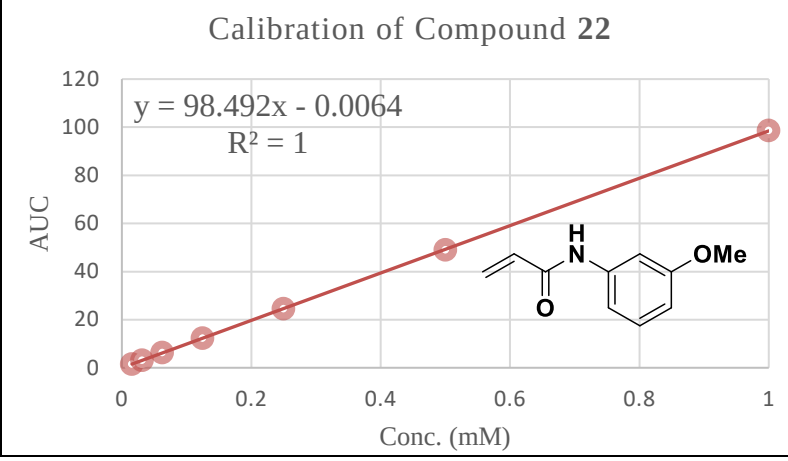
Conc. (mM)	AUC (UV trace)	Result
0.01563	1.5	
0.03125	3.1	
0.0625	6.3	
0.125	12.3	
0.25	24.6	
0.5	49	
1	98.6	

Table S2. Calibration curve of compound **13** in 20% DMSO/buffer (pH 7.4).

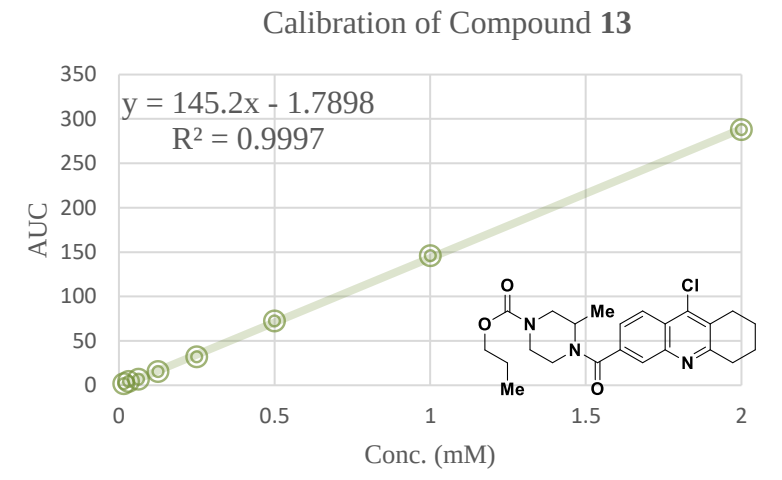
Conc. (mM)	AUC (UV trace)	Result
0.01563	1.5	
0.03125	3.9	
0.0625	6.5	
0.125	15.3	
0.25	31.7	
0.5	72.1	
1	145.6	
2	287.6	

Table S3. Calibration curve of compound **21** in 20% DMSO/buffer (pH 7.4).

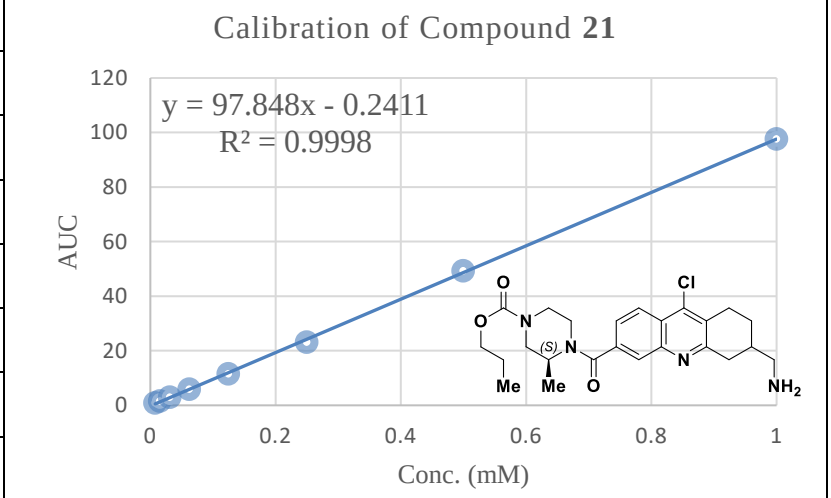
Conc. (mM)	AUC (UV trace)	Result
0.007813	0.9039	
0.01563	1.6	
0.03125	3	
0.0625	5.9	
0.125	11.5	
0.25	23.2	
0.5	49.3	
1	97.6	

Table S4. Data for pseudo 1st-order reaction between **22** and GSH.

t (min)	AUC (**22**)	AUC (indoprofen)	Normalized AUC (**22**)[a]	[**22**] (mM)[b]	ln [**22**]
0	113.3	20.5	114	1.158	0.1463
60	78	20.6	78.4	0.7961	-0.2281
120	59.5	20.4	60.4	0.6133	-0.4889
180	48.6	20.4	49.3	0.5006	-0.6919
240	39.6	20.5	40	0.4062	-0.9009
300	32.6	20.6	32.8	0.3331	-1.099
360	29.9	21	29.5	0.2996	-1.205
420	26.5	21.4	25.6	0.2600	-1.347

Indoprofen used as inert internal standard. Average AUC of indoprofen was calculated to be 20.7. [a]Normalization of experimental AUC was computed by using average AUC (indoprofen). [b]Concentration of **22** was calculated based on calibration curve and experimental AUC.

Figure S1. Rate of consumption of compound **22** in excess GSH (ln [**22**] vs. t).

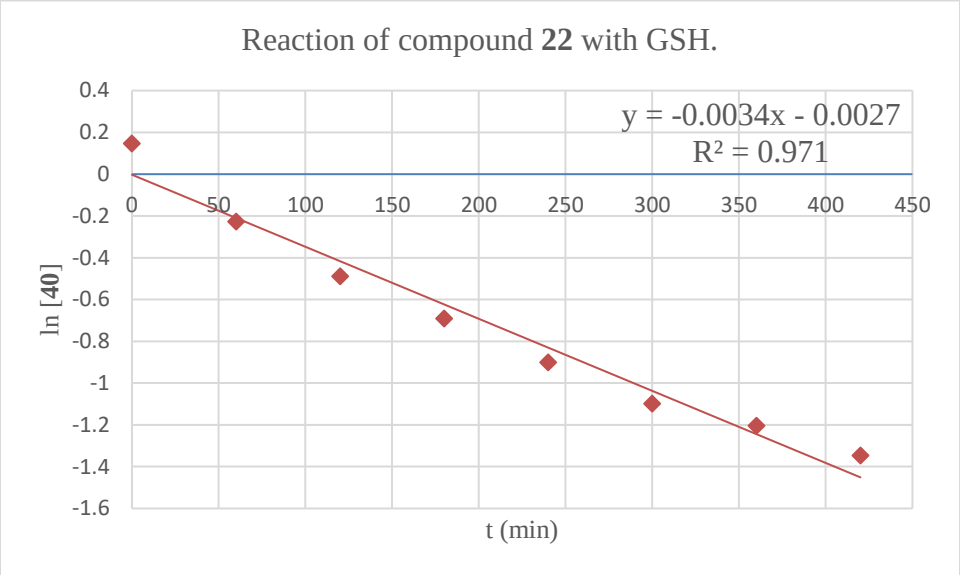


Table S5. Data for pseudo 1st-order reaction between **13** and GSH.

t (min)	AUC (**13**)	AUC (indoprofen)	Normalized AUC (**13**)[a]	[**13**] (mM)[b]	ln [**13**]
0	180.8	20.5	186	1.293	0.2572
60	133.4	21	134	0.9352	-0.0670
120	128.1	21.3	127	0.8870	-0.1199
180	123.4	20.8	125	0.8732	-0.1356
240	119.6	21.1	120	0.8388	-0.1758
300	121.1	21.4	119	0.8319	-0.1841
360	120.3	21.4	119	0.8319	-0.1841
420	120.5	21.5	118	0.8250	-0.1924

Average AUC of indoprofen was calculated to be 21.1. [a]Normalization of experimental AUC was computed by using average AUC (indoprofen). [b]Concentration of **13** was calculated based on calibration curve and experimental AUC.

Figure S2. Rate of consumption of compound **13** with excess GSH (ln [**13**] vs. t).

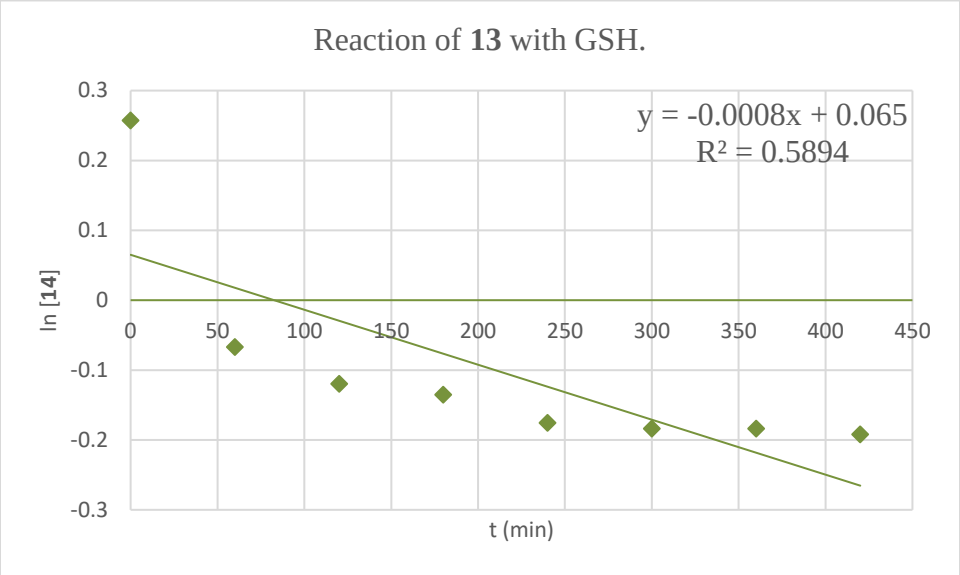


Table S6. Data for pseudo 1st-order reaction between **21** and GSH.

t (min)	AUC (**21**)	AUC (indoprofen)	Normalized AUC (**21**)[a]	[**21**] (mM)[b]	ln [**21**]
0	157.7	21.8	156	1.597	0.4680
60	149.1	21.4	150	1.535	0.4288
120	142.2	21.1	146	1.495	0.4018
180	139.6	21.5	140	1.433	0.3599
240	134.6	21	138	1.413	0.3456
300	134.7	21.6	135	1.382	0.3236
360	132	22.2	128	1.311	0.2705
420	131.4	22.1	128	1.311	0.2705

Average AUC of indoprofen was calculated to be 21.6. [a]Normalization of experimental AUC was computed by using average AUC (indoprofen). [b]Concentration of **21** was calculated based on calibration curve and experimental AUC.

Figure S3. Rate of consumption of compound **21** with excess GSH (ln [**21**] vs. t).

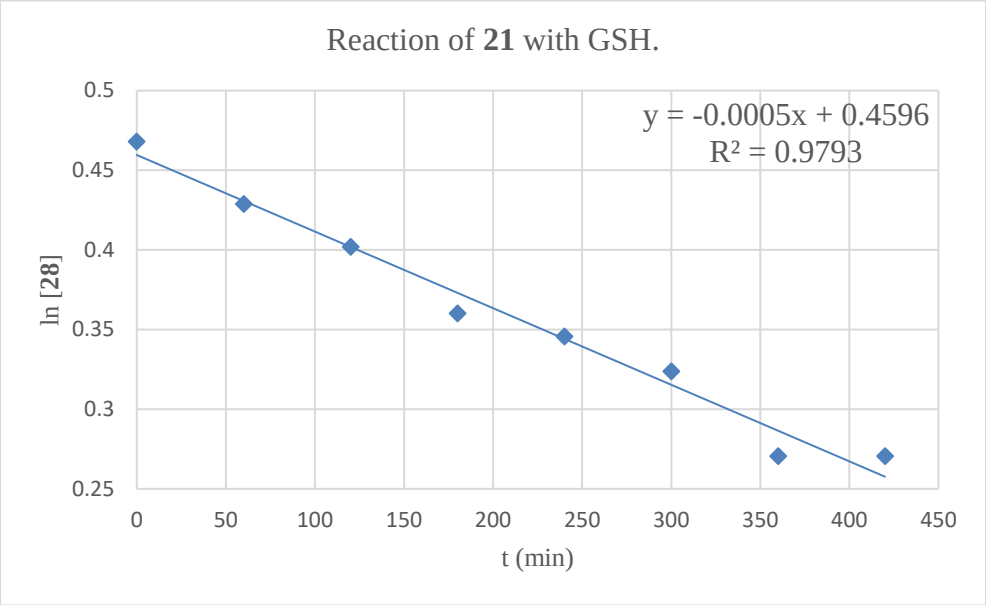


Table S7. X-ray data collection and refinement statistics for compound **21**.

Resolution range (Å)	31.6 - 2.1 (2.2 - 2.1) [a]
Space group	P 21 21 21
Unit cell	61.428 66.181 107.648 90 90 90
Unique reflections	24821 (2375)
Multiplicity	4.8 (4.4)
Completeness (%)	99.10 (96.08)
Mean I/sigma(I)	9.37 (5.2)
Wilson B-factor	21.86
R-merge[b](%)	0.16(0.31)
R-work[c](%)	0.1982 (0.2219)
R-free[d](%)	0.2292 (0.2812)
Number of non-hydrogen atoms	3569

macromolecules	3376
ligands	34
solvent	159
Protein residues	423
RMS(bonds) (Å)	0.002
RMS(angles) (°)	0.48
Ramachandran favored (%)	99.05
Ramachandran allowed (%)	0.95
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	2.37
Average B-factor	23.88
macromolecules	23.96
ligands	23.76
solvent	22.32

[a]Statistics for the highest-resolution shell are shown in parentheses.

[b]*R*merge = Σ*hkl*Σ*i* |*Ii*(*hkl*) − <*I*(*hkl*)> |/Σ*hkl*Σ*i* *Ii*(*hkl*), where *Ii*(*hkl*) and <*I*(*hkl*)> are the intensity of measurement *i* and the mean intensity for the reflection with indices *hkl*, respectively.

[c]*R*work = Σ*hkl*[||*F*obs| − *k*/*F*calc||]/Σhkl[|*F*obs|];

[d]*R*free = Σ*hkl*⊂T [||*F*obs| − *k*/*F*calc||]/Σ *hkl*⊂T[|*F*obs|]; *hkl*⊂T – test set.

Table S8. X-ray data collection and refinement statistics for compound **29**.

Resolution range (Å)	41.7 - 2.3 (2.4 - 2.3) [a]
Space group	P 21 21 21
Unit cell (Å)	61.212 66.232 107.397 90 90 90
Unique reflections	18661 (1758)
Multiplicity	6.9 (5.8)
Completeness (%)	99.44 (95.44)
Mean I/sigma(I)	11.81 (5.66)
Wilson B-factor	25.53
R-meas[e] (%)	0.147 (0.299)
R-work[c](%)	0.2730 (0.2749)
R-free[d](%)	0.3220 (0.3861)
Number of non-hydrogen atoms	3498
macromolecules	3360
ligands	38
water	100
Protein residues	423
RMS(bonds) (Å)	0.012
RMS(angles) **(°)**	1.52
Ramachandran favored (%)	97
Ramachandran allowed (%)	2.76
Ramachandran outliers (%)	0.24
Clashscore	11.19

Average B-factor	29.70
macromolecules	29.70
ligands	46.90
solvent	24.50

[a]Statistics for the highest-resolution shell are shown in parentheses.

[b]*R*merge = Σ*hkl*Σ*i* |*Ii*(*hkl*) − <*I*(*hkl*)> |/Σ*hkl*Σ*i* *Ii*(*hkl*), where *Ii*(*hkl*) and <*I*(*hkl*)> are the intensity of measurement *i* and the mean intensity for the reflection with indices *hkl*, respectively.

[c]*R*work = Σ*hkl*[||*F*obs| − *k*/*F*calc||]/Σhkl[|*F*obs|];

[d]*R*free = Σ*hkl*⊂T [||*F*obs| − *k*/*F*calc||]/Σ *hkl*⊂T[|*F*obs|]; *hkl*⊂T – test set.

[e]Rmeas = $\Sigma_{hkl}[N/(N-1)]^{1/2}\Sigma_i|I_i(hkl) - [I(hkl)]|/\Sigma hkl\Sigma_i I_i(hkl)$.

Figure S4. Biochemical IC_{50} of compound **29**.

SMYD3 IC_{50} = 0.0117 µM (Confidence Interval 0.01105 to 0.01230)

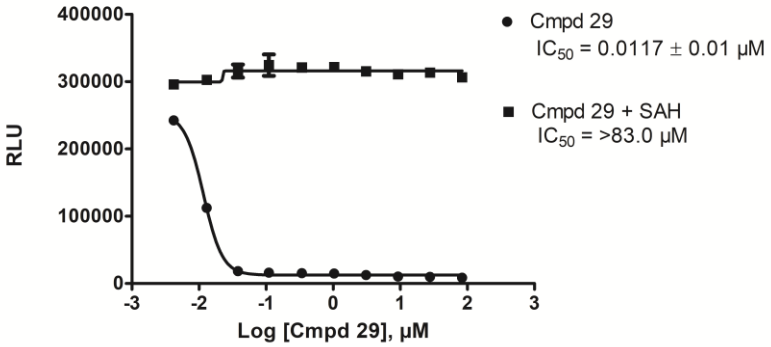


Figure S5. Plot of k_{obs} vs [compound **29**] for k_{inact}/K_I.

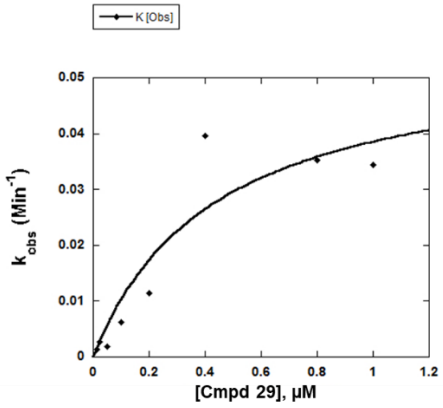


Table S9. Quantification of methylated MAP3K2 using NanoPro 100.

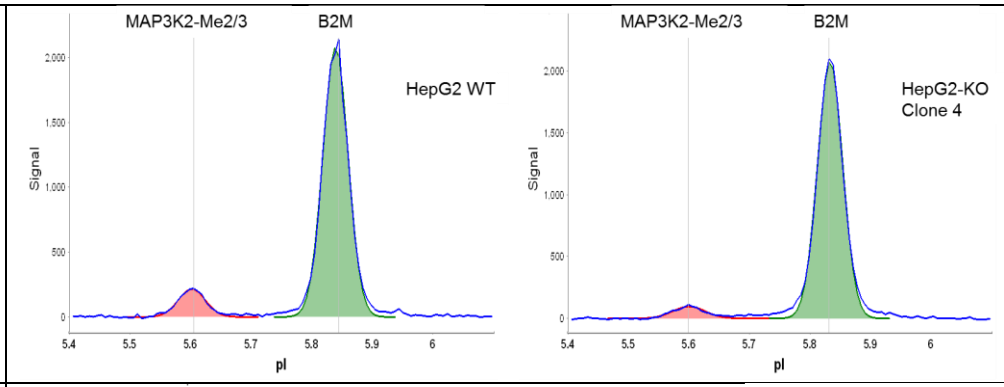
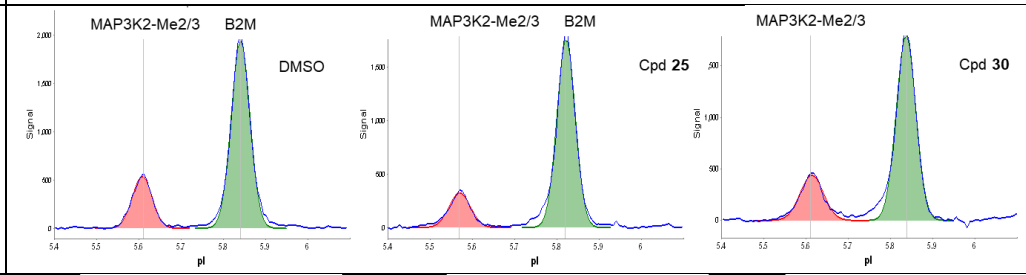
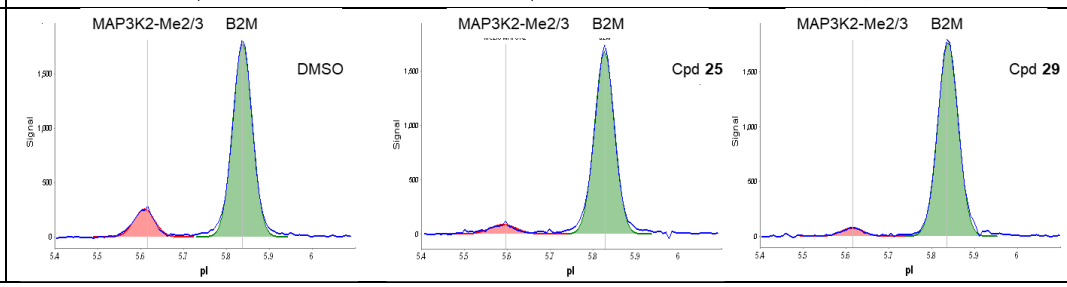
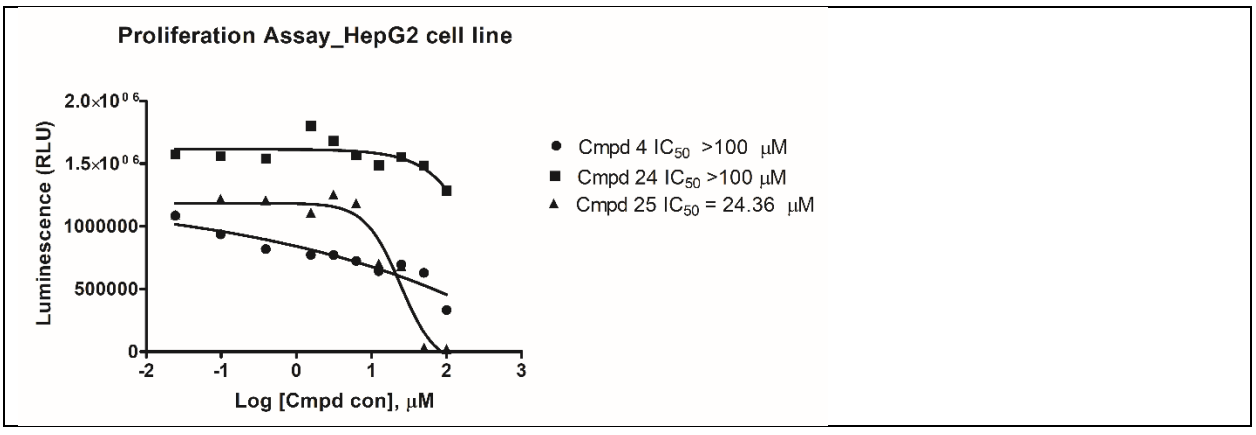
HepG2 wild type vs SMYD3-knockout HepG2 Clone 4	
Compound **25** and **30** vs DMSO control	
Compound **25** and **29** vs DMSO control	

Table S10. Antiproliferative activity of selected compounds against HepG2 in 2D cell culture.



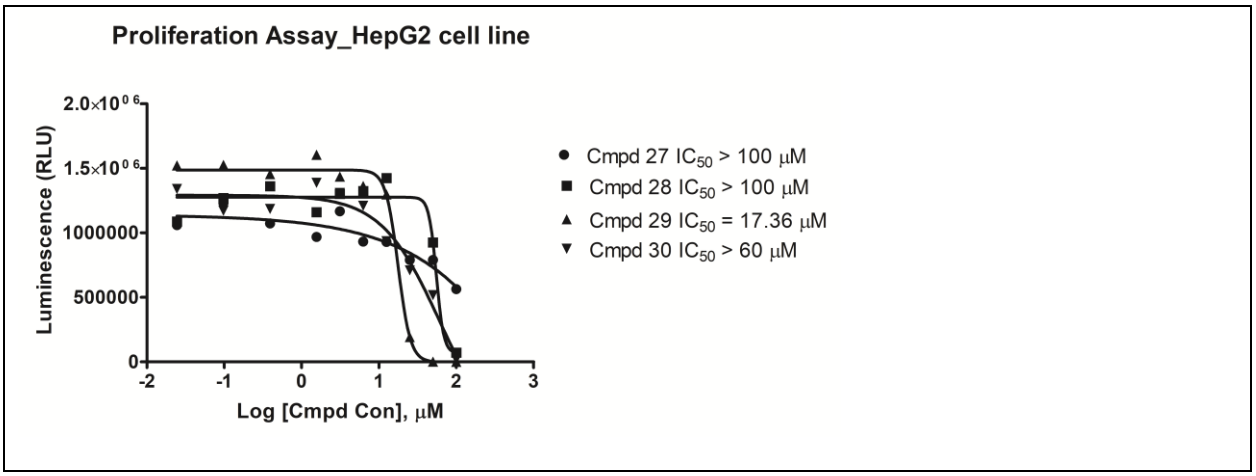
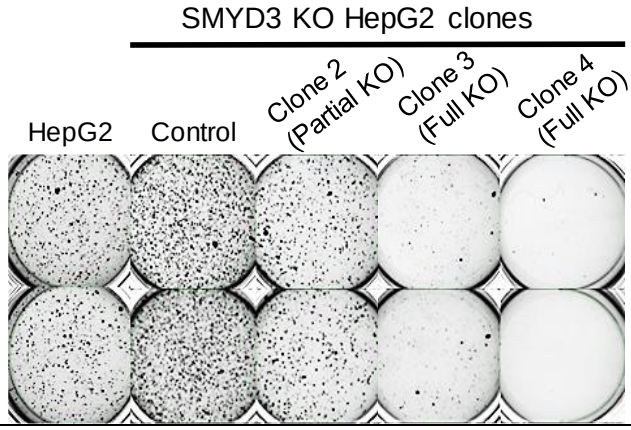
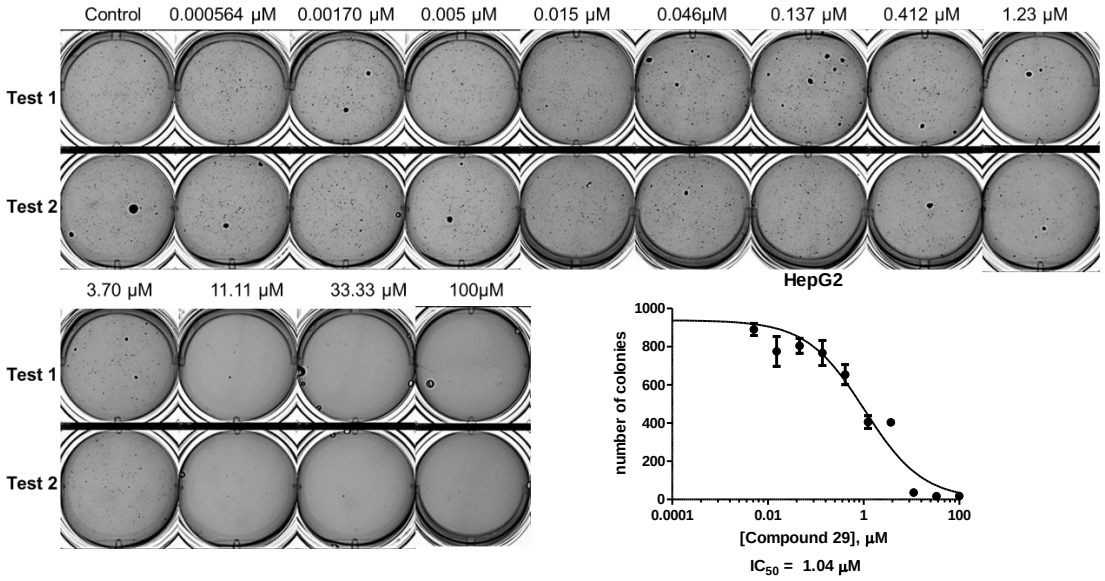


Table S11. Antiproliferative activity of SMYD3 KO clones and selected compounds against HepG2 in 3D assay.

HepG2 Control + SMYD3 KO Clones	
Cpd **29**	

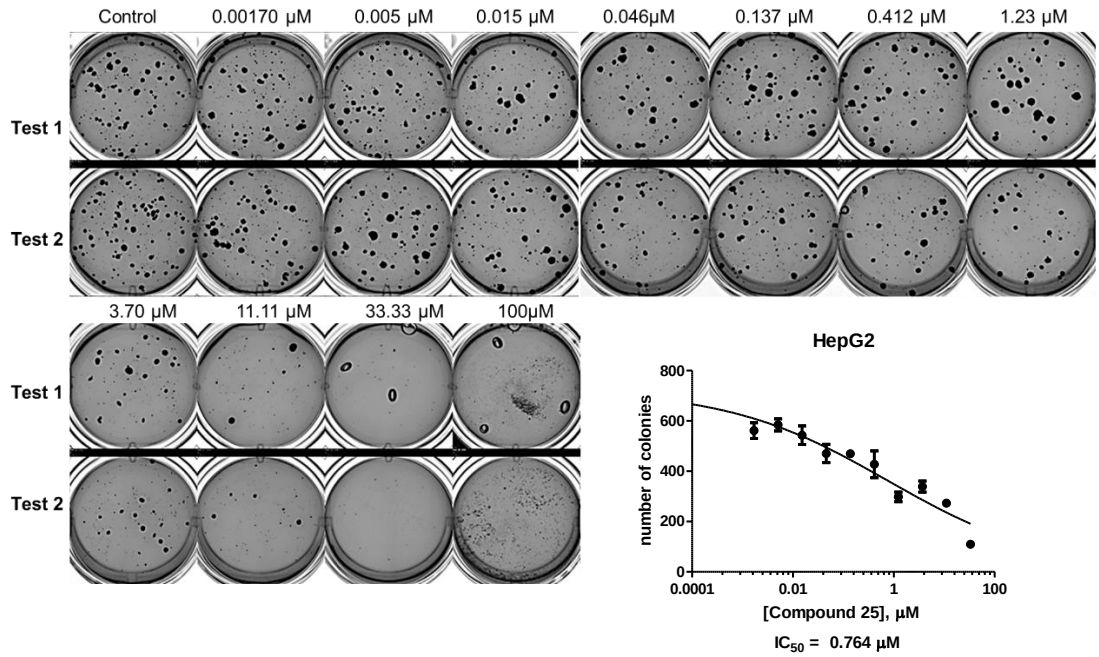
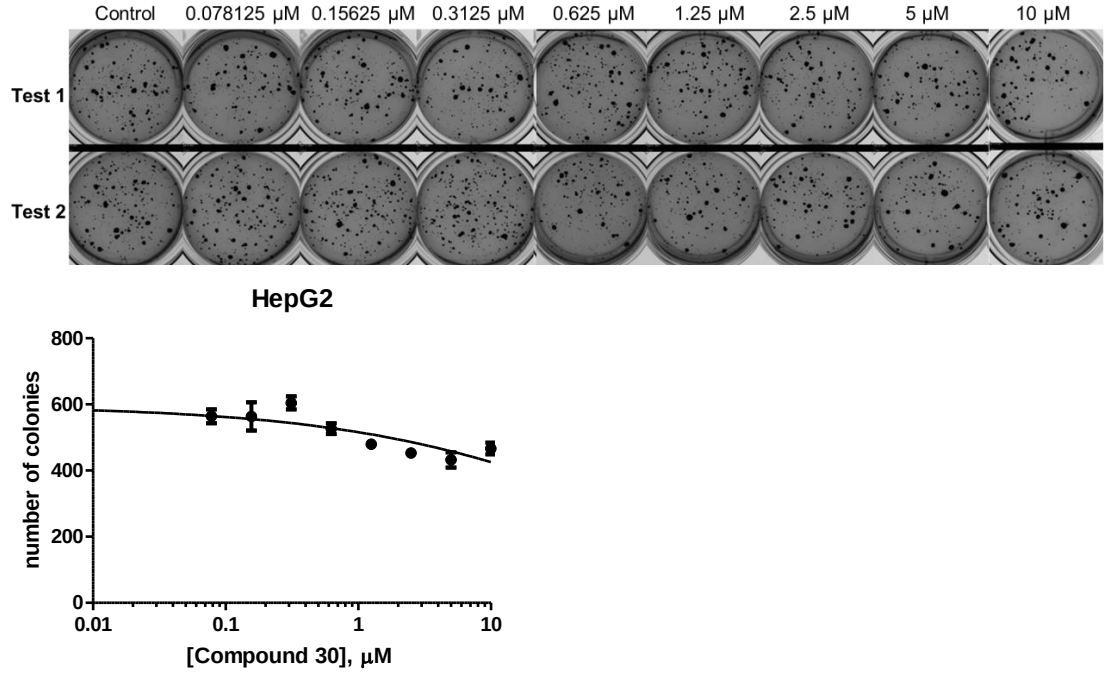
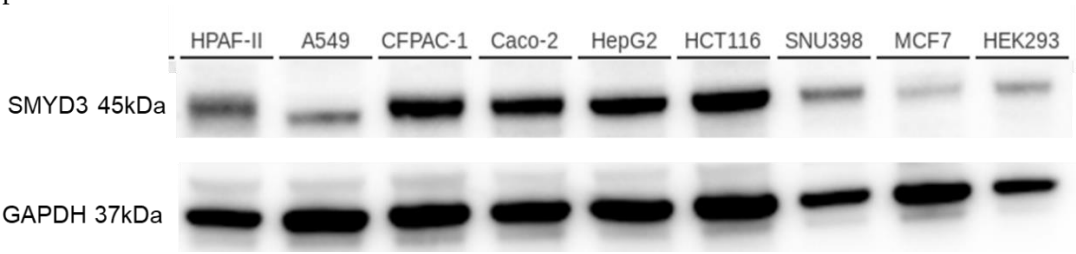
Cpd **25**	 Control 0.00170 µM 0.005 µM 0.015 µM 0.046µM 0.137 µM 0.412 µM 1.23 µM Test 1 Test 2 3.70 µM 11.11 µM 33.33 µM 100µM Test 1 Test 2 HepG2 number of colonies 800 600 400 200 0 0.0001 0.01 1 100 [Compound 25], µM IC50 = 0.764 µM
Cpd **30**	 Control 0.078125 µM 0.15625 µM 0.3125 µM 0.625 µM 1.25 µM 2.5 µM 5 µM 10 µM Test 1 Test 2 HepG2 number of colonies 800 600 400 200 0 0.01 0.1 1 10 [Compound 30], µM

Table S12. Western blot analysis of SMYD3 protein.

SMYD3 Expression levels in human cells.



Western-blot analysis of SMYD3 protein using compounds **35** and **39** vs DMSO control in HepG2 cells.

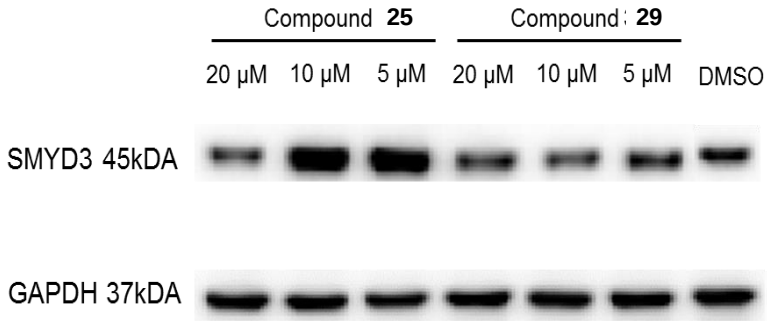
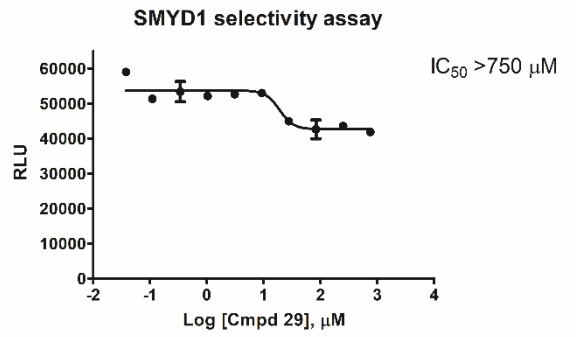
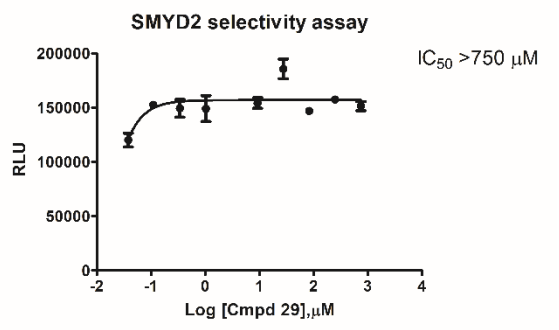
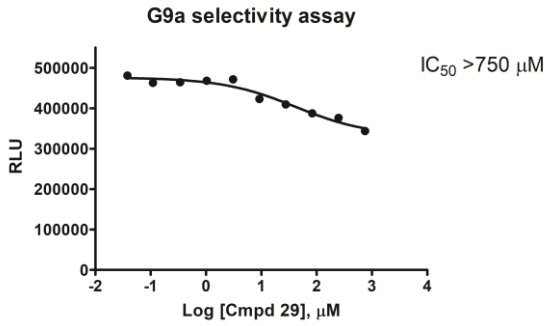
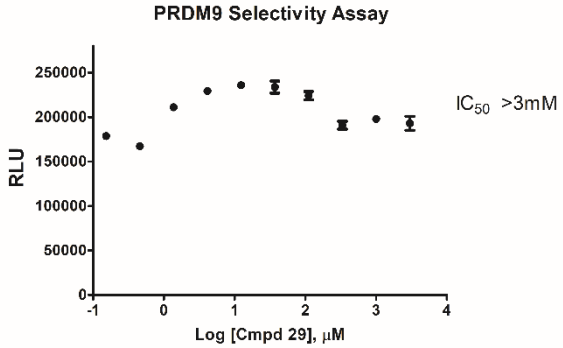
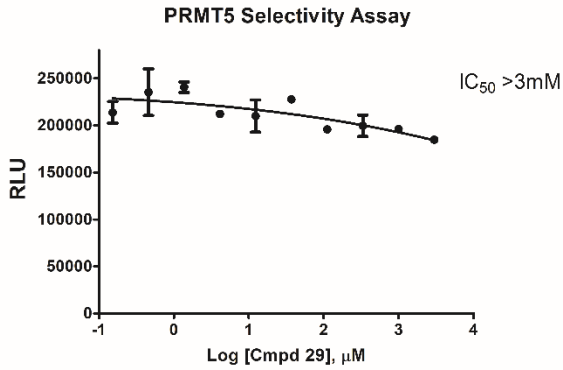


Table S13. Selectivity data for compound **29**.

Histone methyltransferase	Data
SMYD1	
SMYD2	

G9a	
PRDM9	
PRMT5	

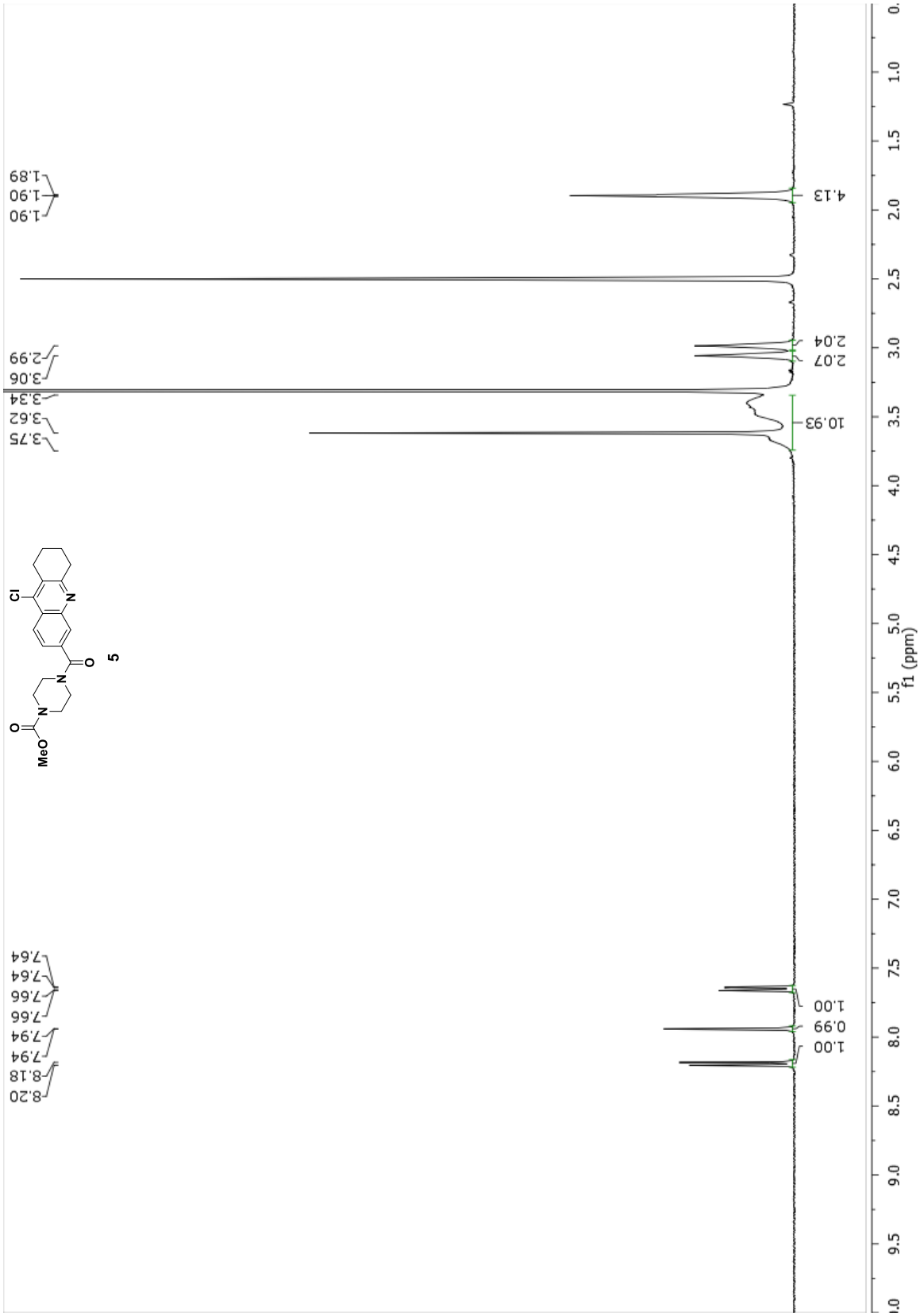
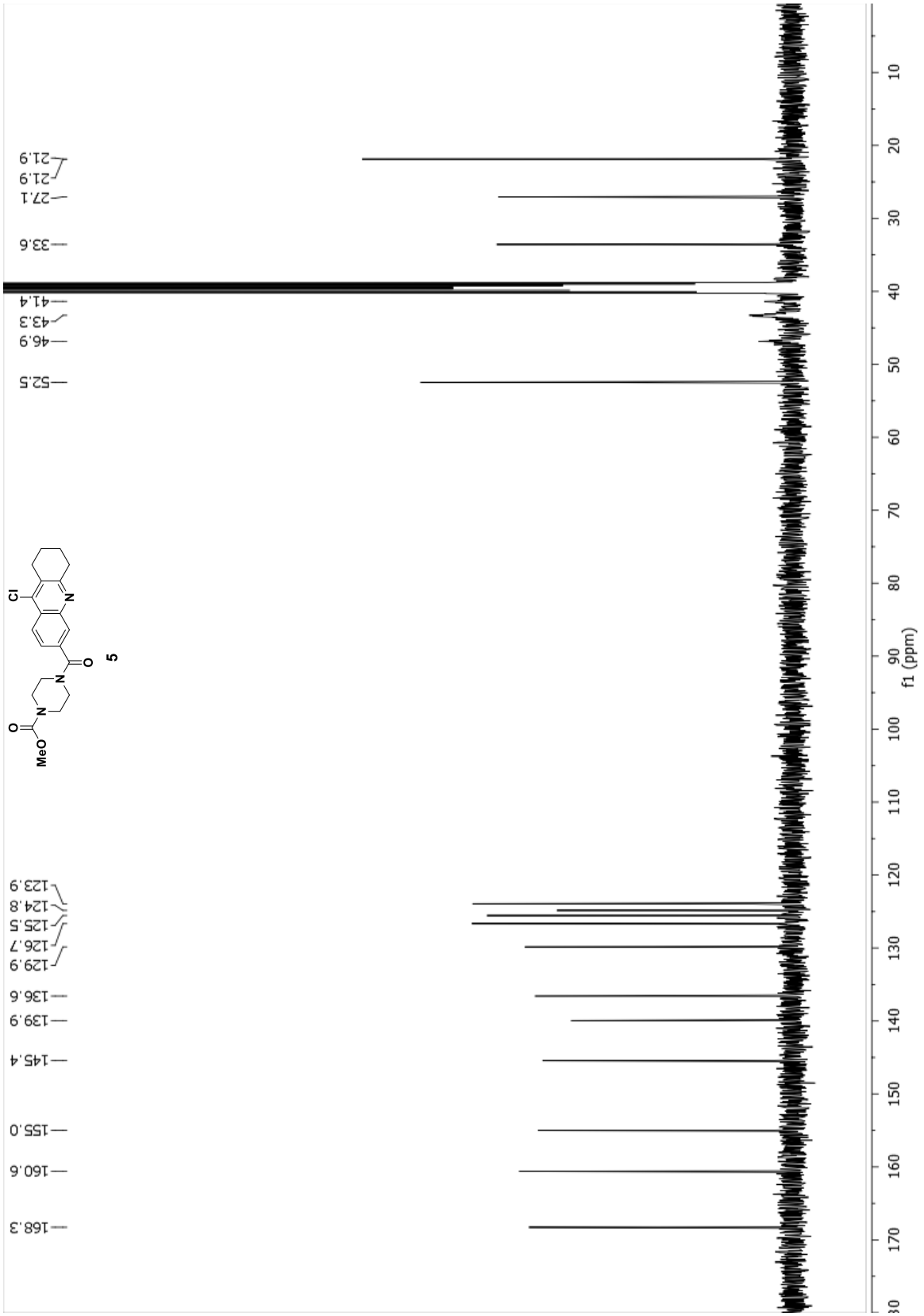


Figure S6. ^{1}H NMR (400 MHz, DMSO-d_6) of **5**.

Figure S7. ^{13}C NMR (101 MHz, DMSO-d_6) of **5**.



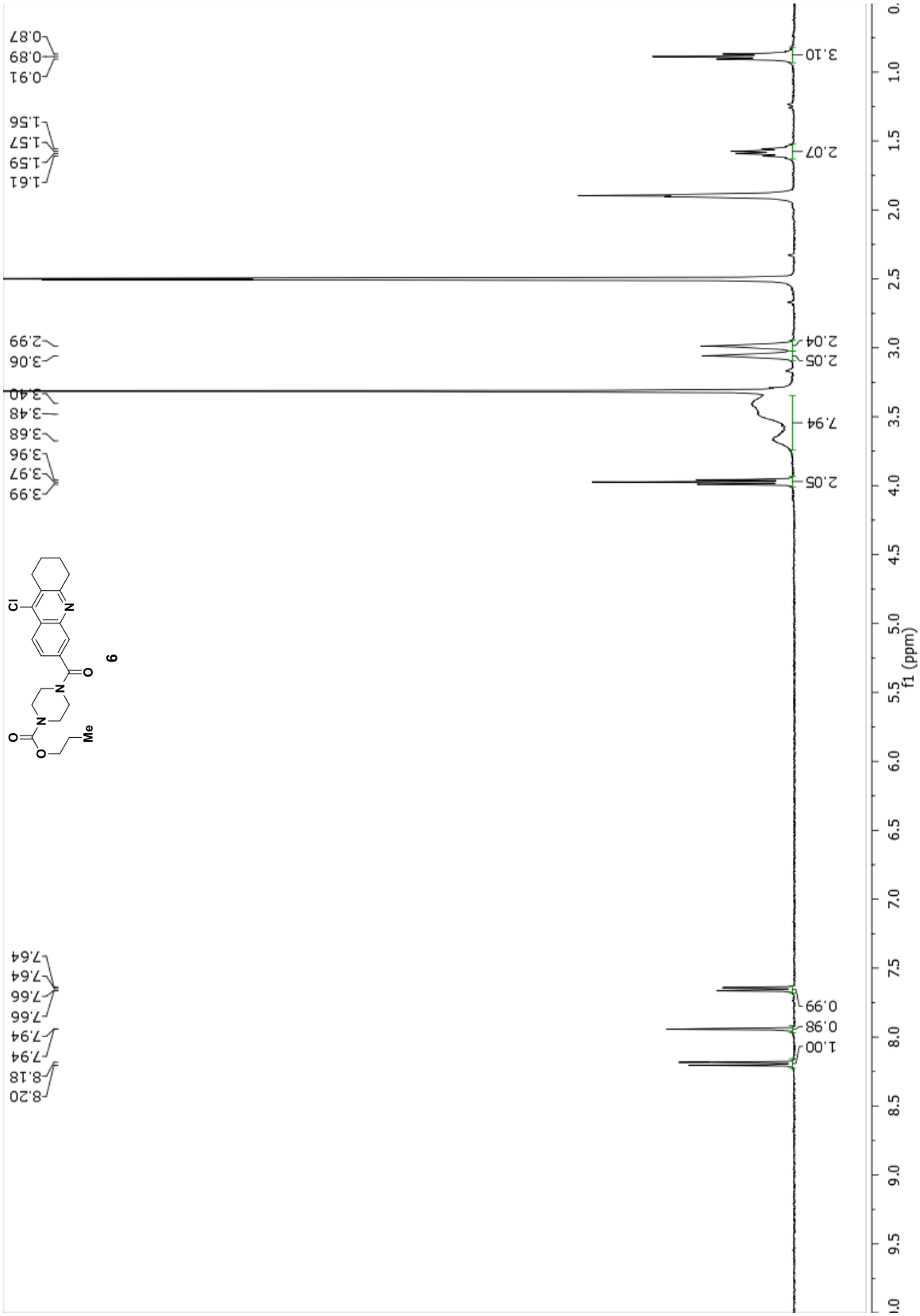


Figure S8. ^{1}H NMR (400 MHz, DMSO-d_6) of **6**.

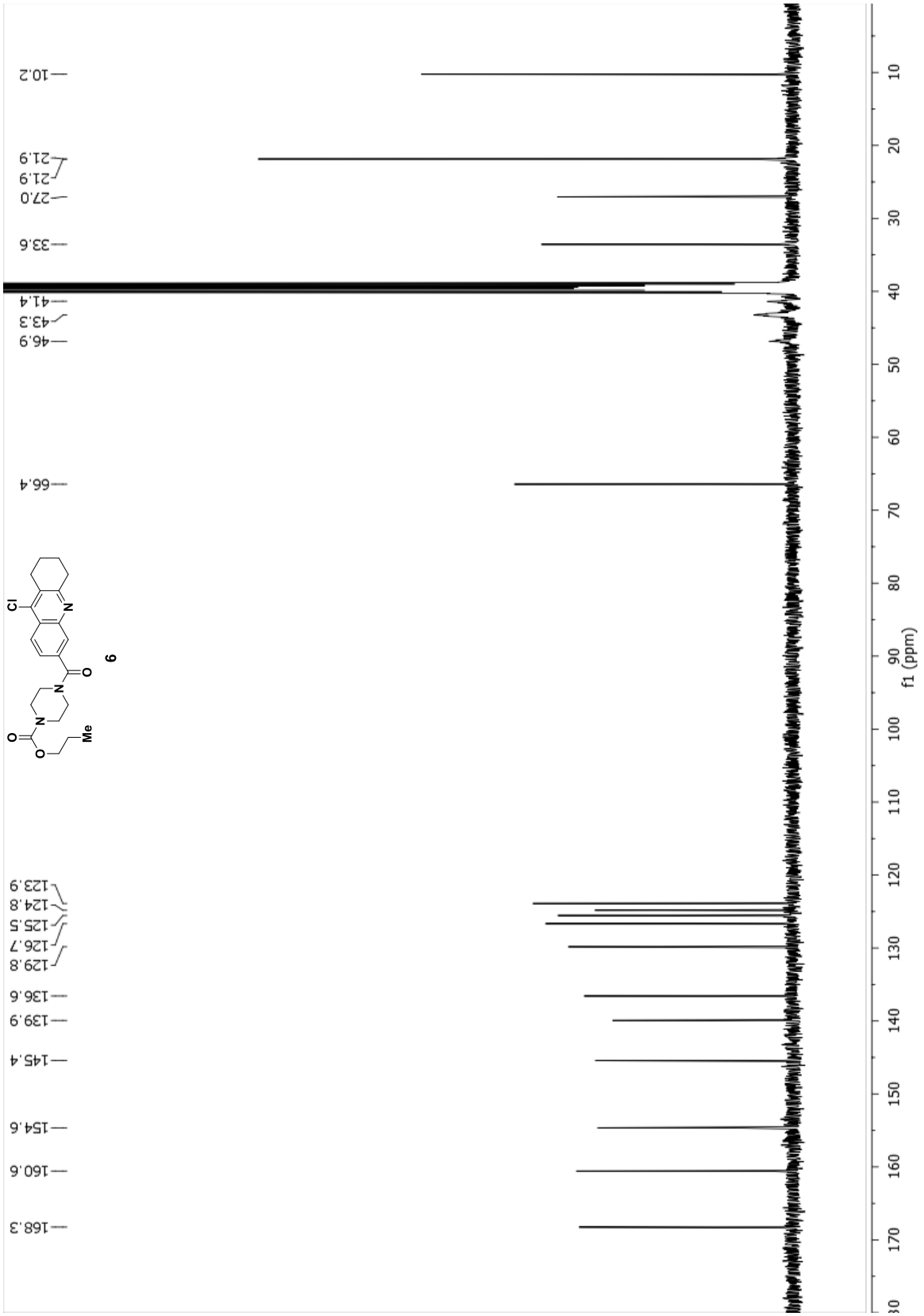


Figure S9. ^{13}H NMR (101 MHz, DMSO-d_6) of **6**.

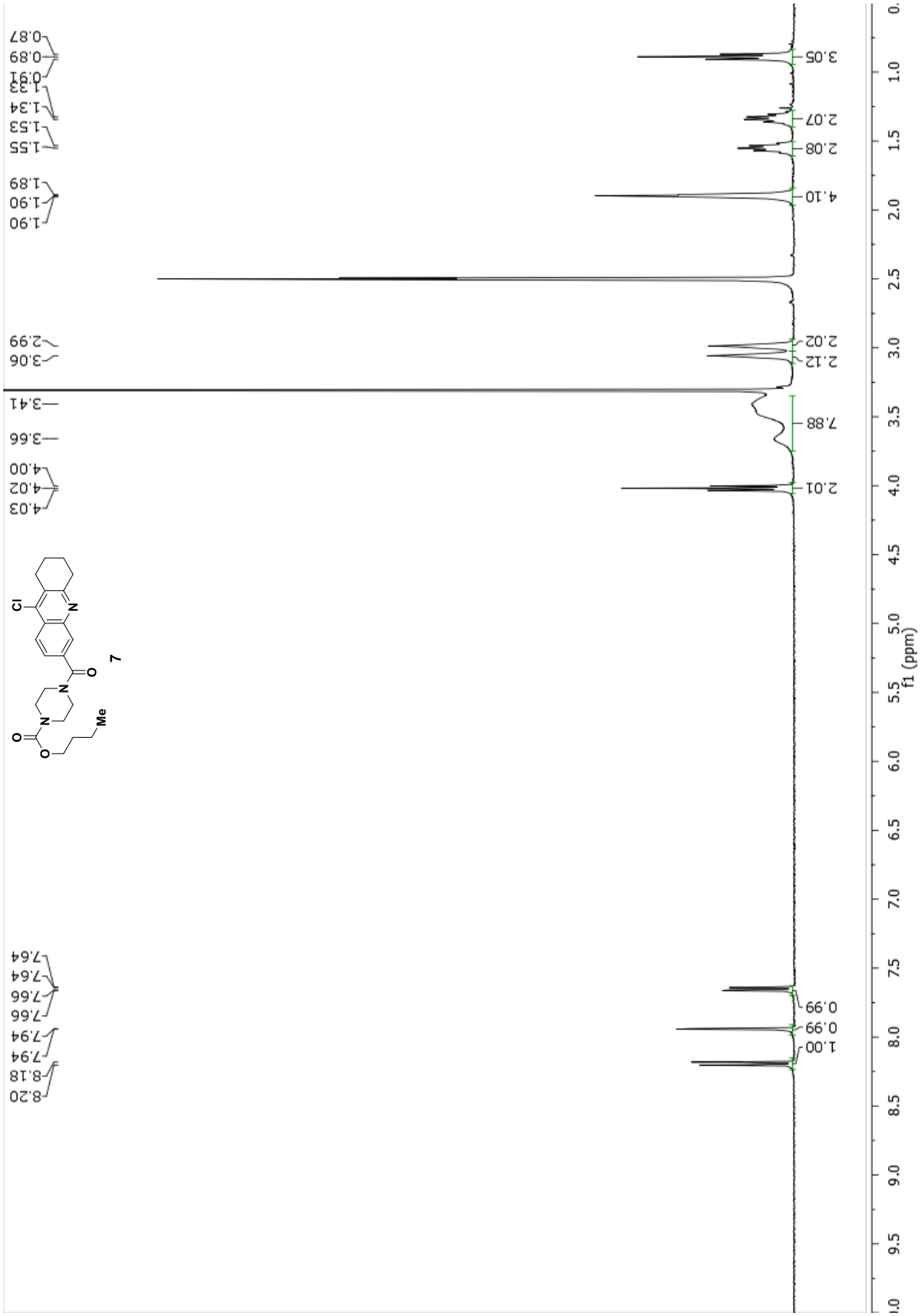


Figure S10. ^{1}H NMR (400 MHz, DMSO-d_6) of **7**.

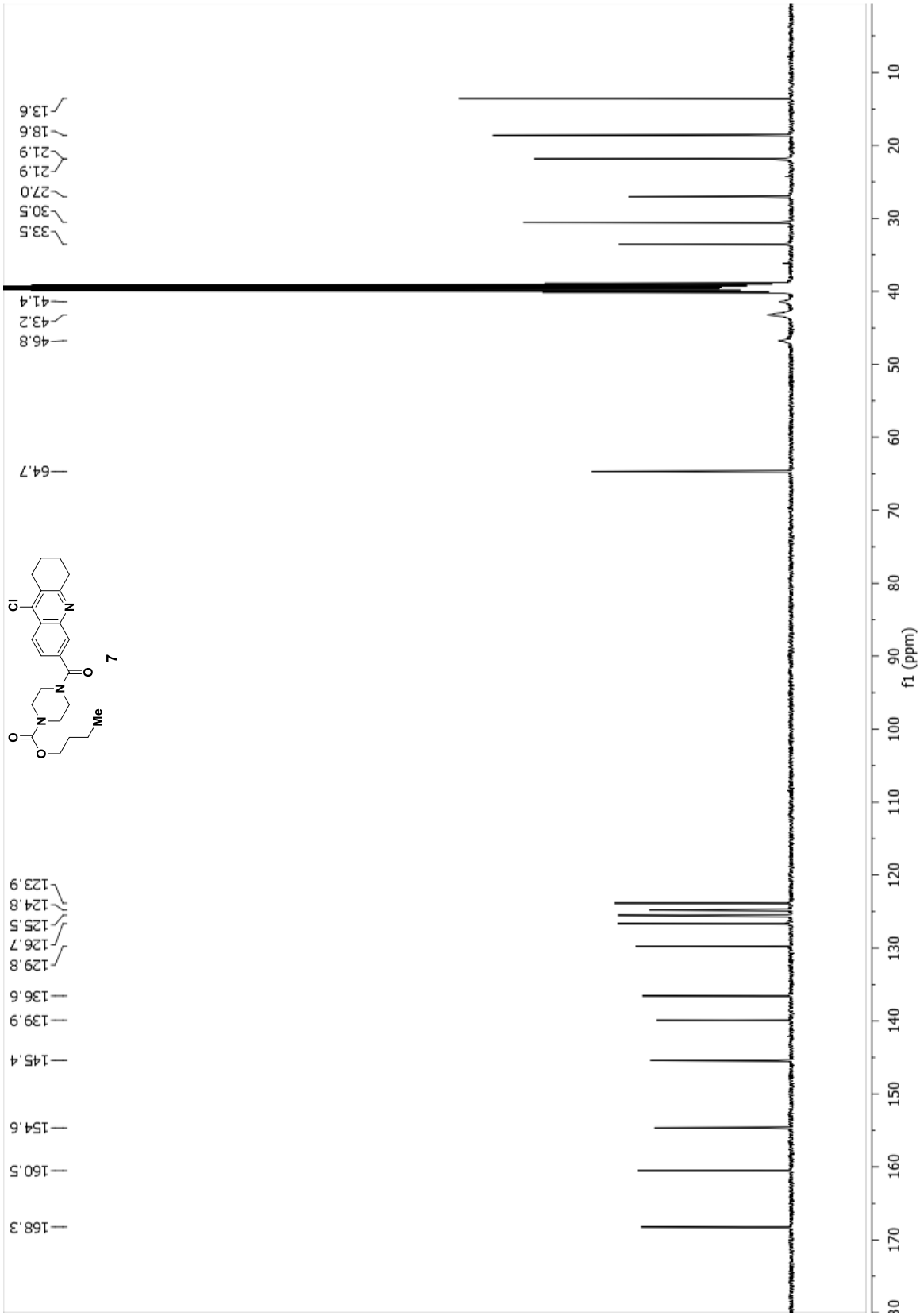


Figure S11. ^{13}C NMR (101 MHz, DMSO-d_6) of **7**.

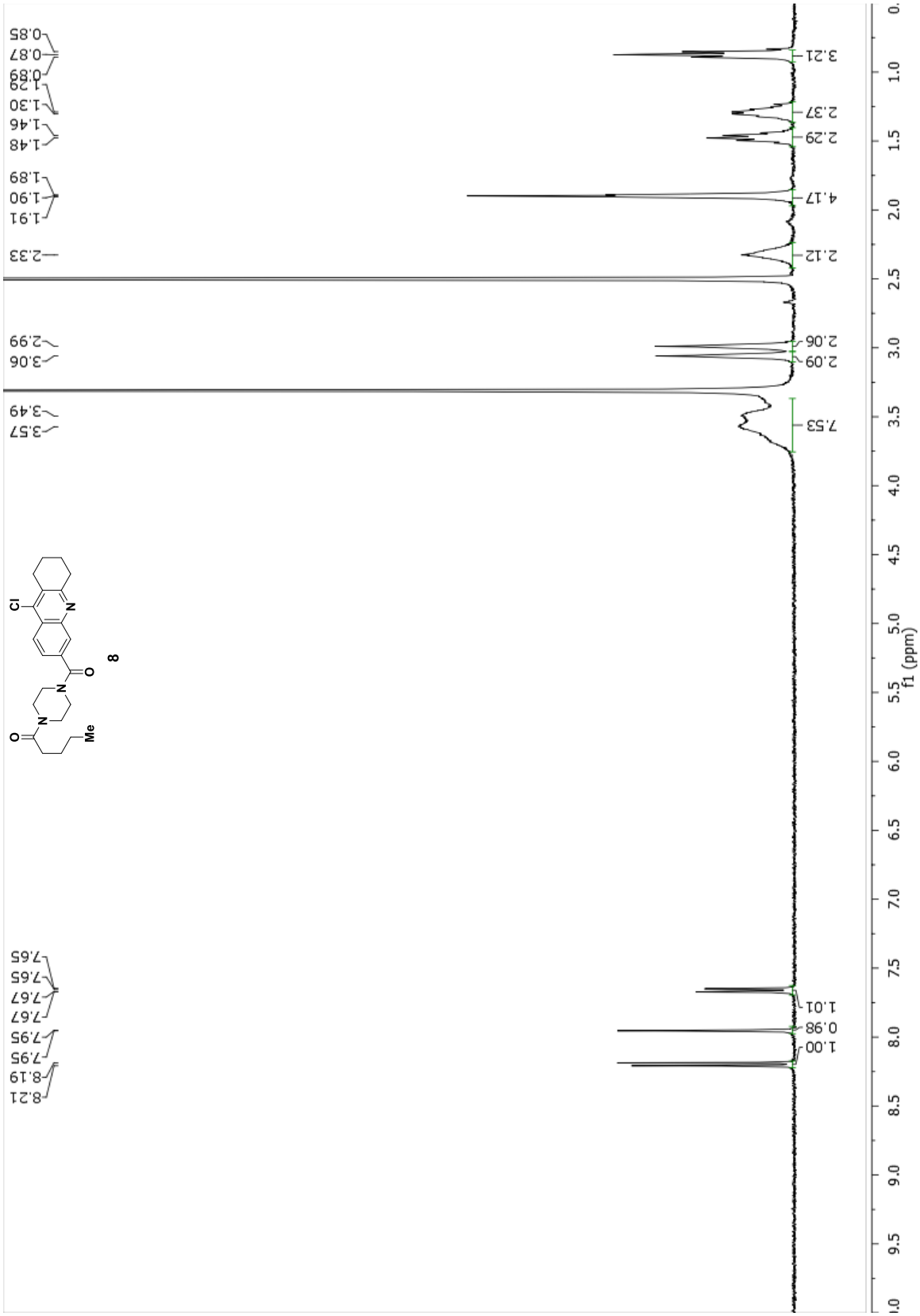


Figure S12. ^{1}H NMR (400 MHz, DMSO-d_6) of **8**.

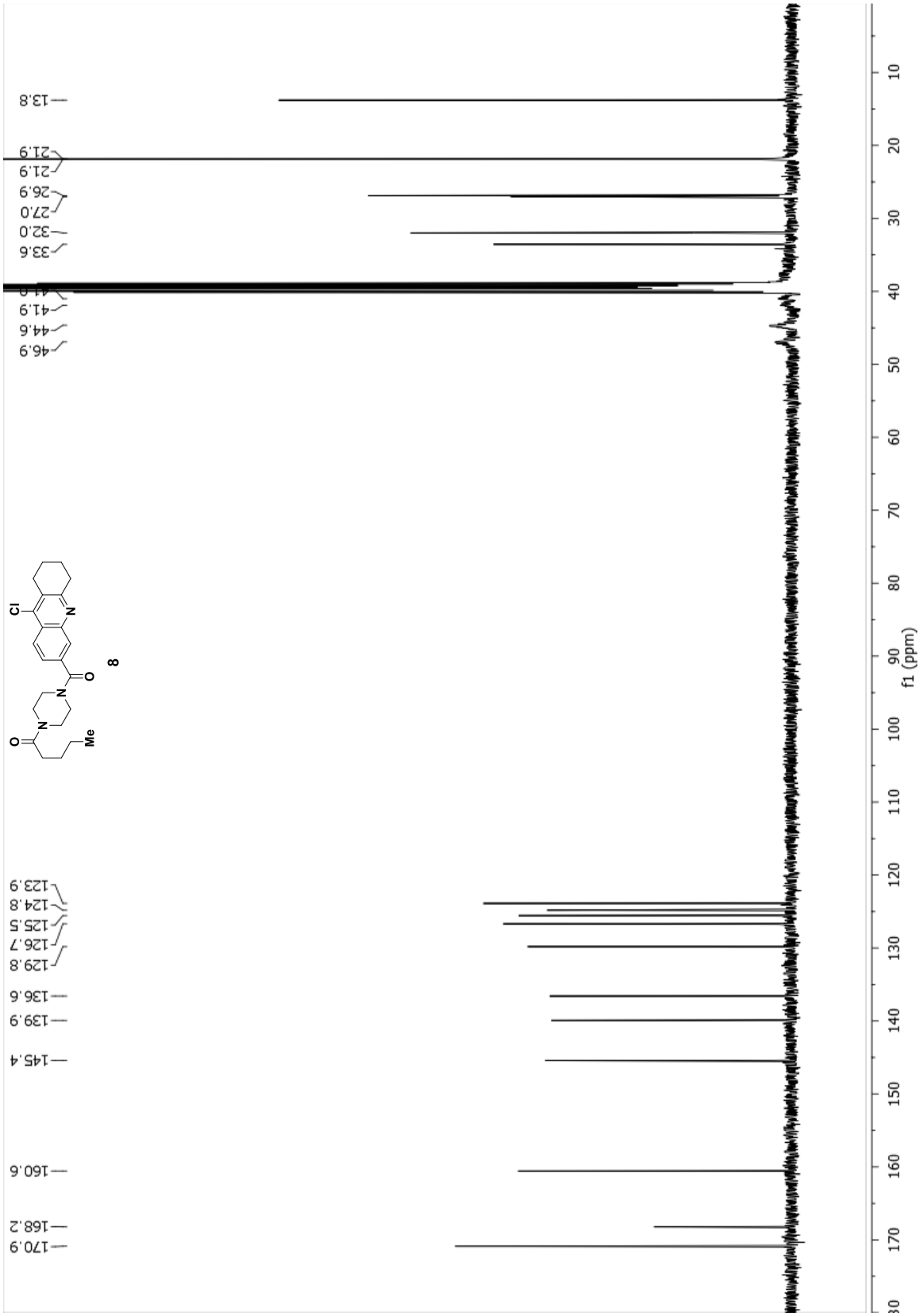


Figure S13. ^{13}C NMR (101 MHz, DMSO-d_6) of **8**.

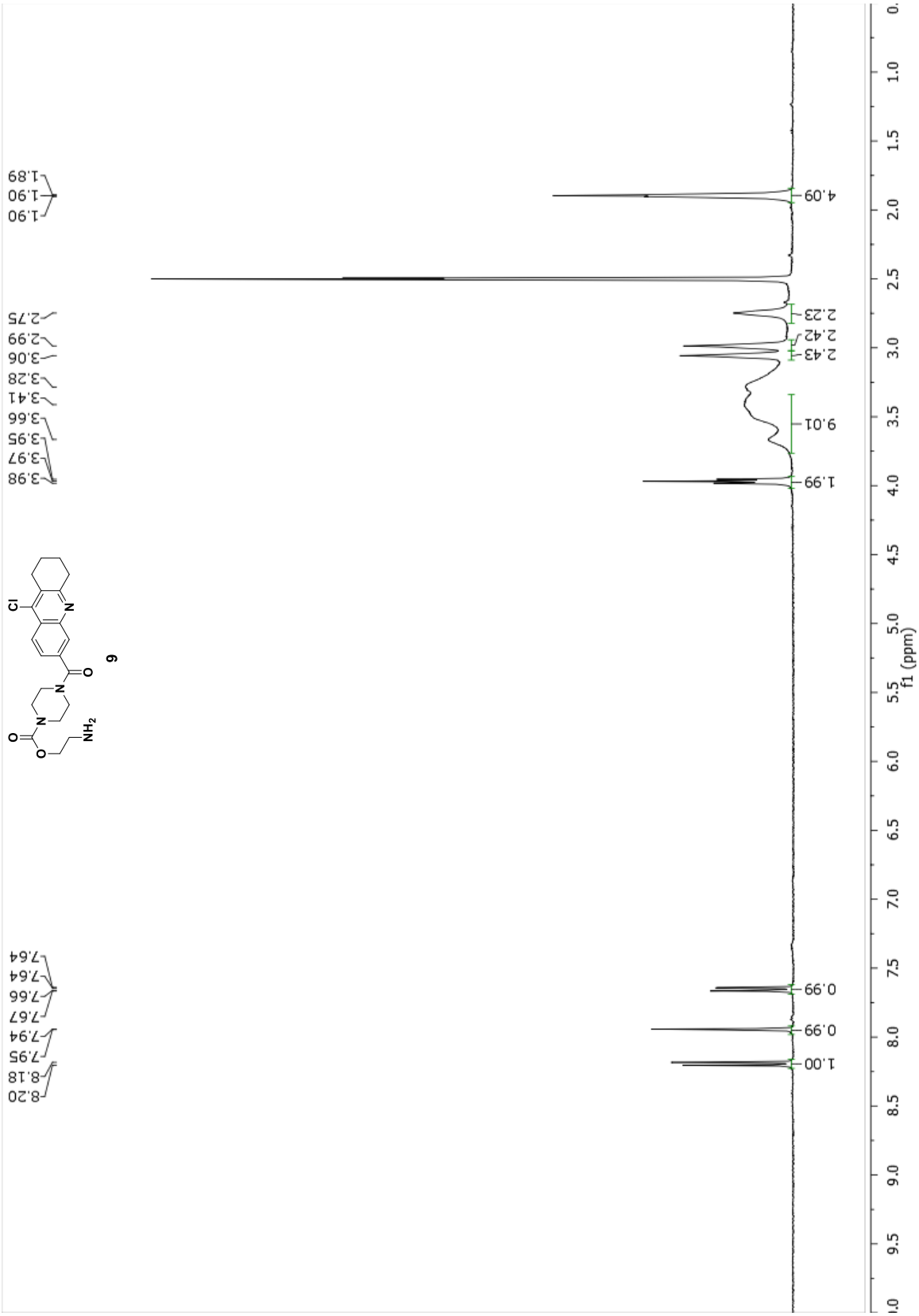
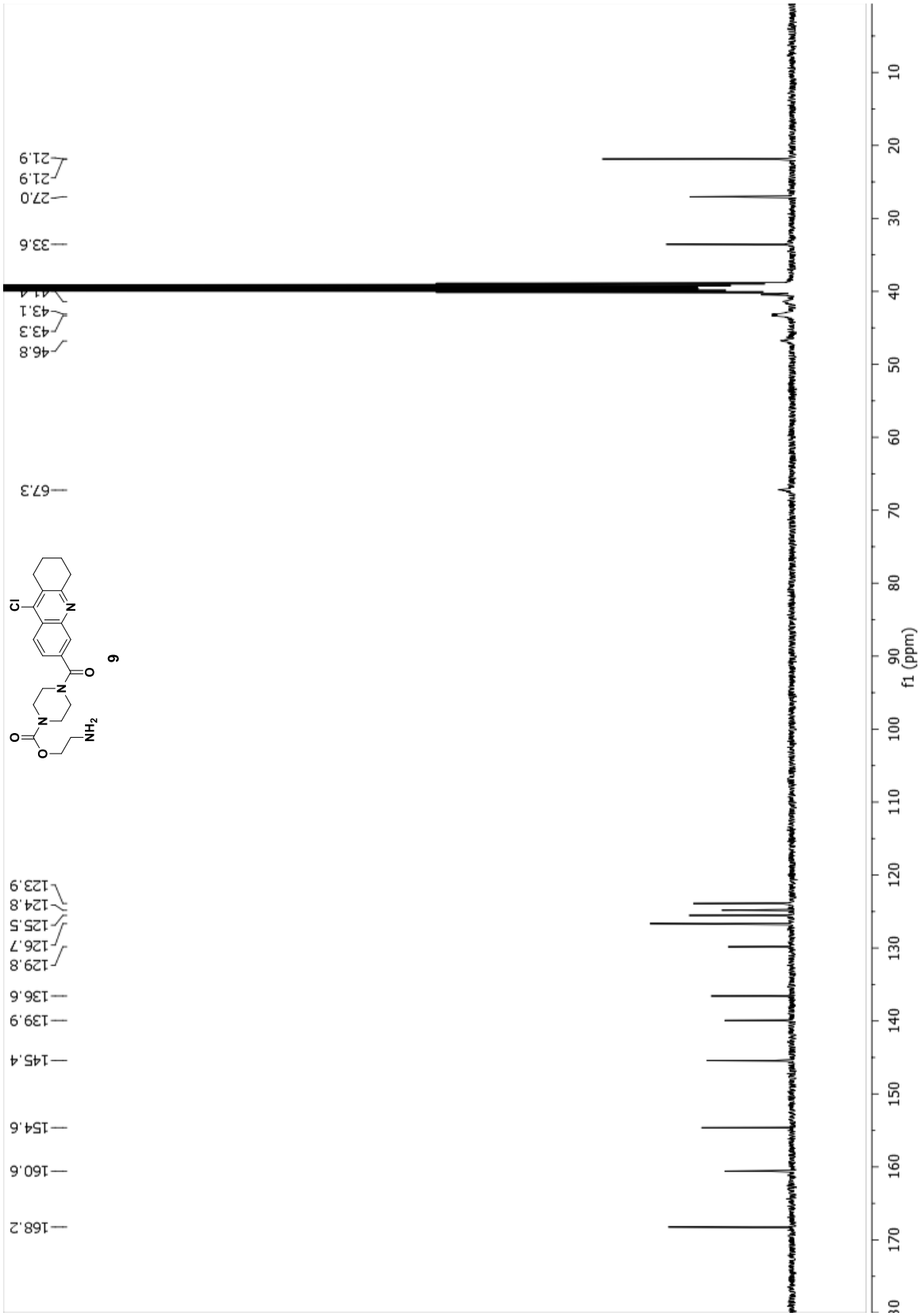


Figure S14. ^{1}H NMR (400 MHz, DMSO-d_6) of **9**.

Figure S15. ^{13}C NMR (101 MHz, DMSO-d_6) of **9**.



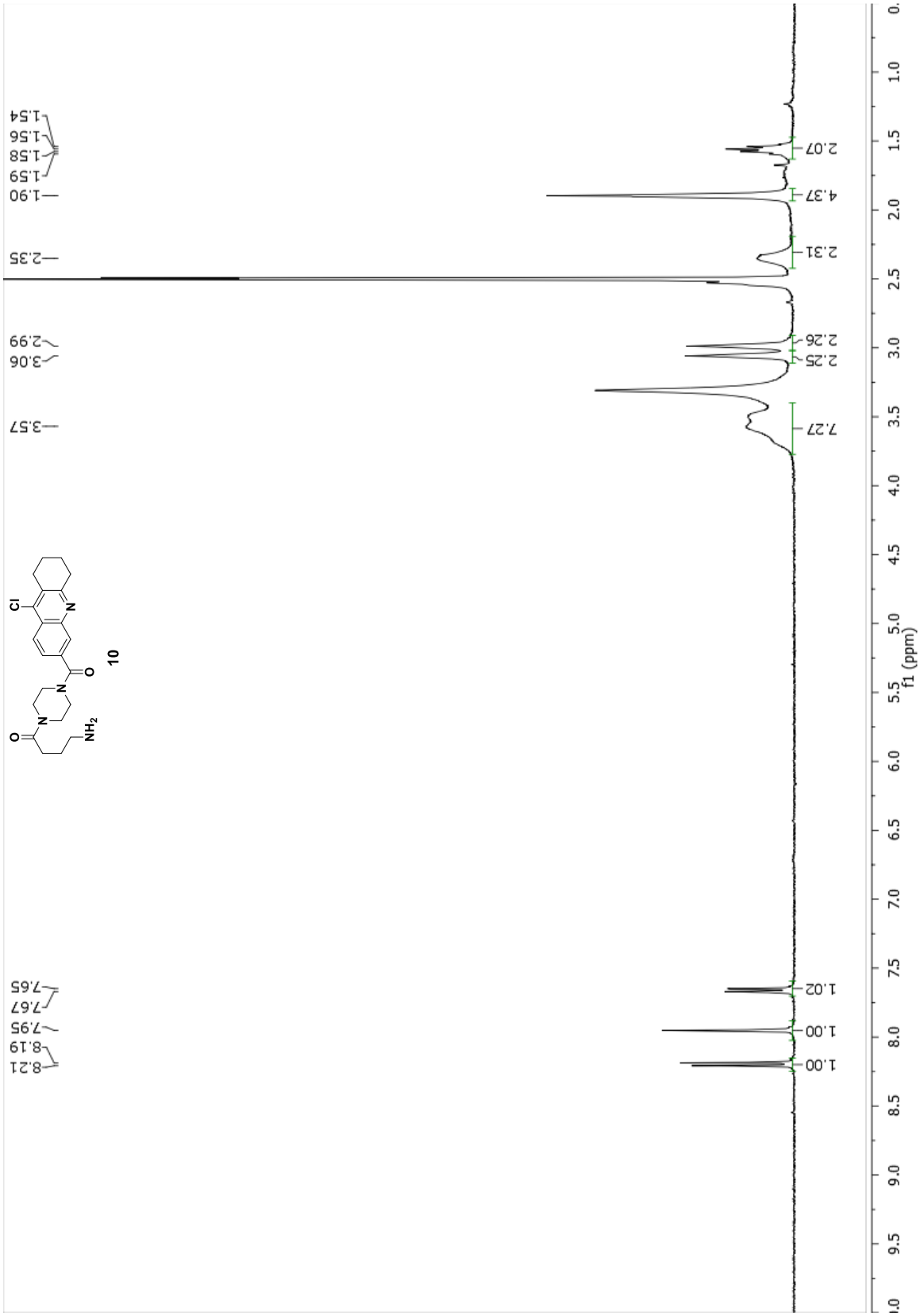


Figure S16. ^{1}H NMR (400 MHz, DMSO-d_6) of **10**.

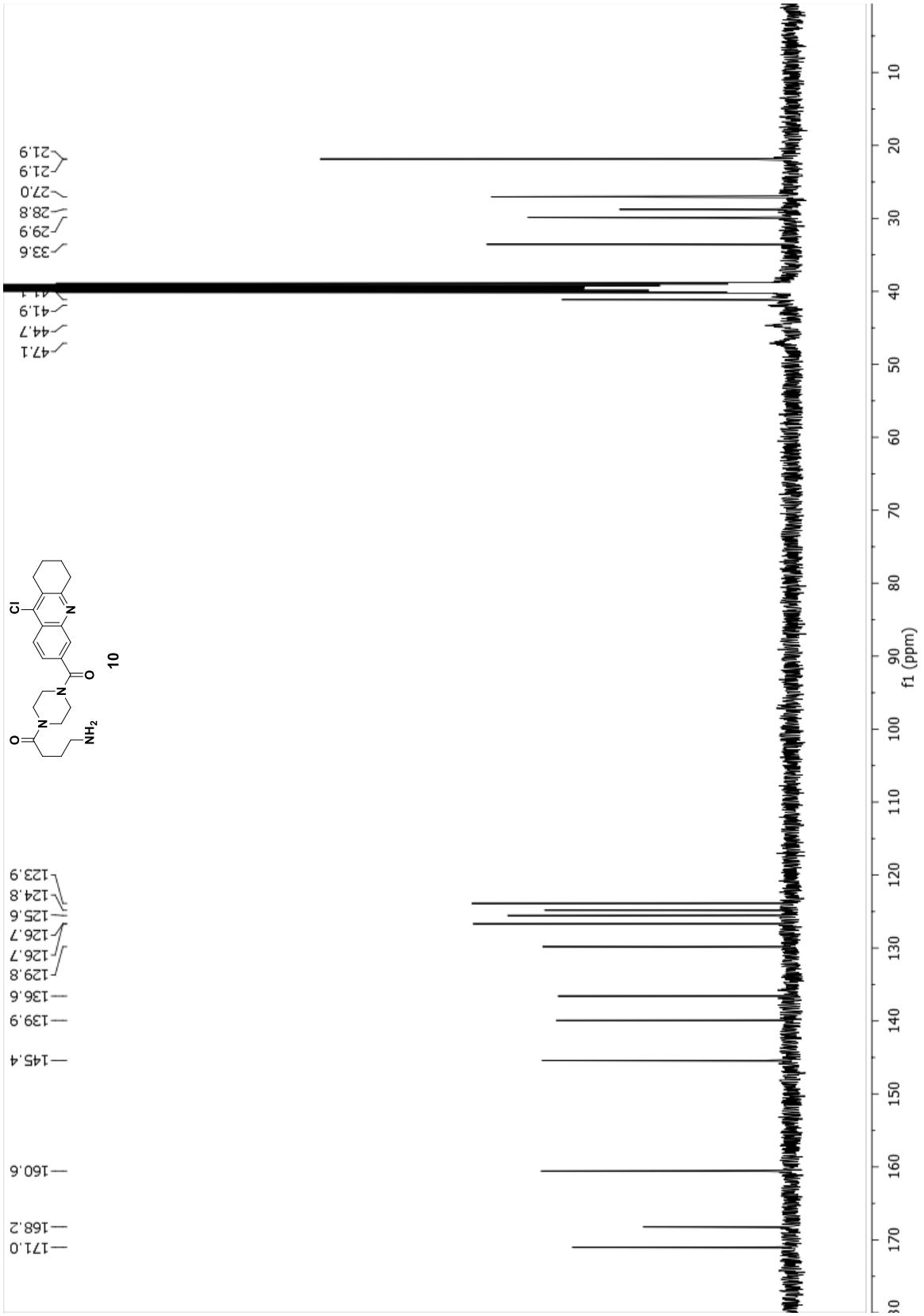


Figure S17. ^{13}C NMR (101 MHz, DMSO-d_6) of **10**.

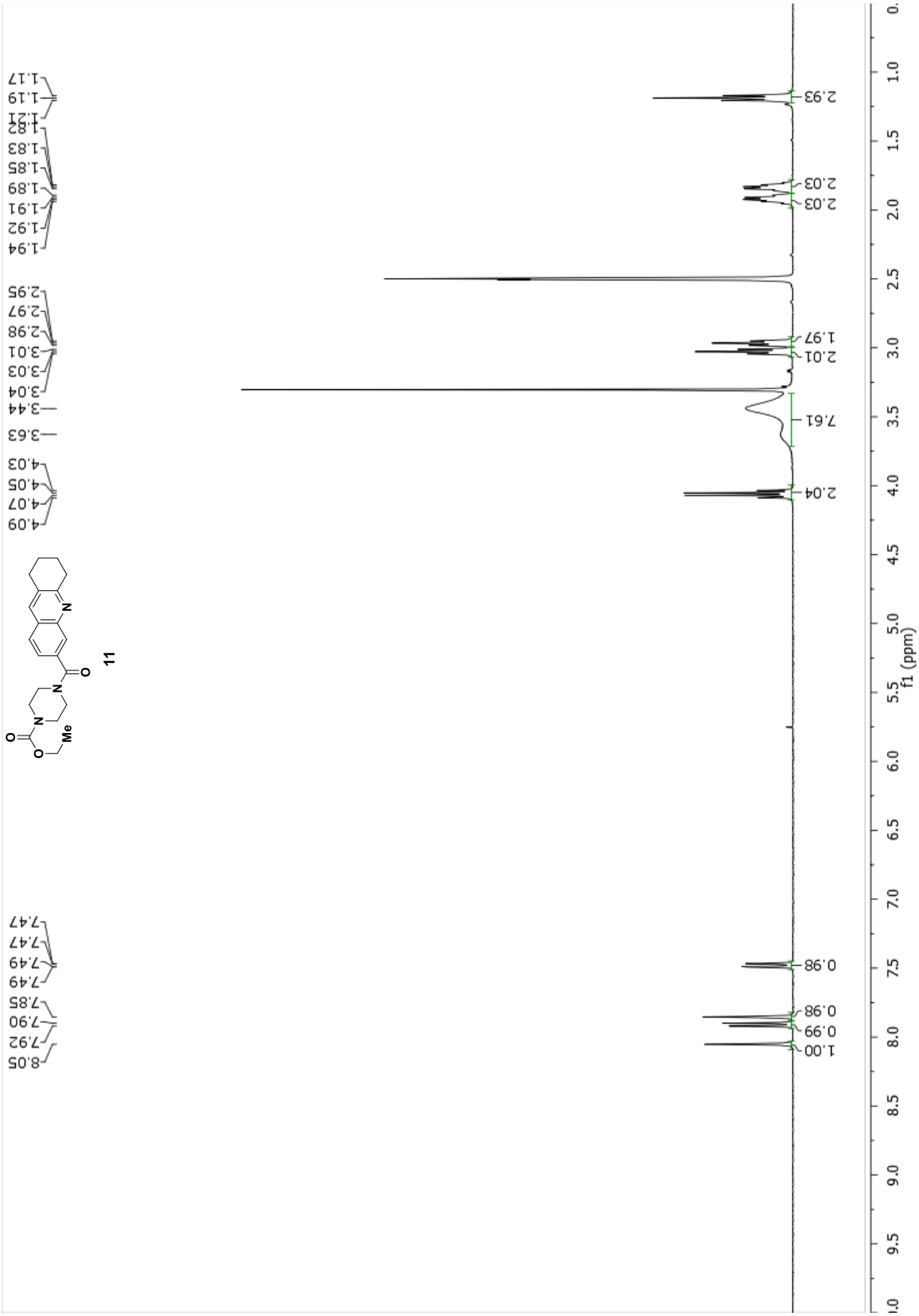


Figure S18. ^{1}H NMR (400 MHz, DMSO-d_6) of **11**.

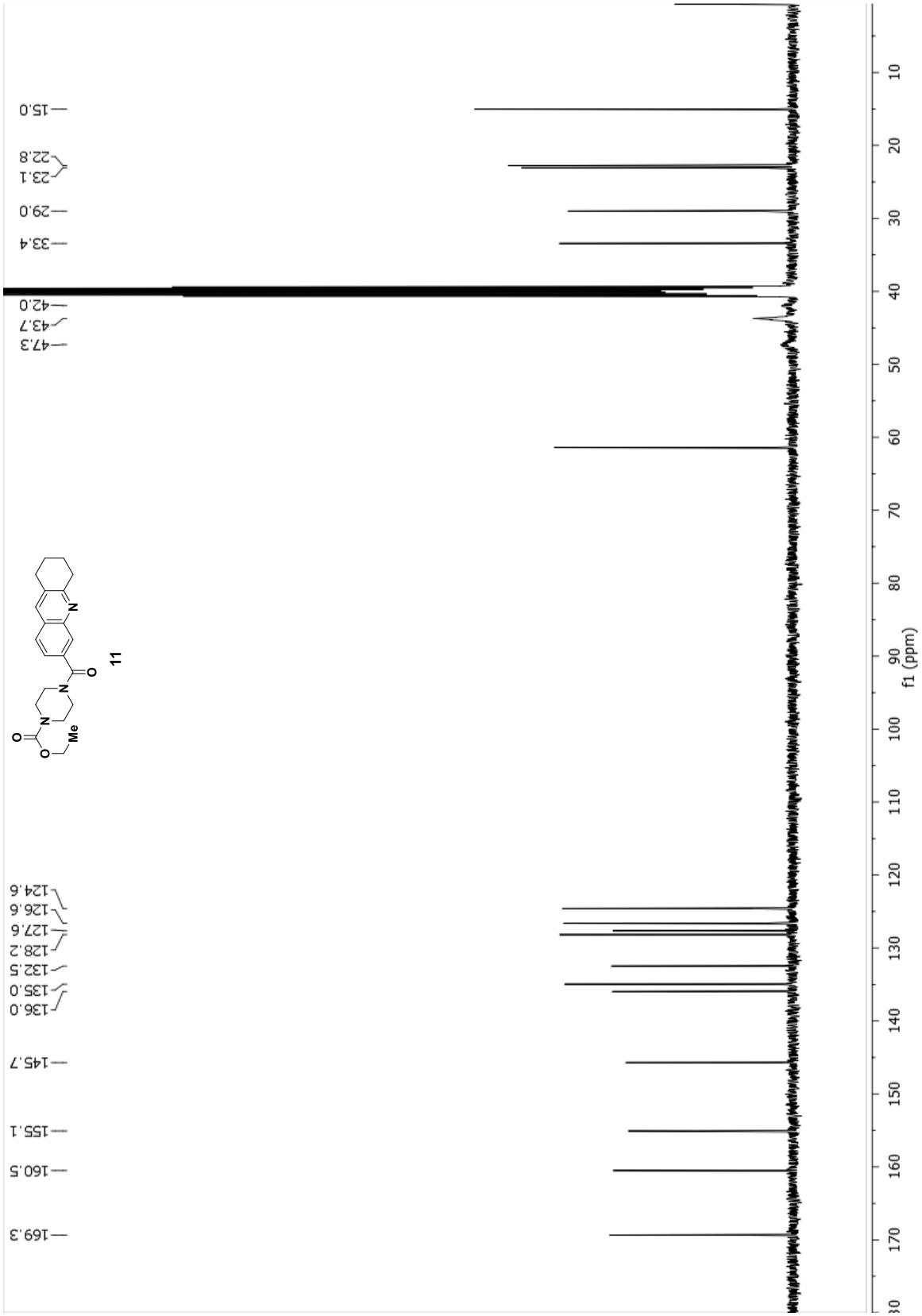


Figure S19. ^{13}C NMR (101 MHz, DMSO-d_6) of **11**.

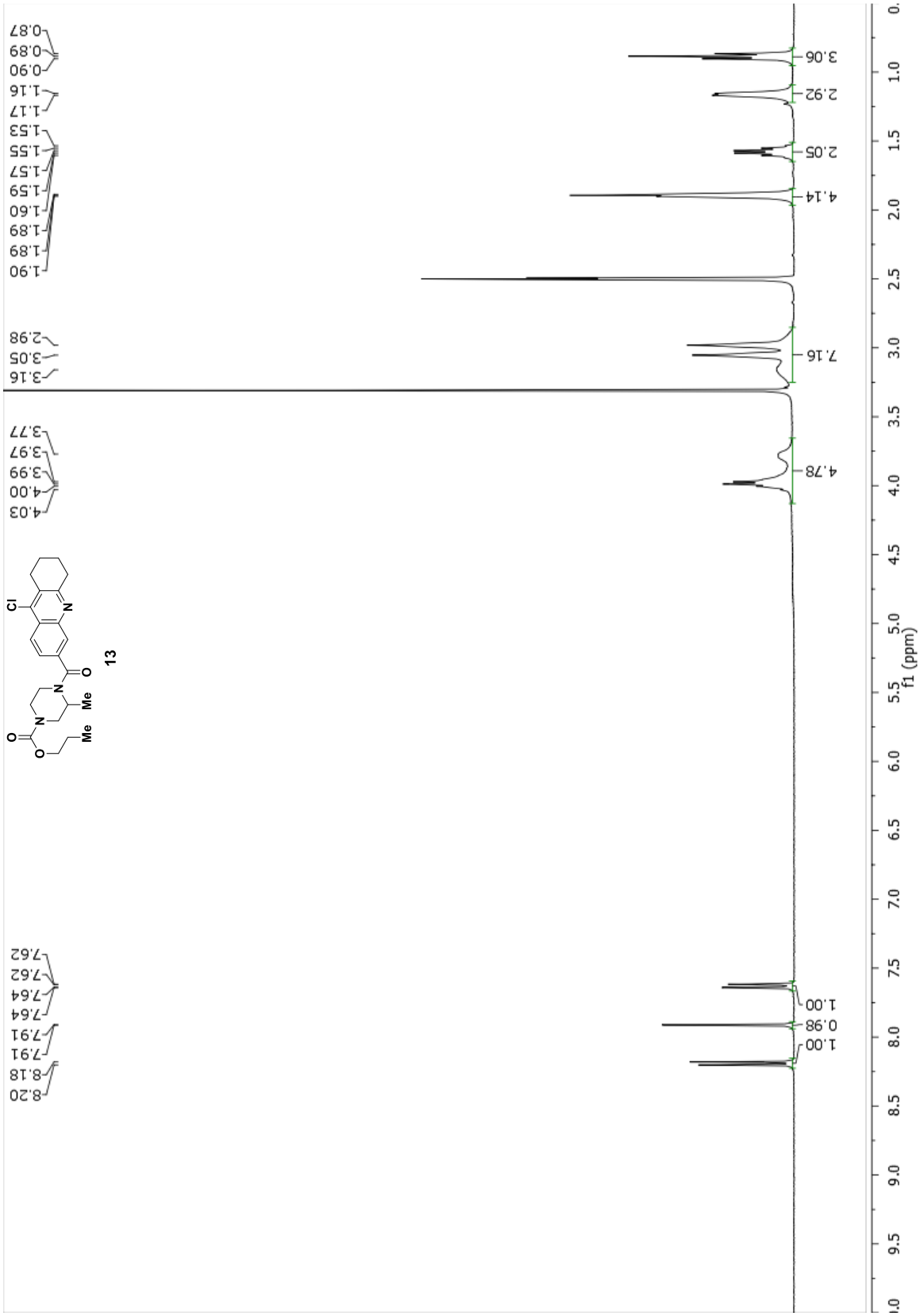
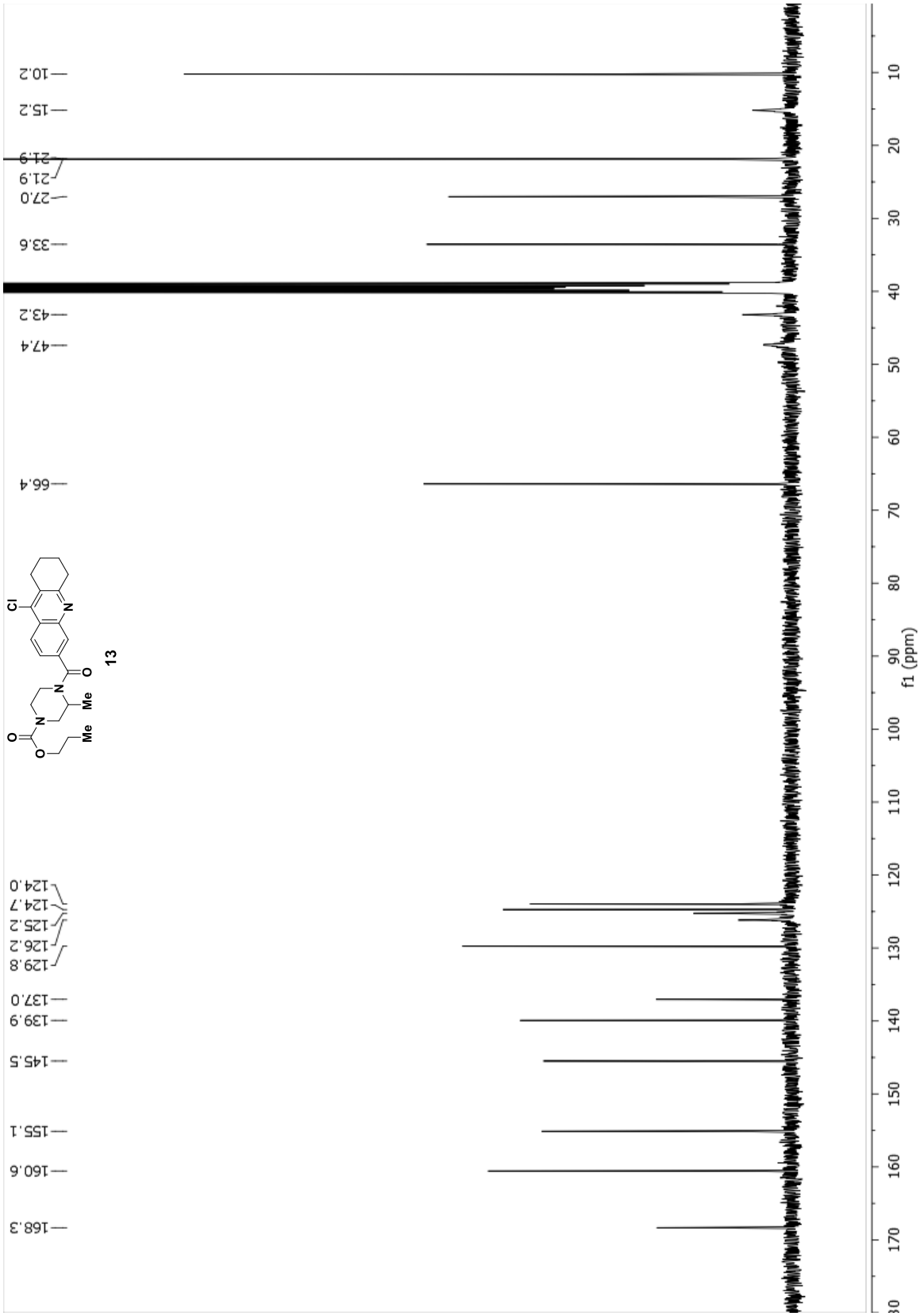


Figure S20. ^{1}H NMR (400 MHz, DMSO-d_6) of **13**.

Figure S21. ^{13}C NMR (101 MHz, DMSO-d_6) of **13**.



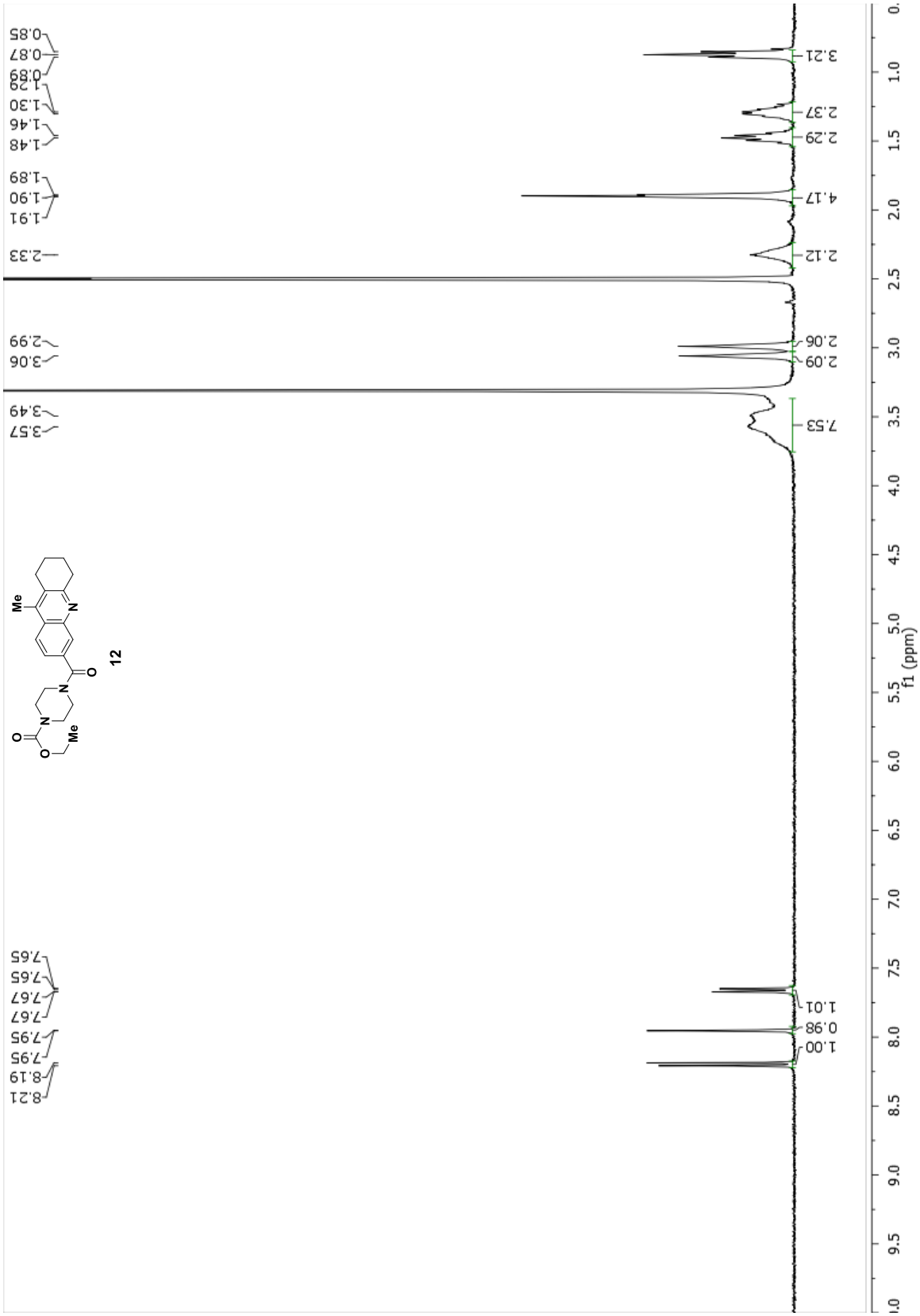


Figure S22. ^{1}H NMR (400 MHz, DMSO-d_6) of **12**.

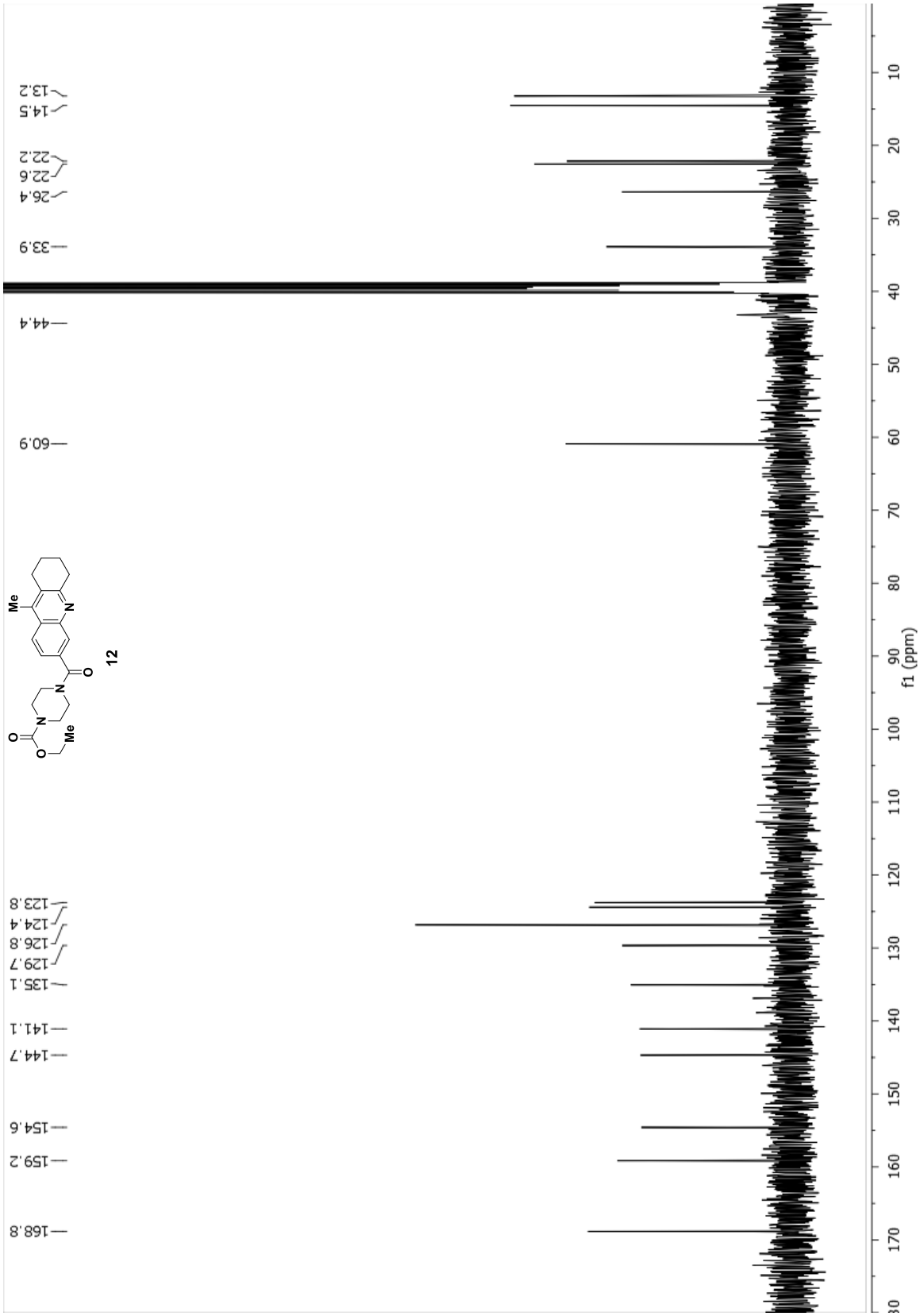


Figure S23. ^{13}C NMR (101 MHz, DMSO-d_6) of **12**.

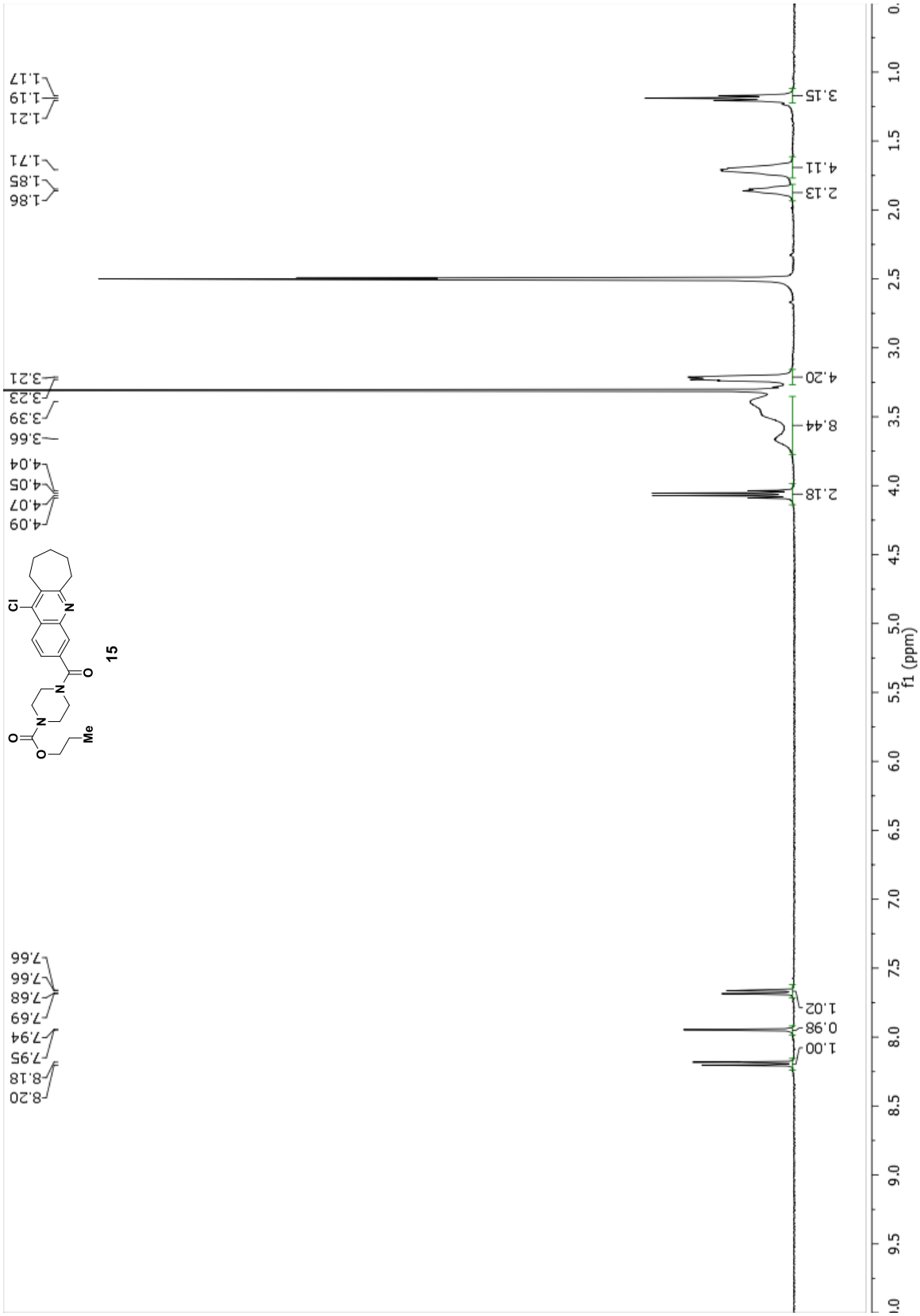


Figure S24. ^{1}H NMR (400 MHz, DMSO-d_{66}) of **15**.

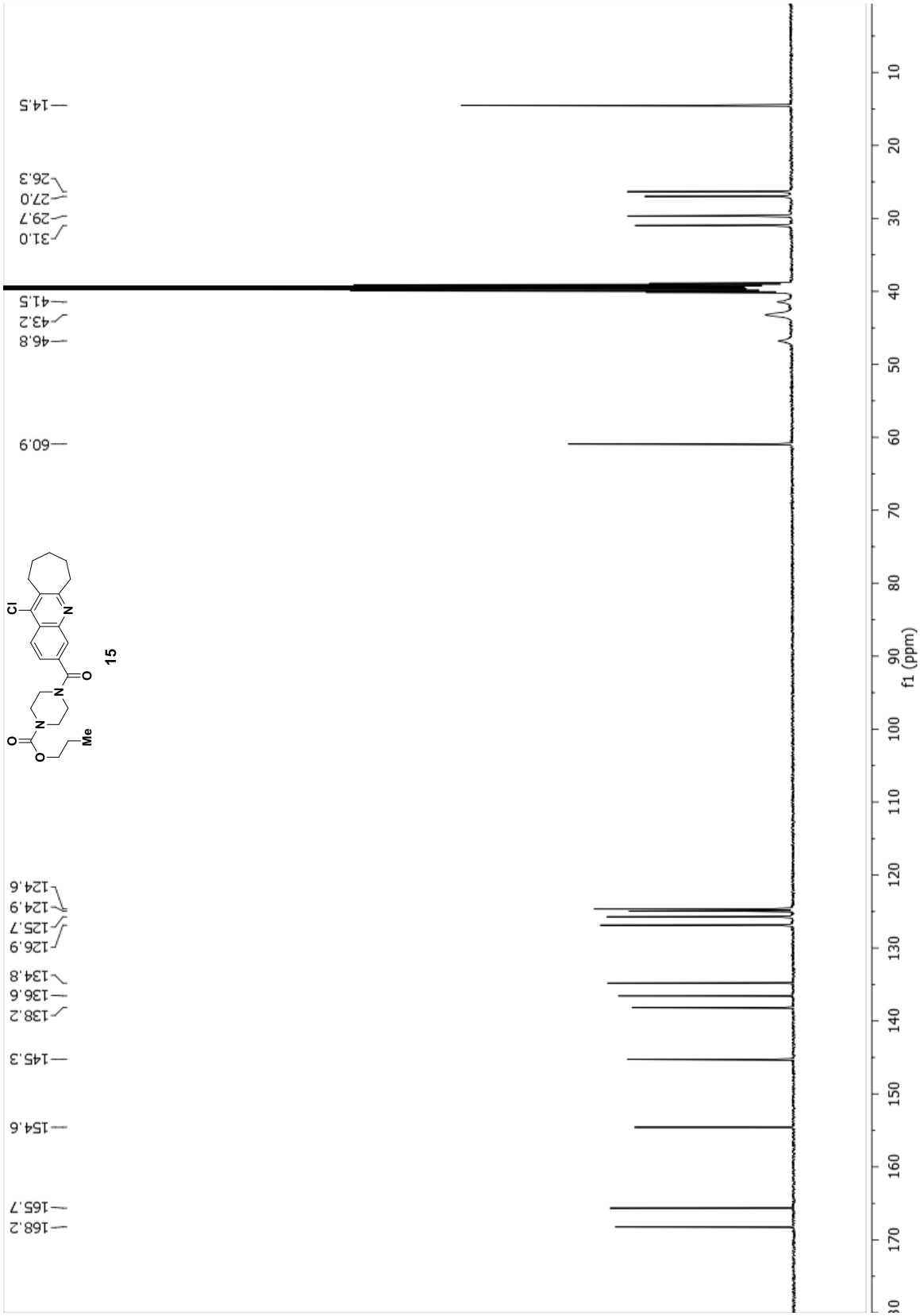


Figure S25. ^{13}C NMR (101 MHz, DMSO-d_6) of **15.**

Figure S26. ^{1}H NMR (400 MHz, DMSO-d_6) of **16**.

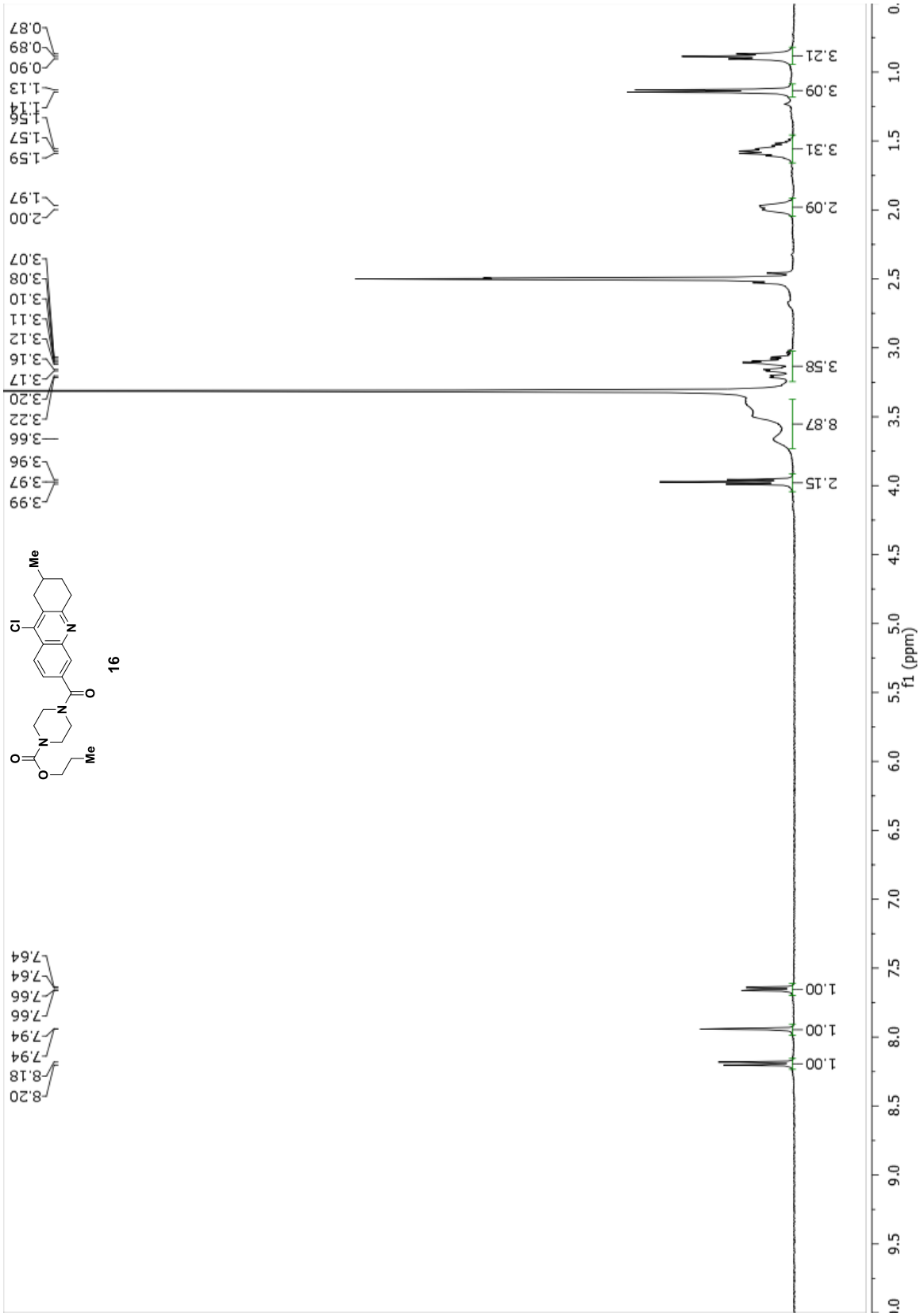
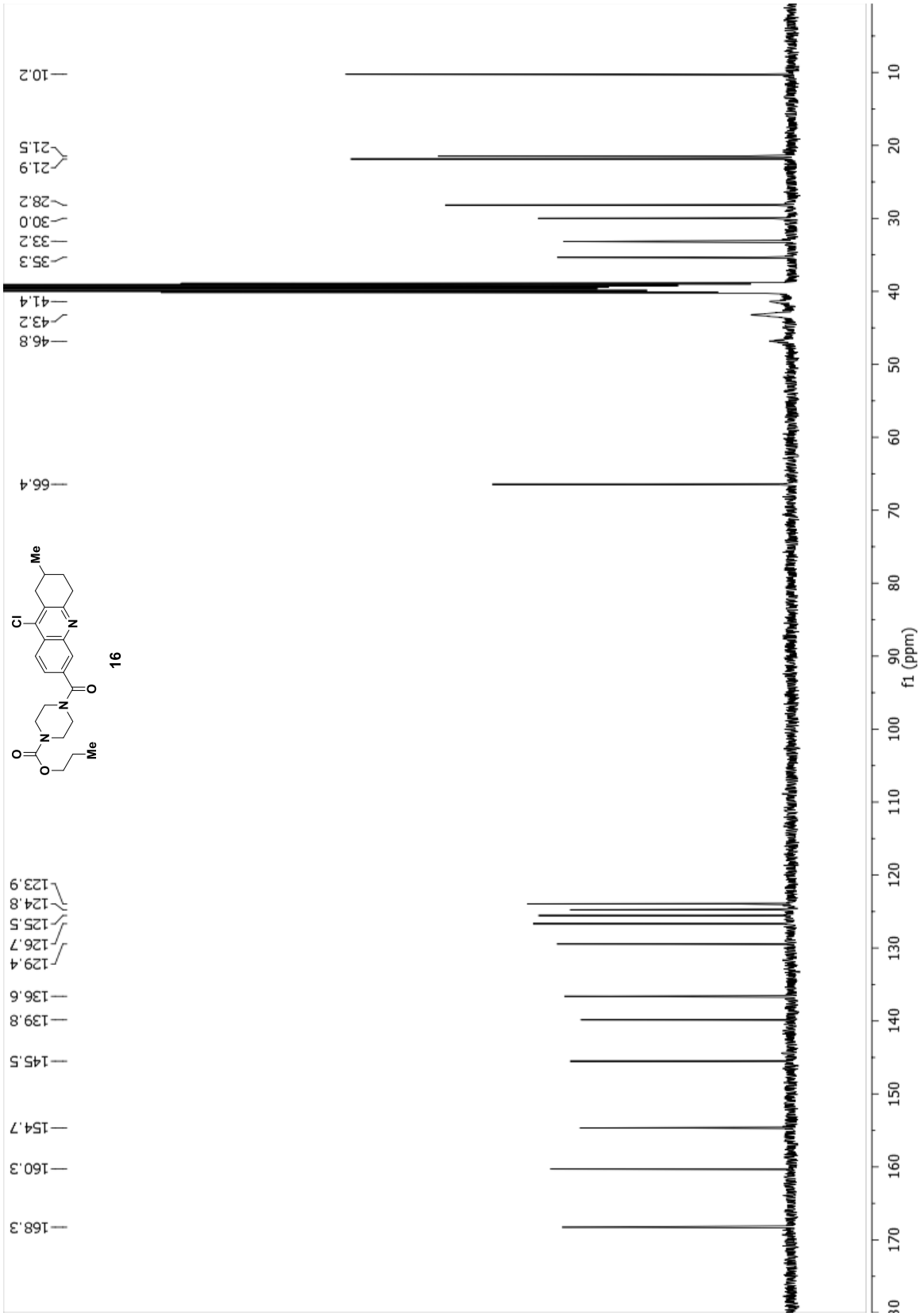


Figure S27. ^{13}C NMR (101 MHz, DMSO-d_6) of **16**.



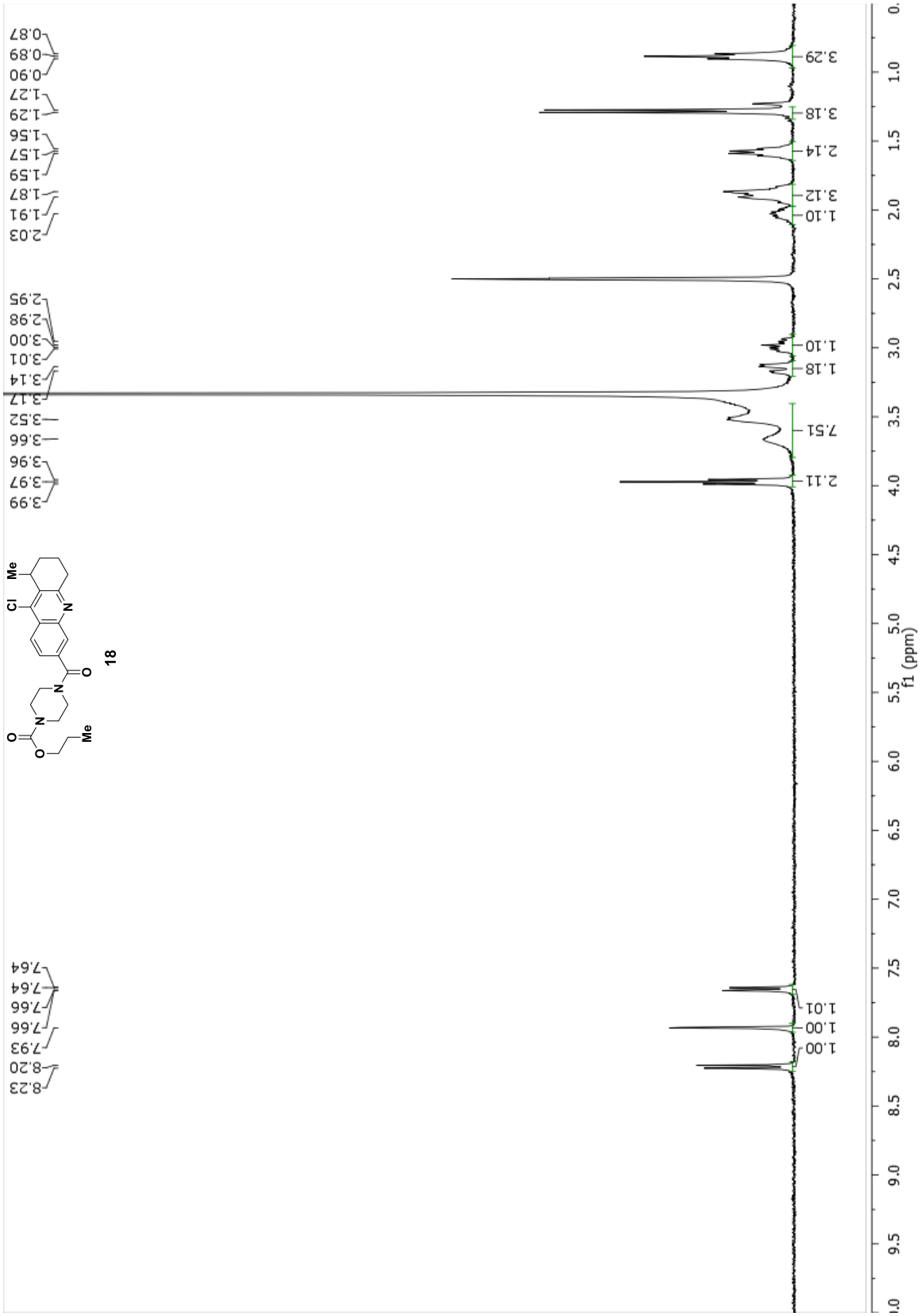


Figure S28. ^{1}H NMR (400 MHz, DMSO-d_6) of **18**.

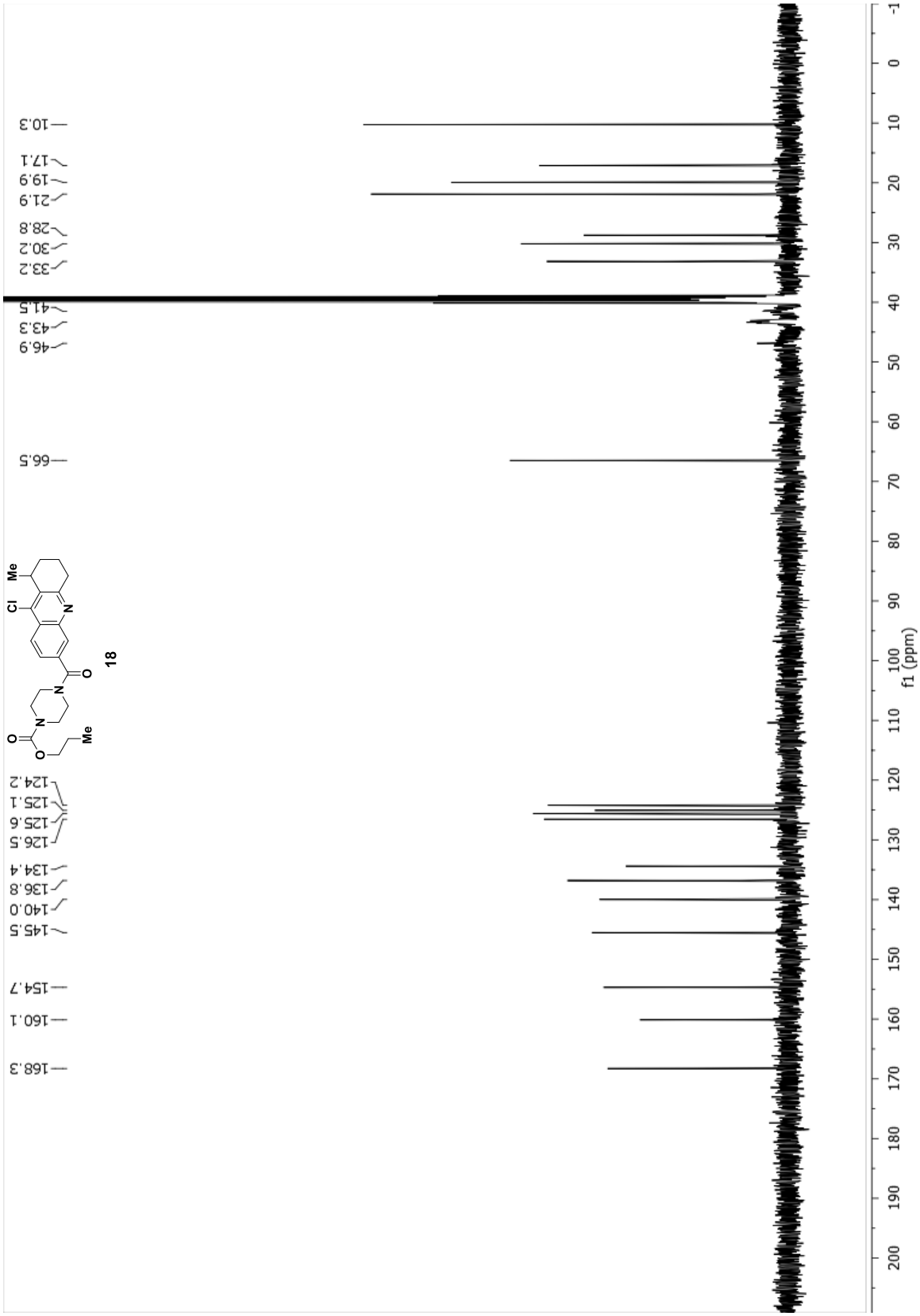


Figure S29. ^{13}C NMR (101 MHz, DMSO-d_6) of **18**.

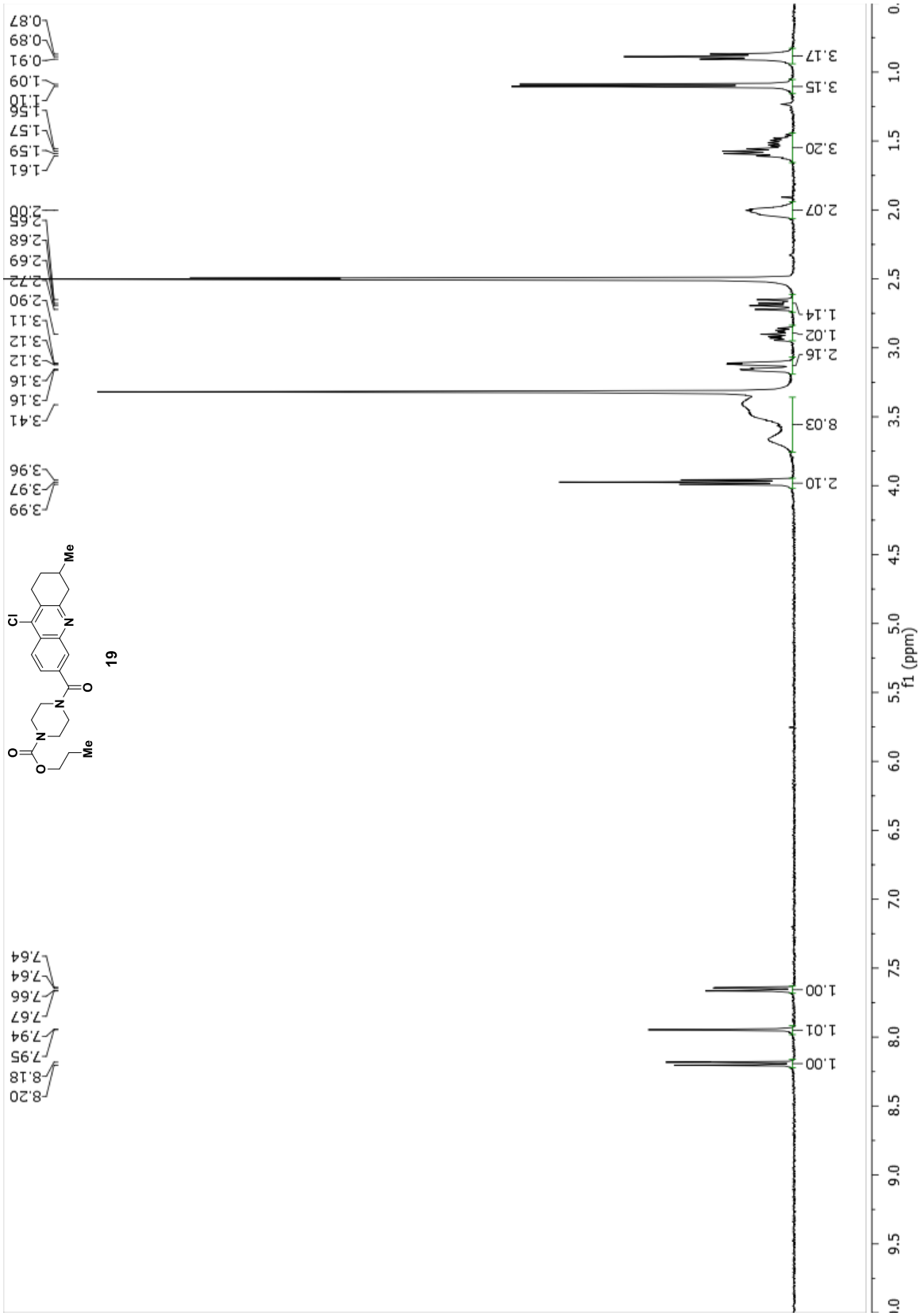
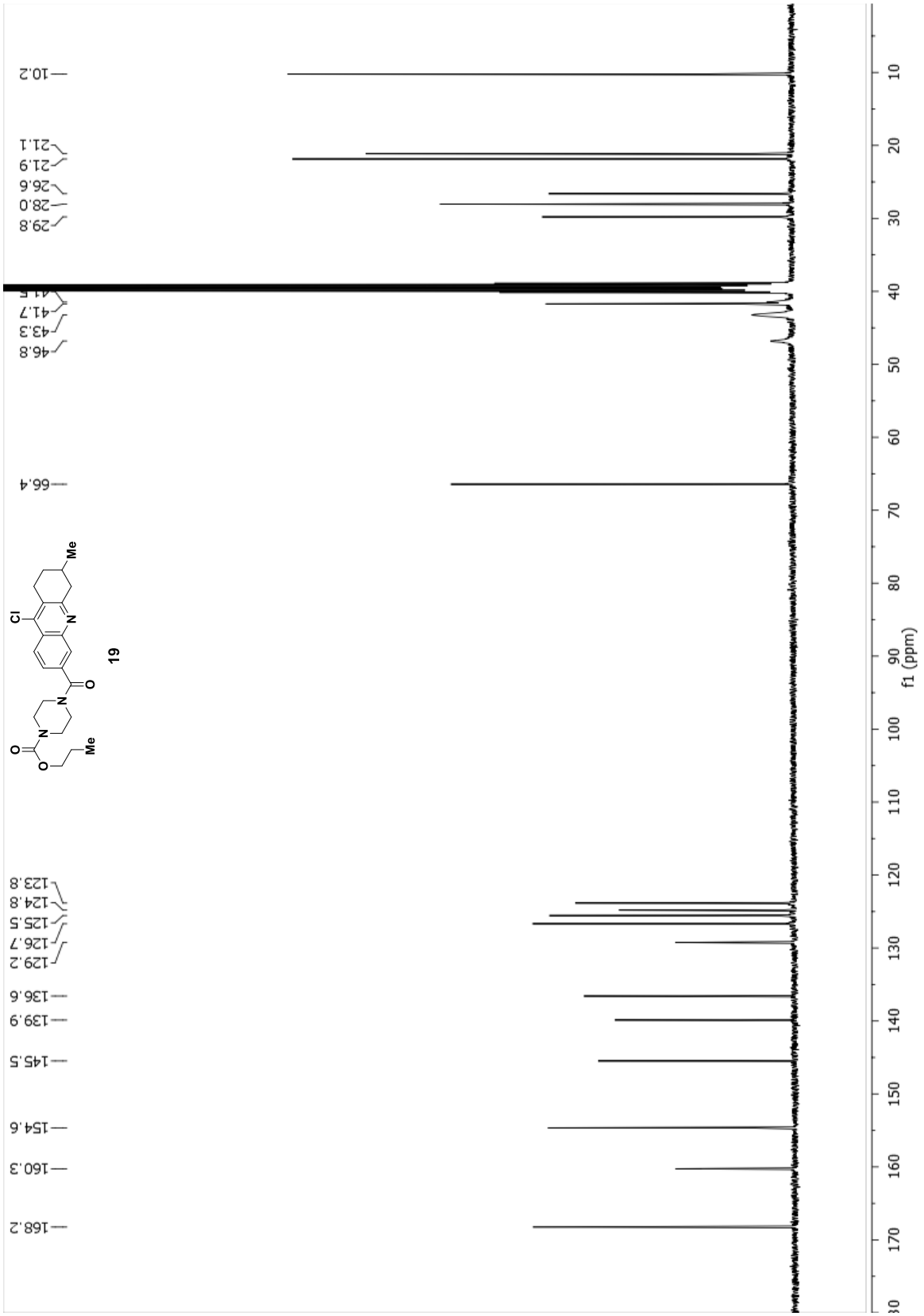


Figure S30. ^{1}H NMR (400 MHz, DMSO-d_6) of **19**.

Figure S31. ^{13}C NMR (101 MHz, DMSO-d_6) of **19**.



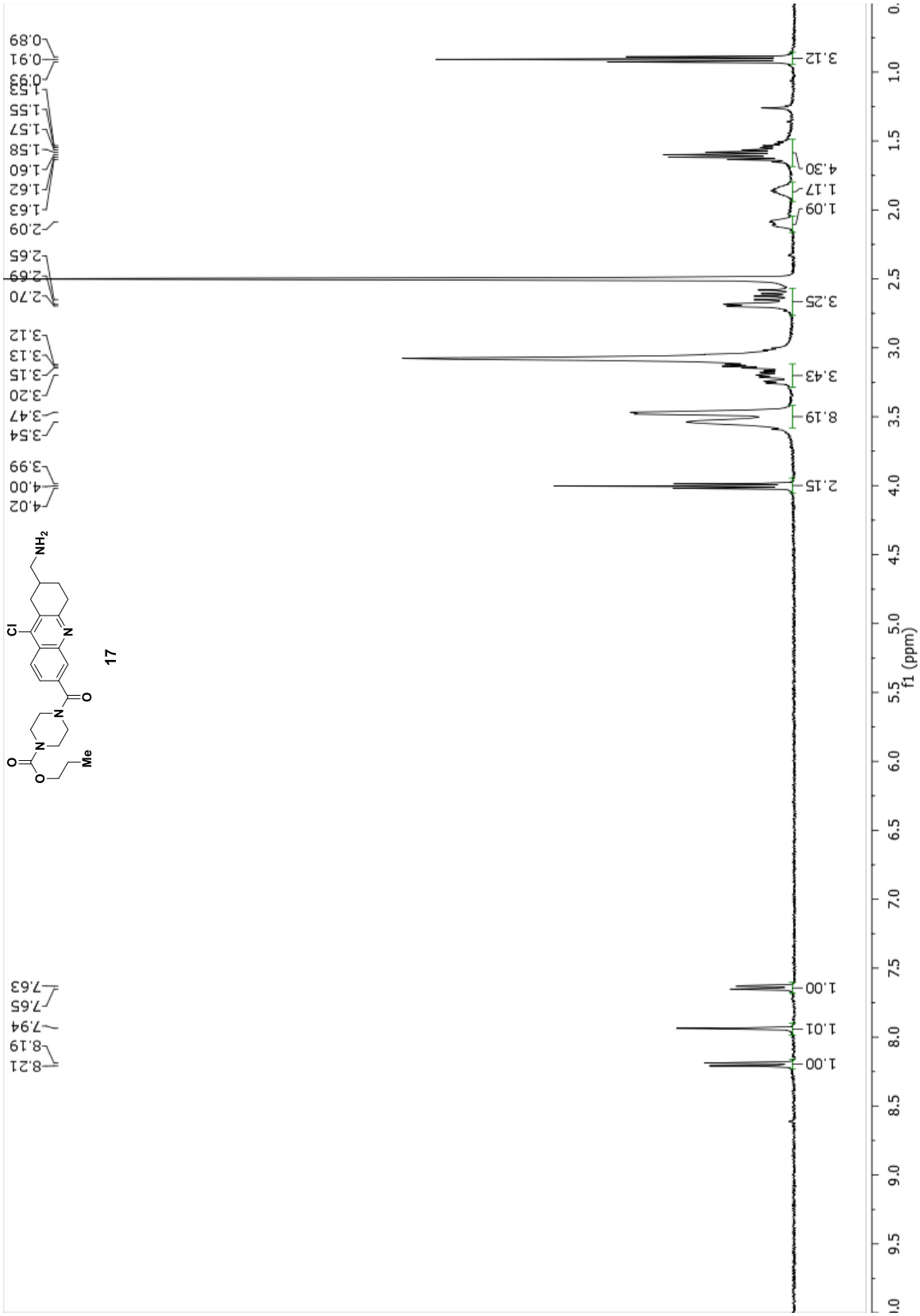


Figure S32. ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) of **17**.

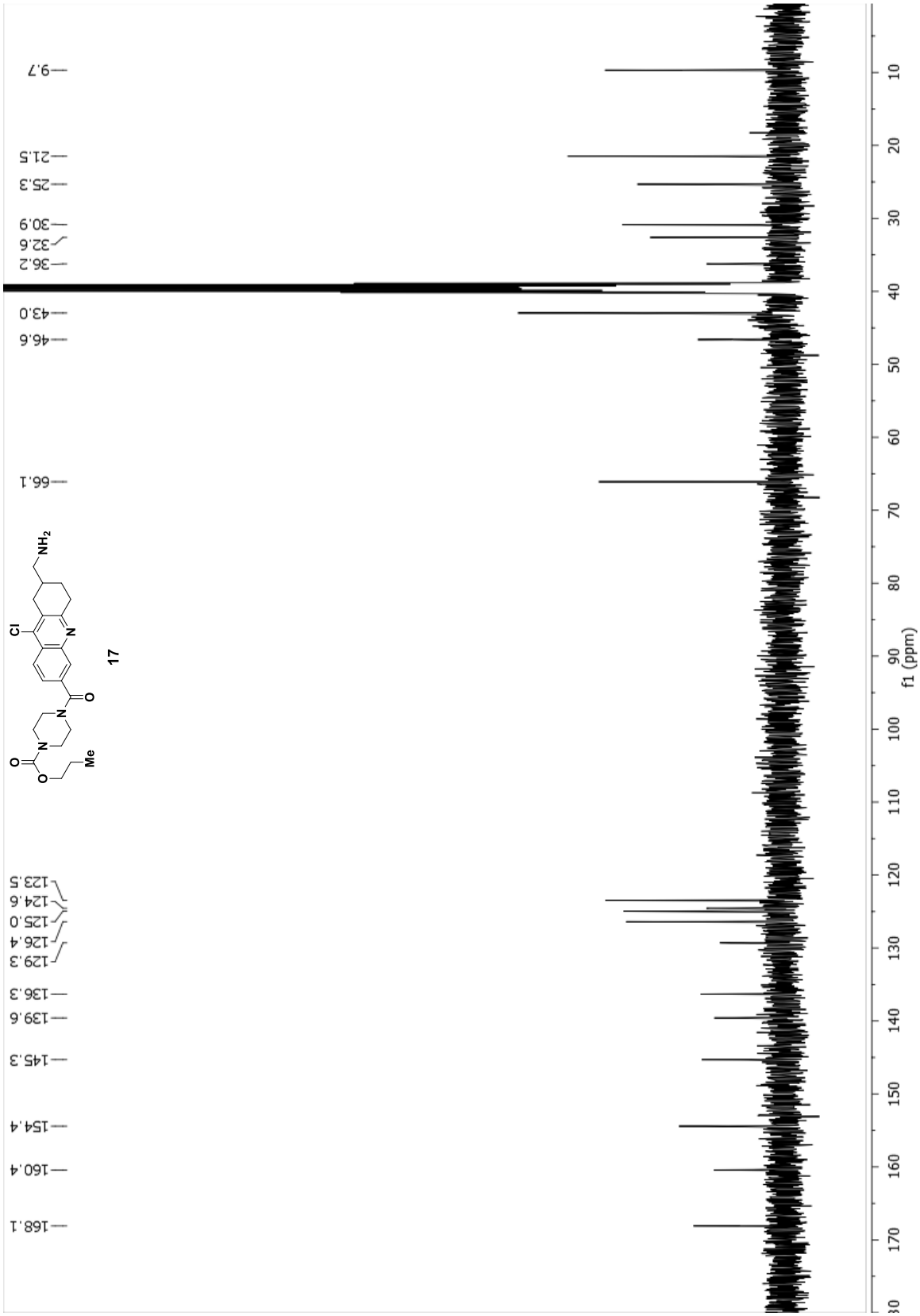


Figure S33. ^{13}C NMR (101 MHz, 80 °C, DMSO-d_6) of **17**.

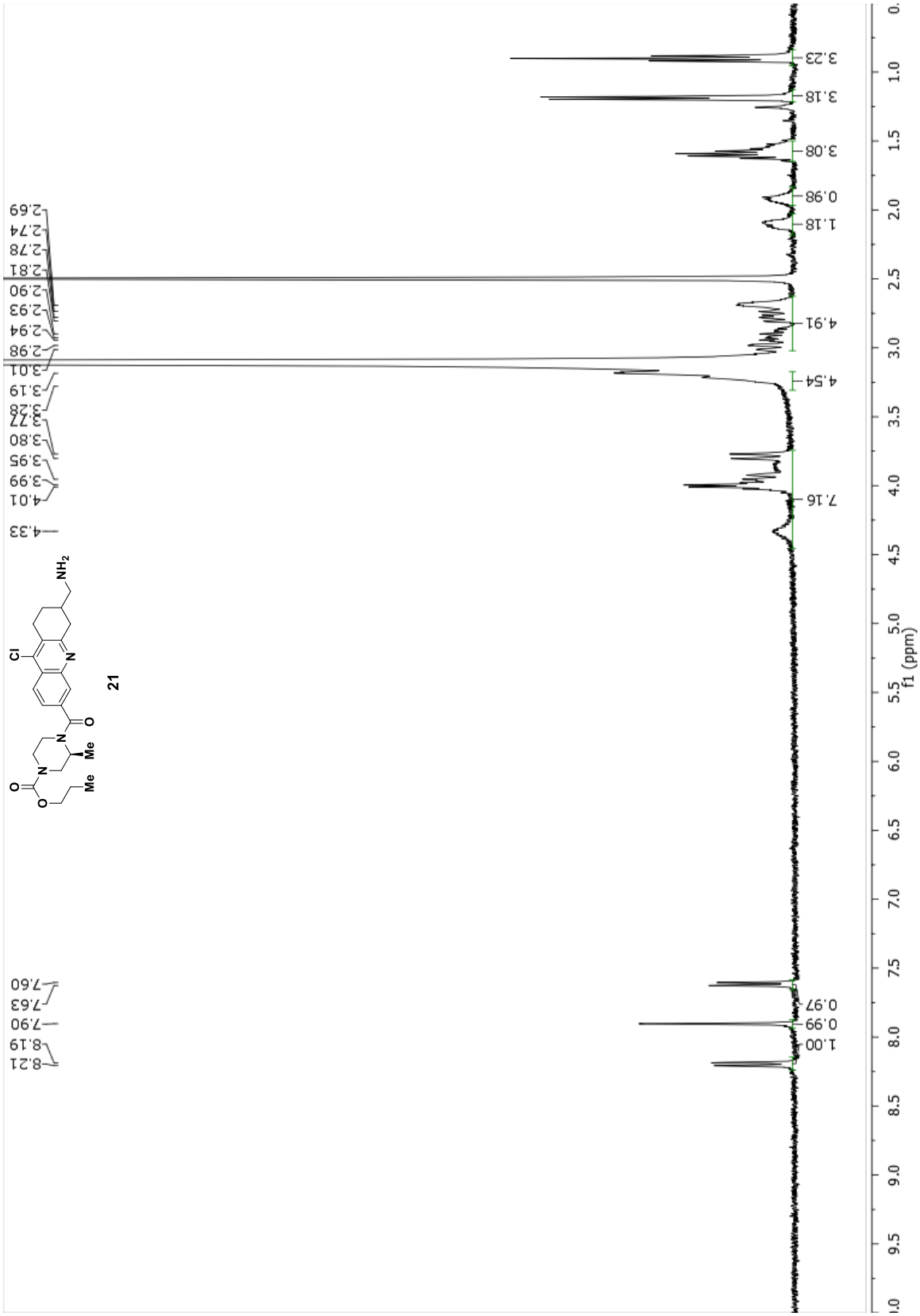


Figure S34. ^{1}H NMR (400 MHz, 80 °C, DMSO-d_6) of **21**.

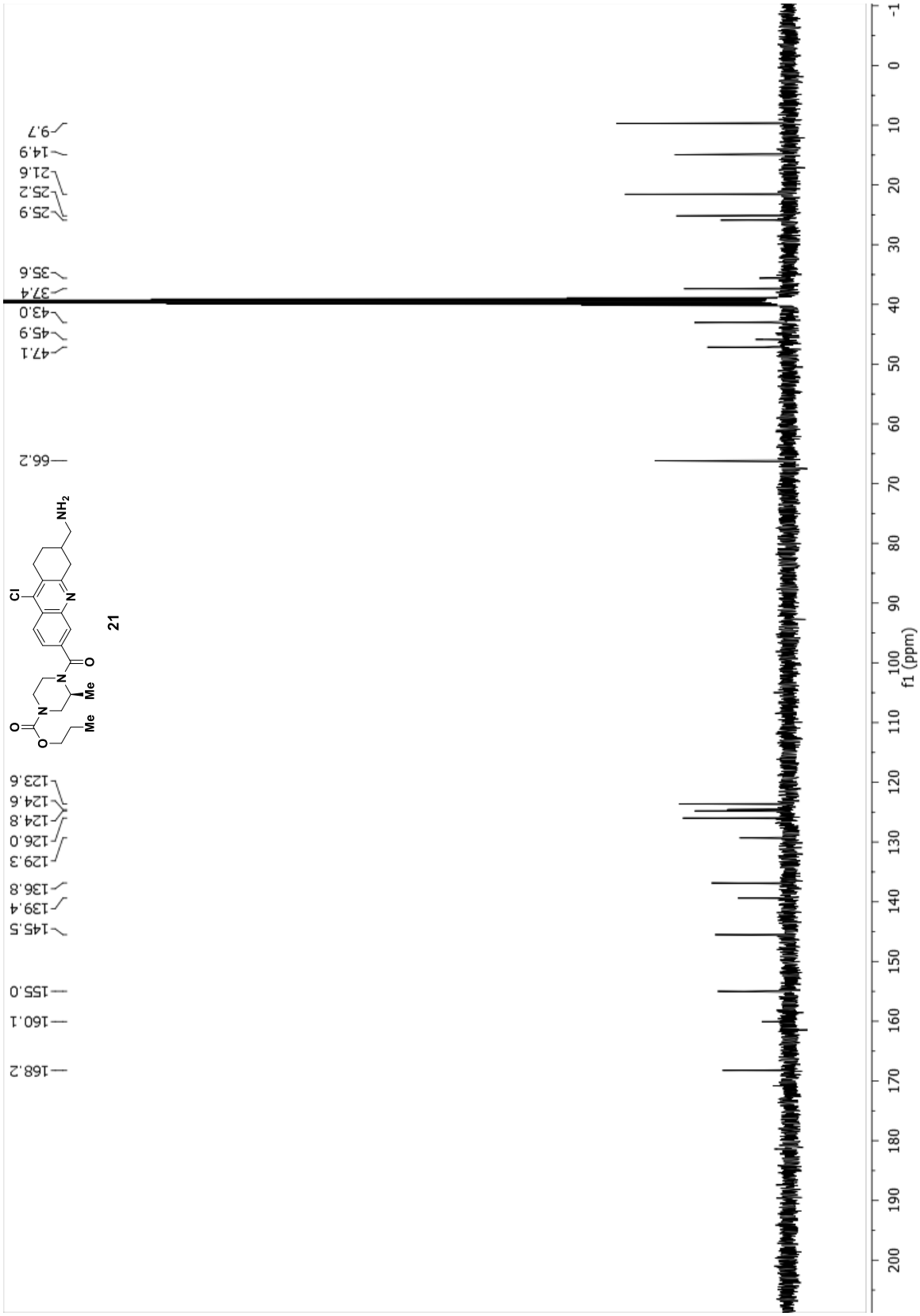


Figure S35. ^{13}C NMR (101 MHz, 80 °C, DMSO-d_6) of **21**.

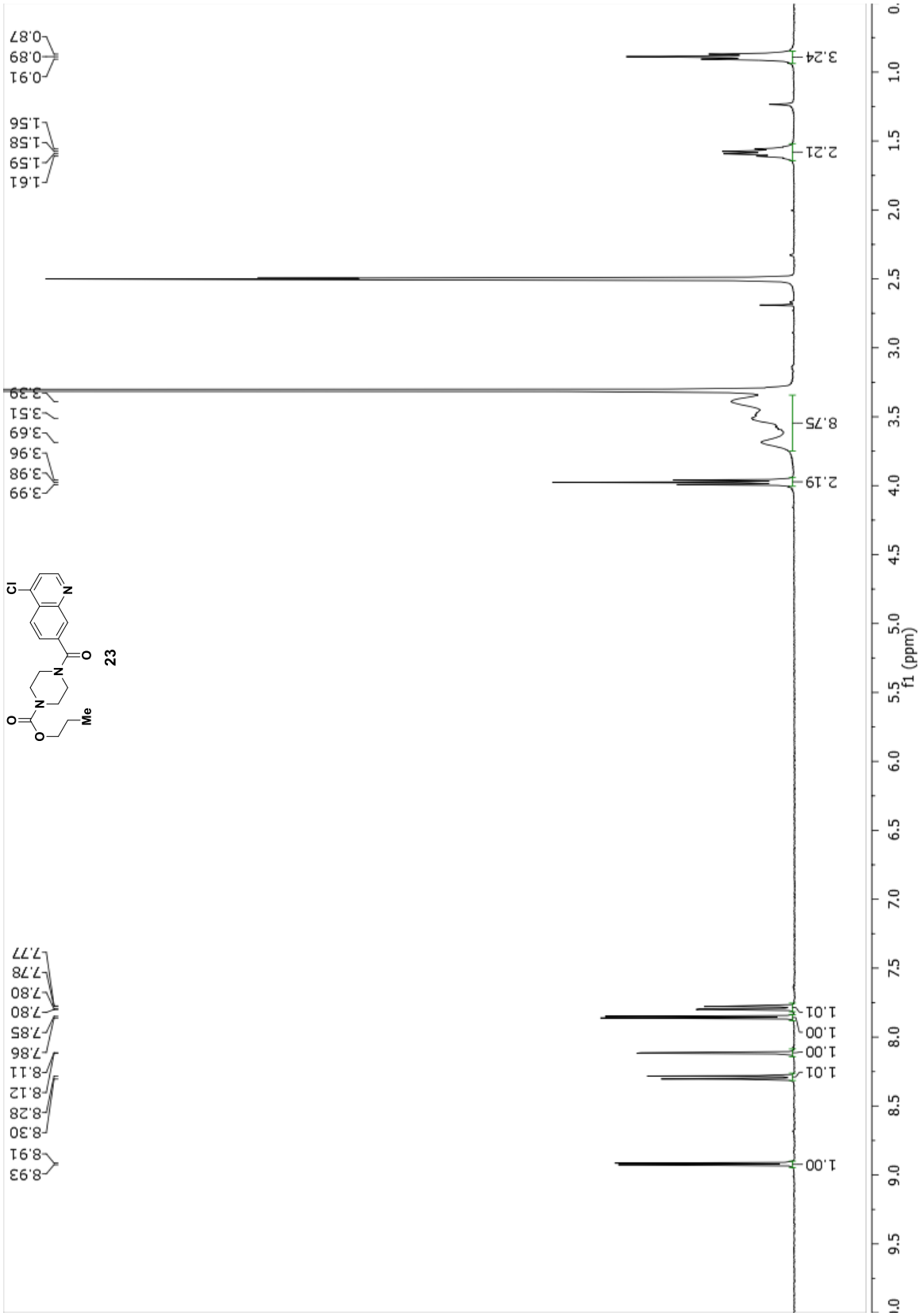
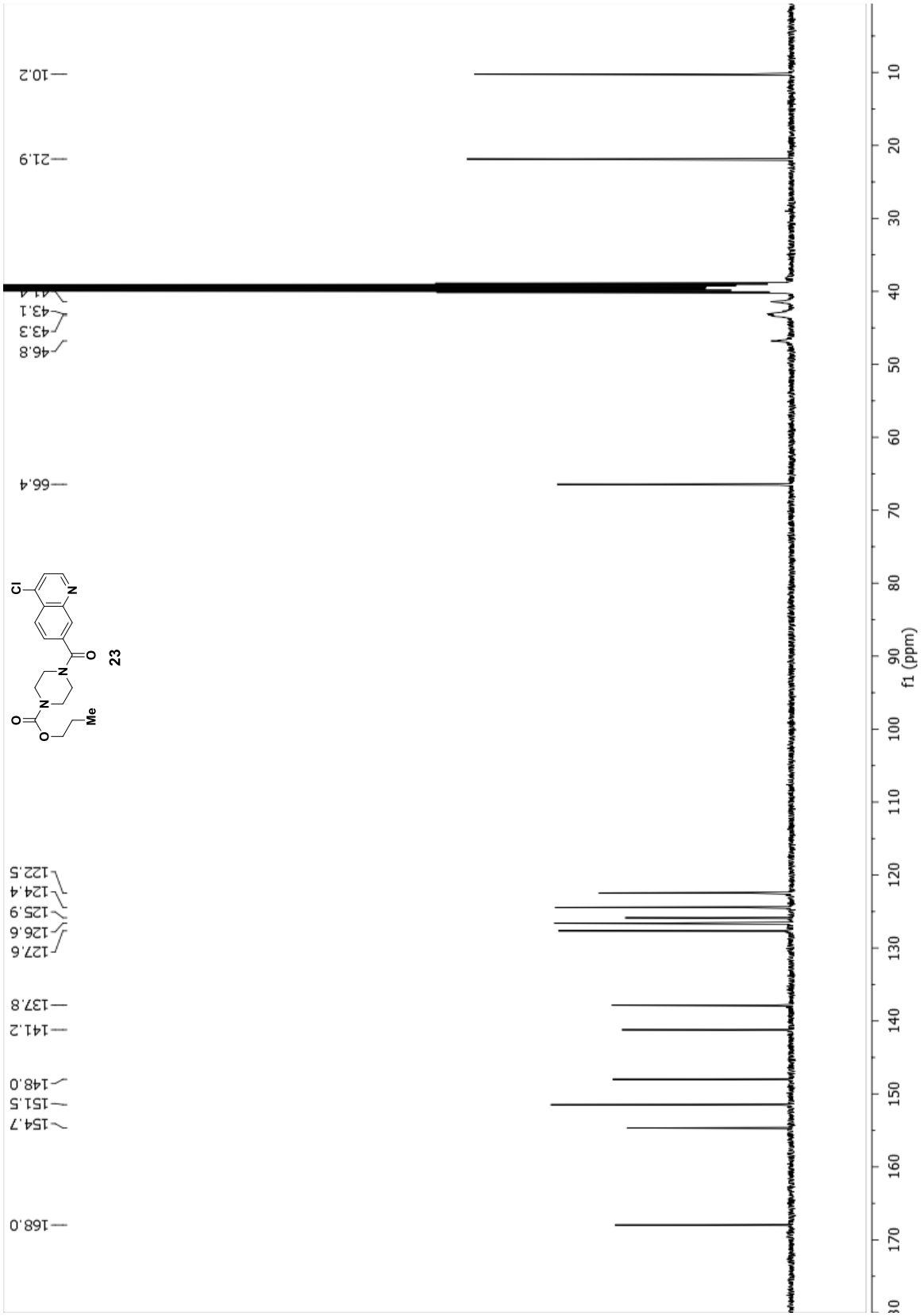


Figure S36. ^{1}H NMR (400 MHz, DMSO-d_6) of **23**.

Figure S37. ^{13}C NMR (101 MHz, DMSO-d_6) of **23**.



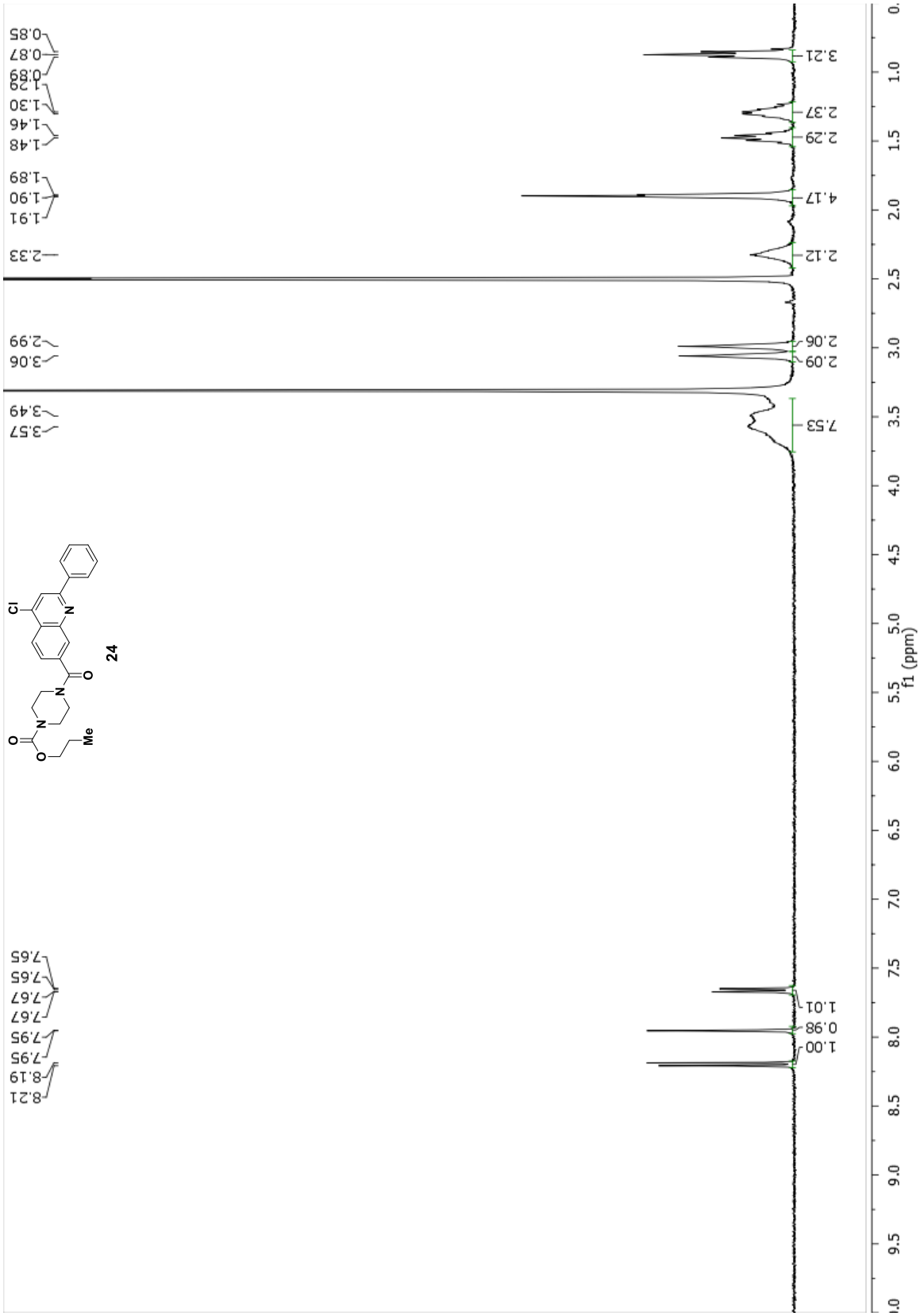


Figure S38. ^{1}H NMR (400 MHz, DMSO-d_6) of **24**.

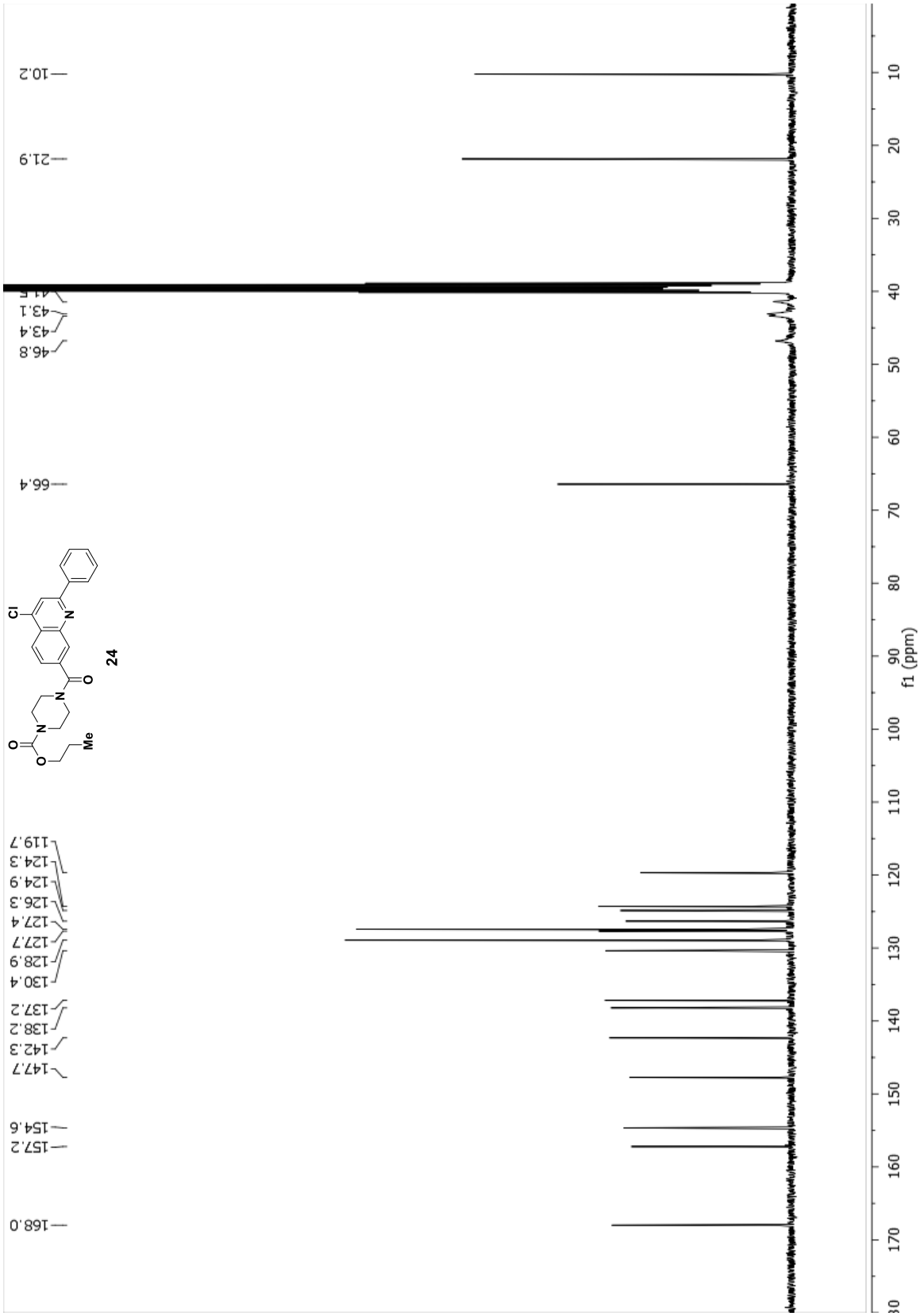


Figure S39. ^{13}C NMR (101 MHz, DMSO-d_6) of **24**.

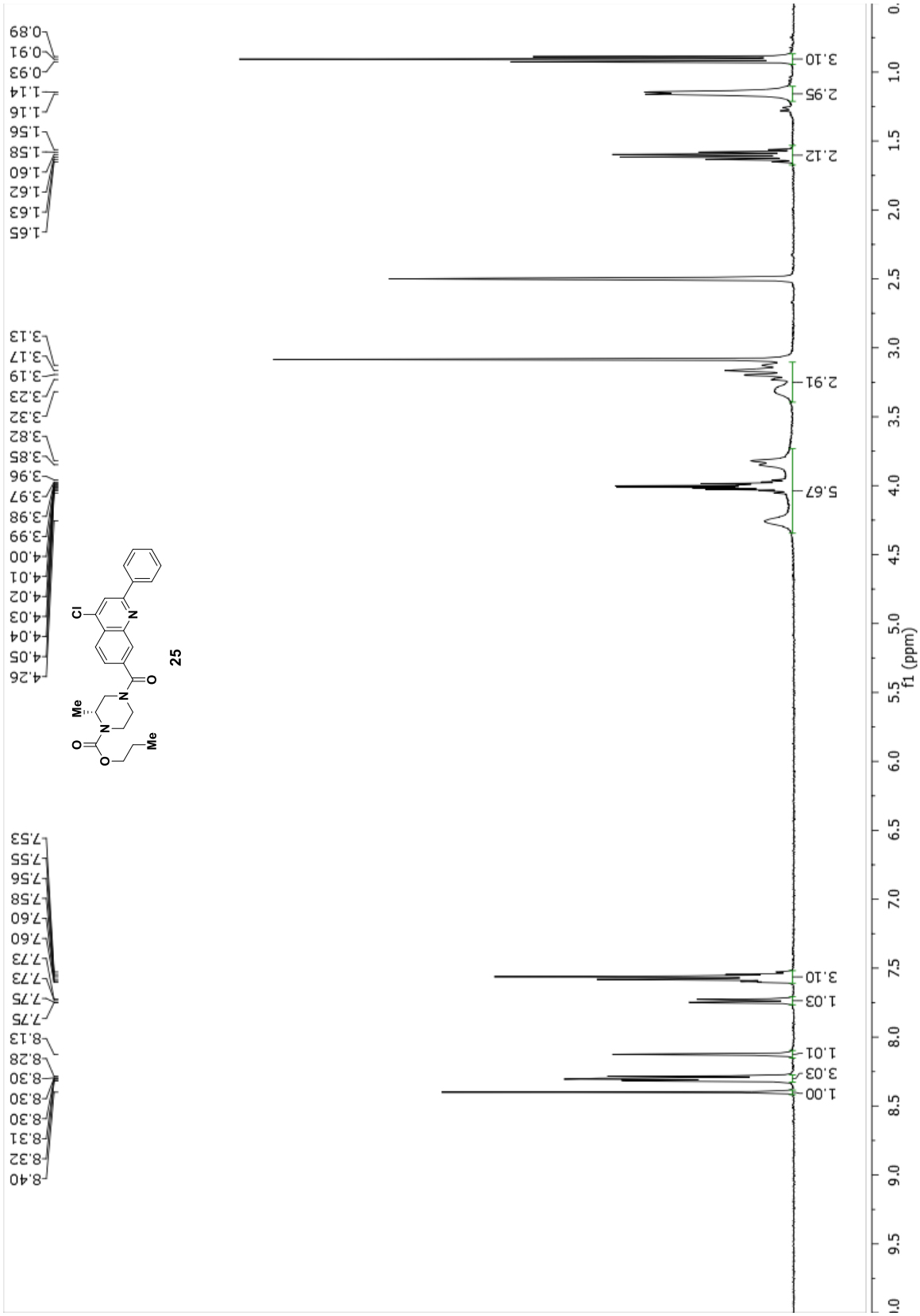
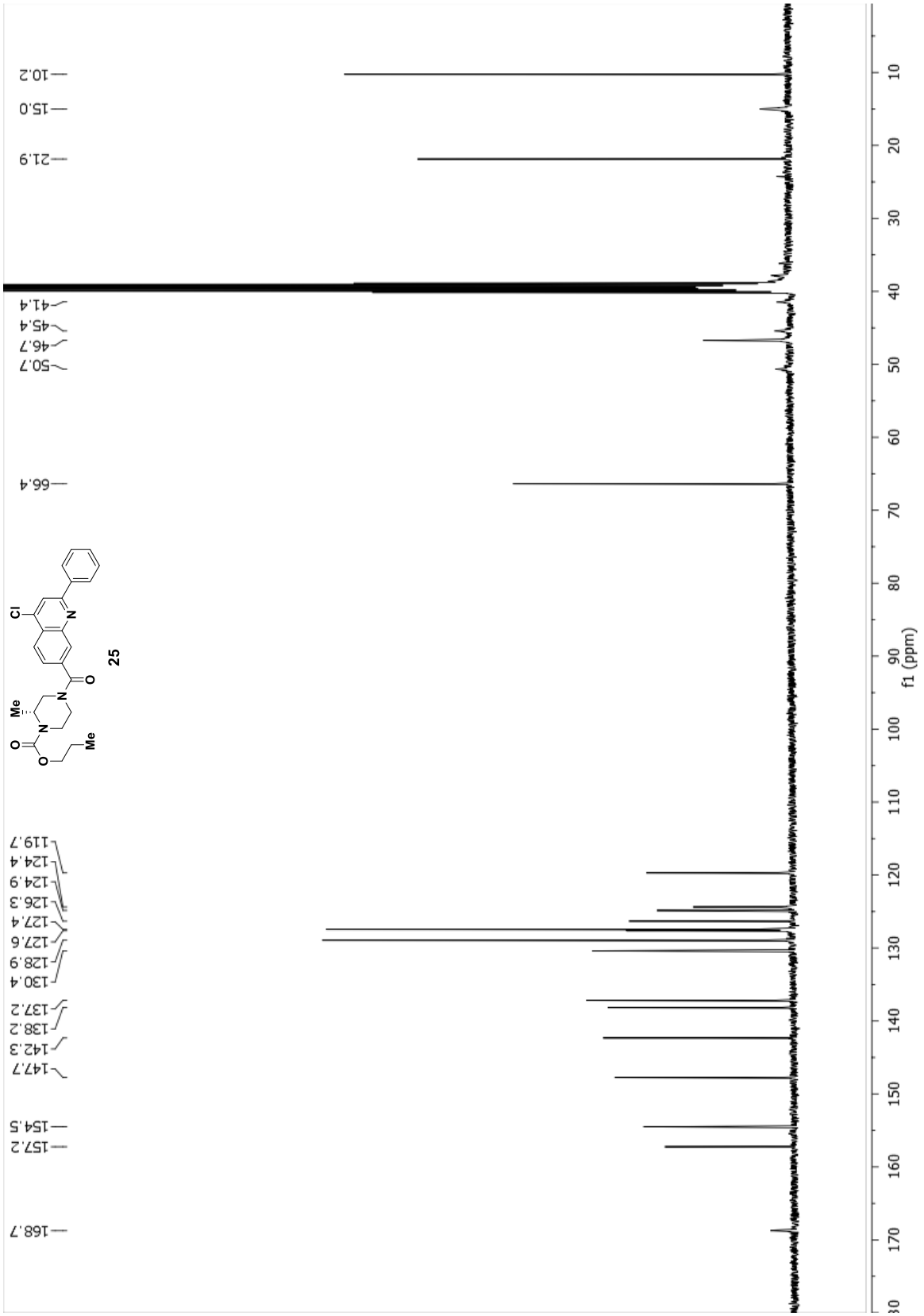


Figure S40. ^{1}H NMR (400 MHz, DMSO-d_6) of **25**.

Figure S41. ^{13}C NMR (101 MHz, DMSO-d_6) of **25**.



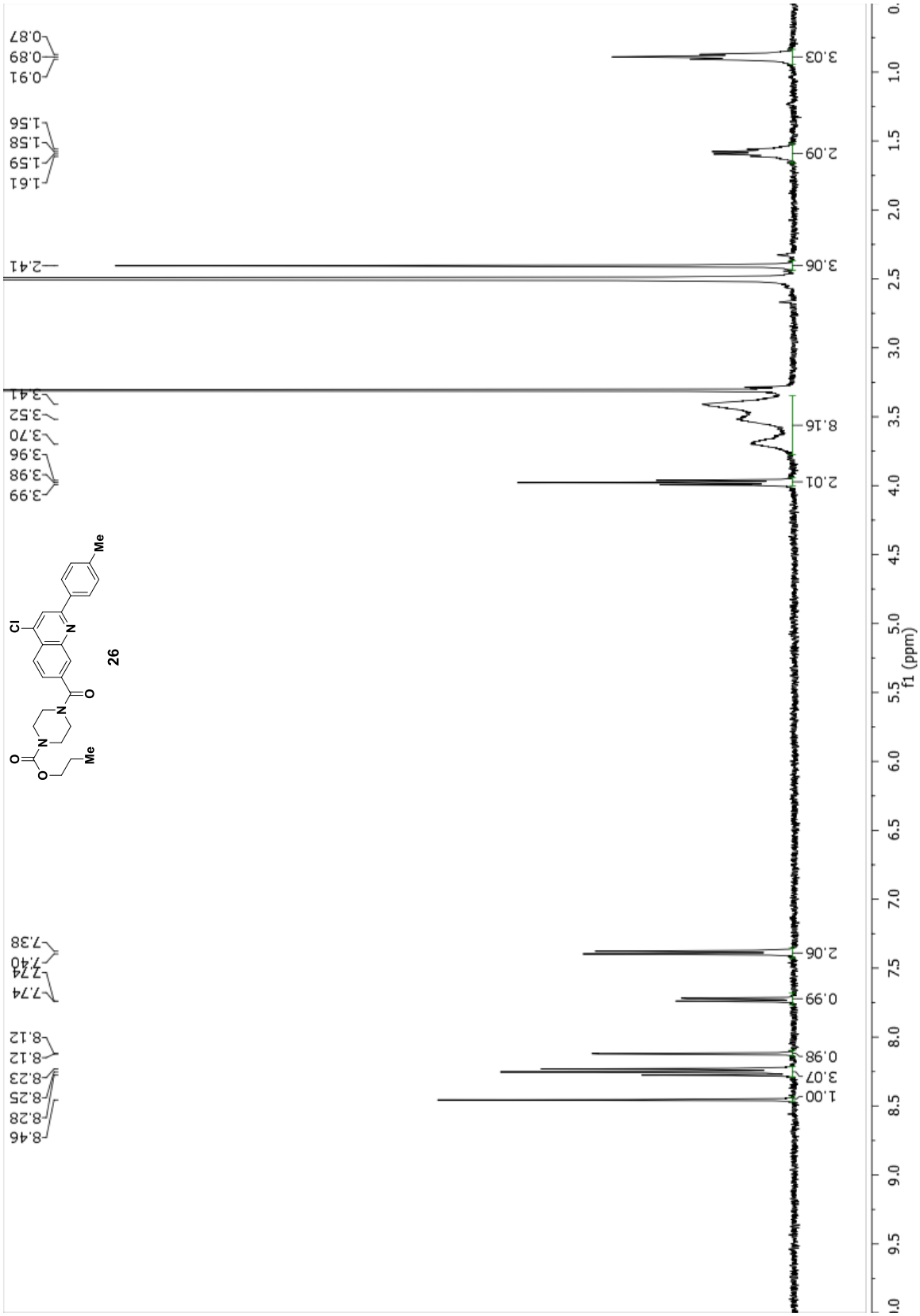
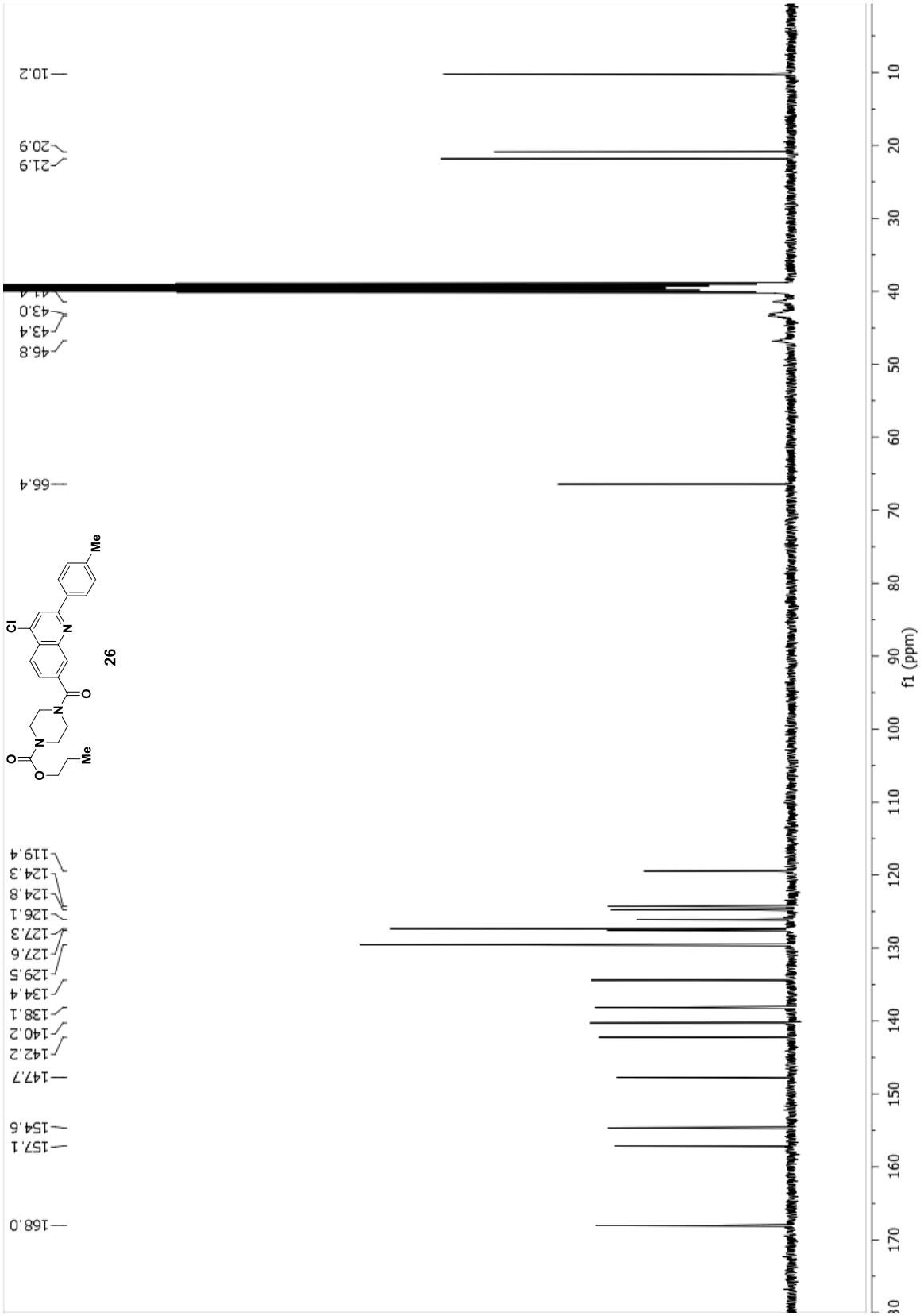


Figure S42. ^{1}H NMR (400 MHz, DMSO-d_6) of **26**.

Figure S43. ^{13}C NMR (101 MHz, DMSO-d_6) of **26**.



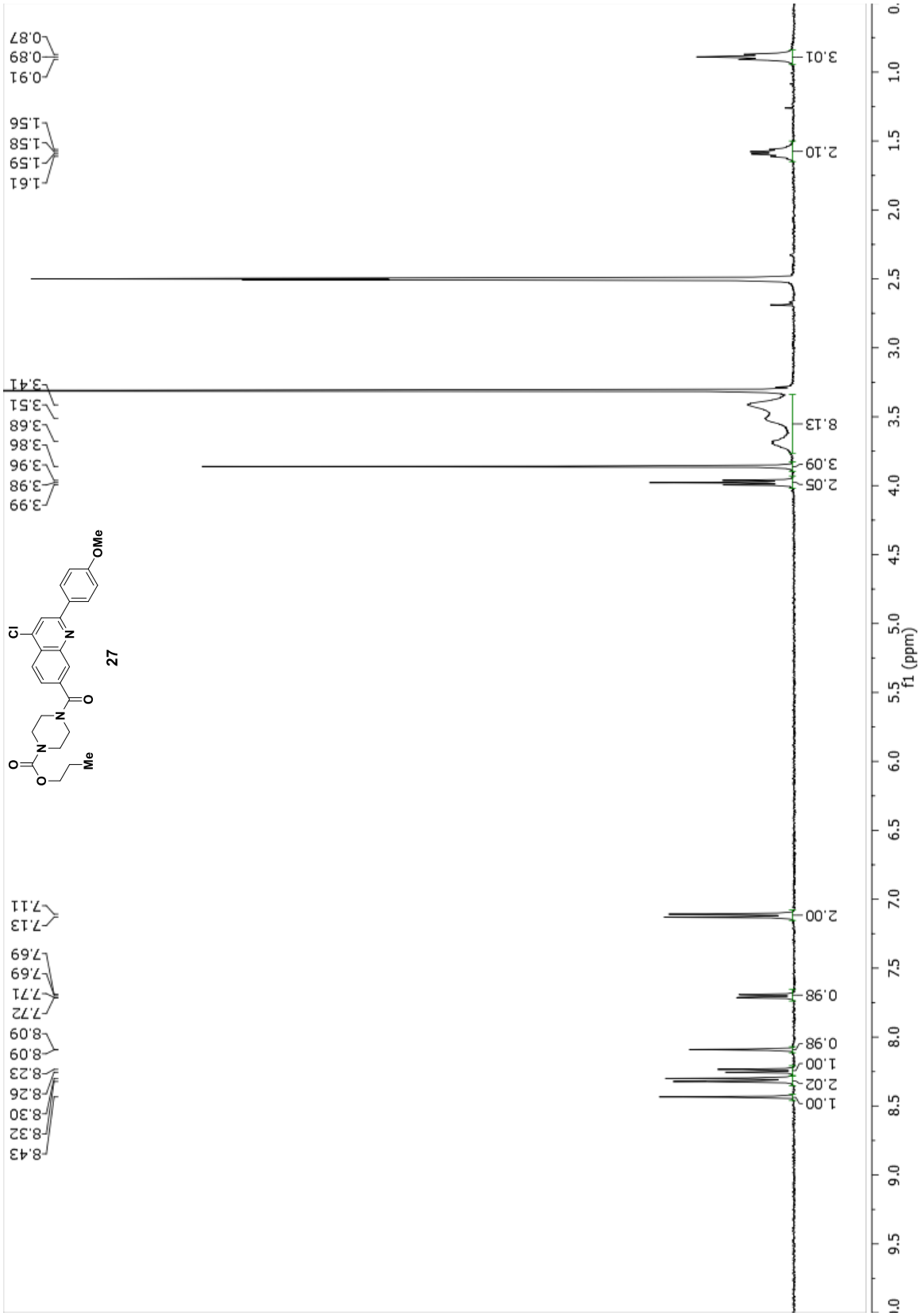
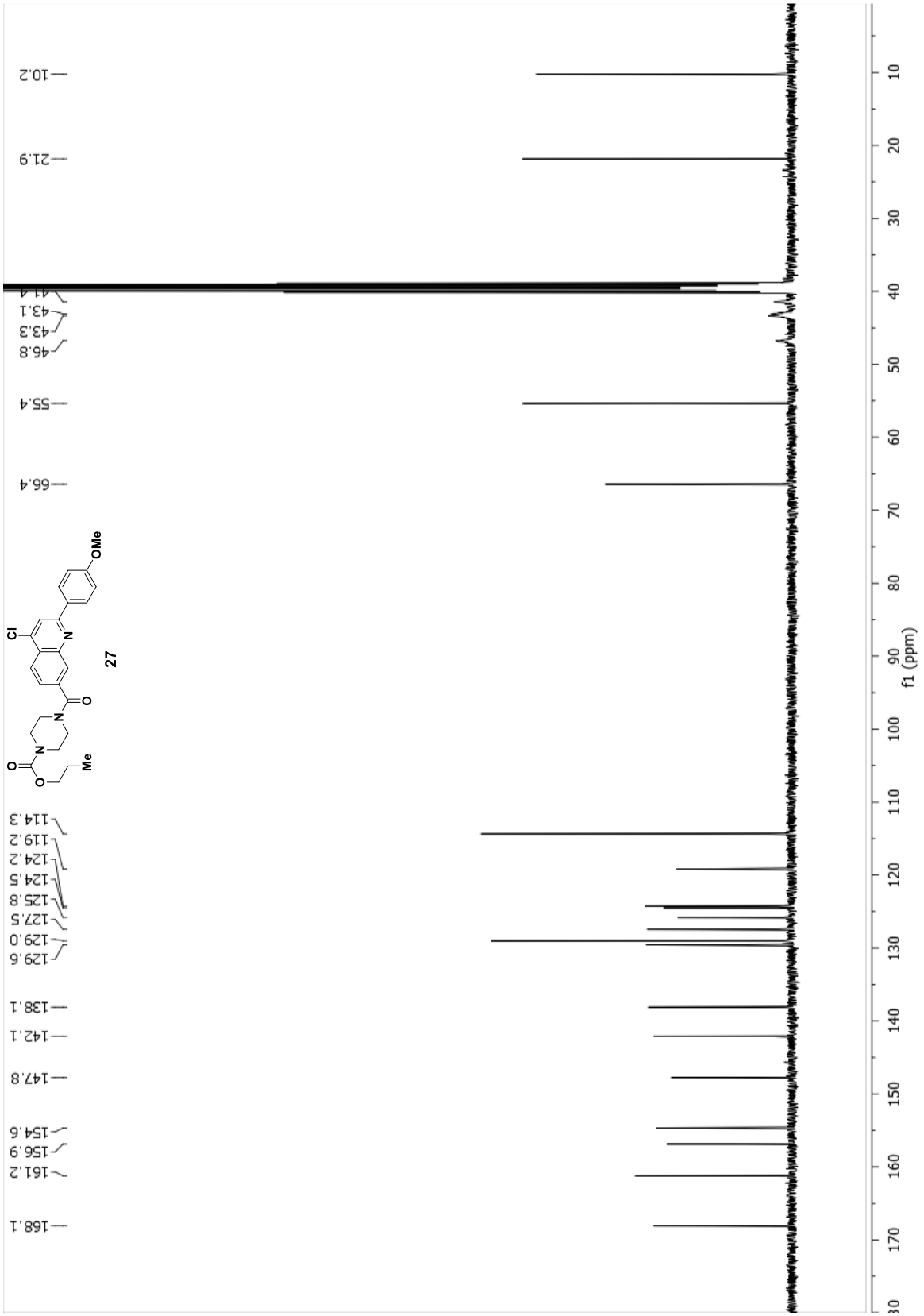


Figure S44. ^{1}H NMR (400 MHz, DMSO-d_6) of **27**.

Figure S45. ^{13}C NMR (101 MHz, DMSO-d_6) of **27**.



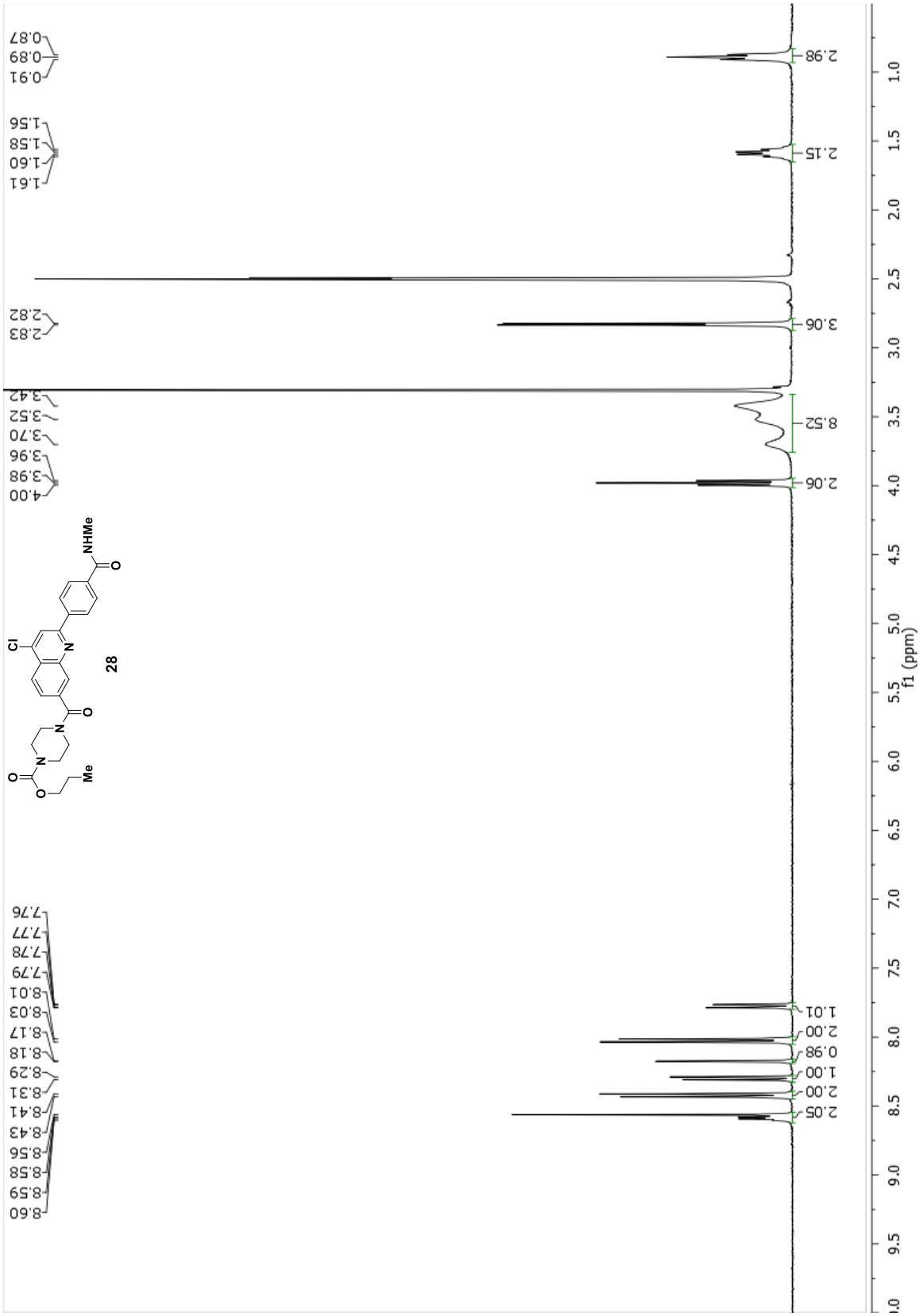


Figure S46. ^{1}H NMR (400 MHz, DMSO-d_6) of **28**.

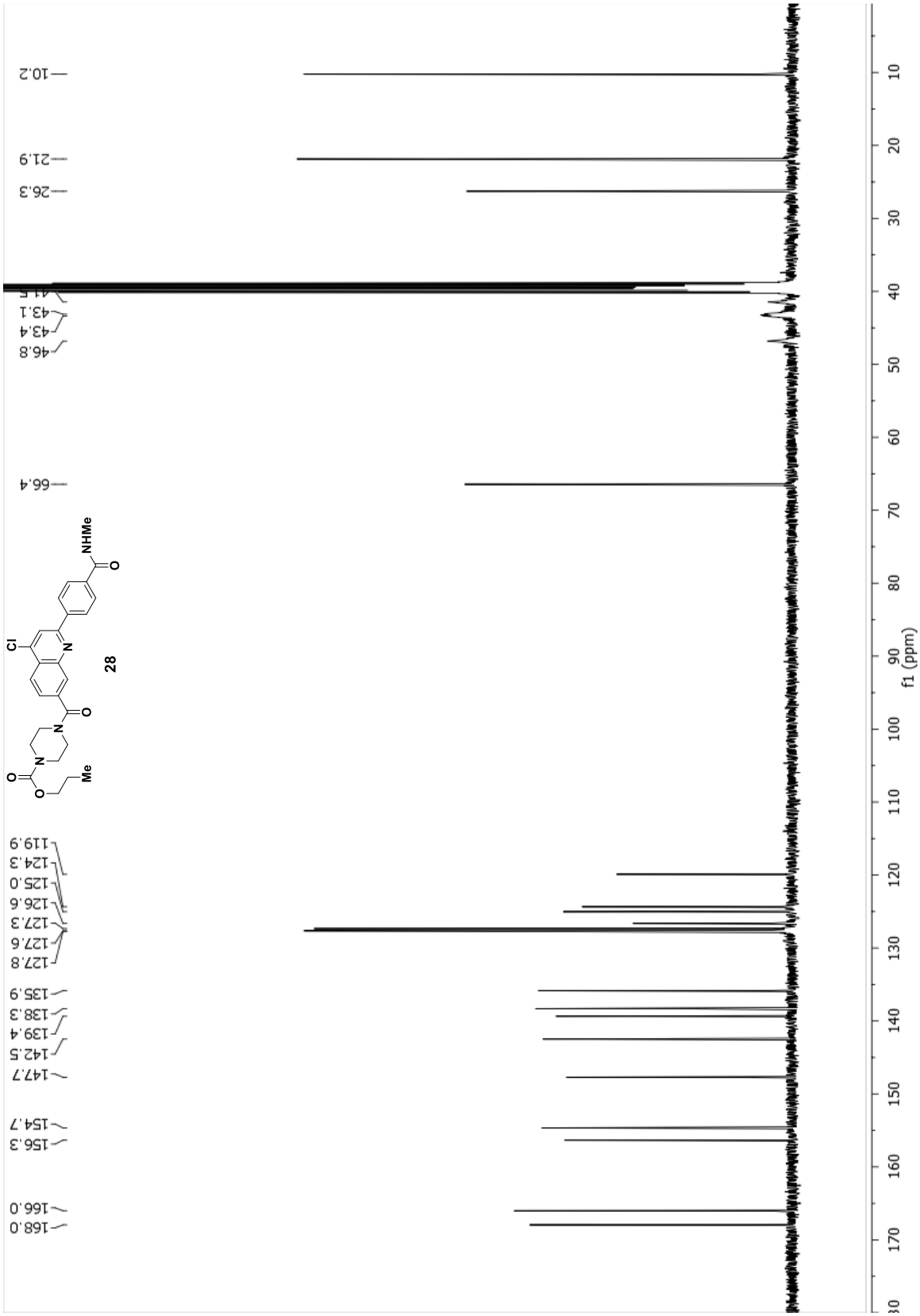
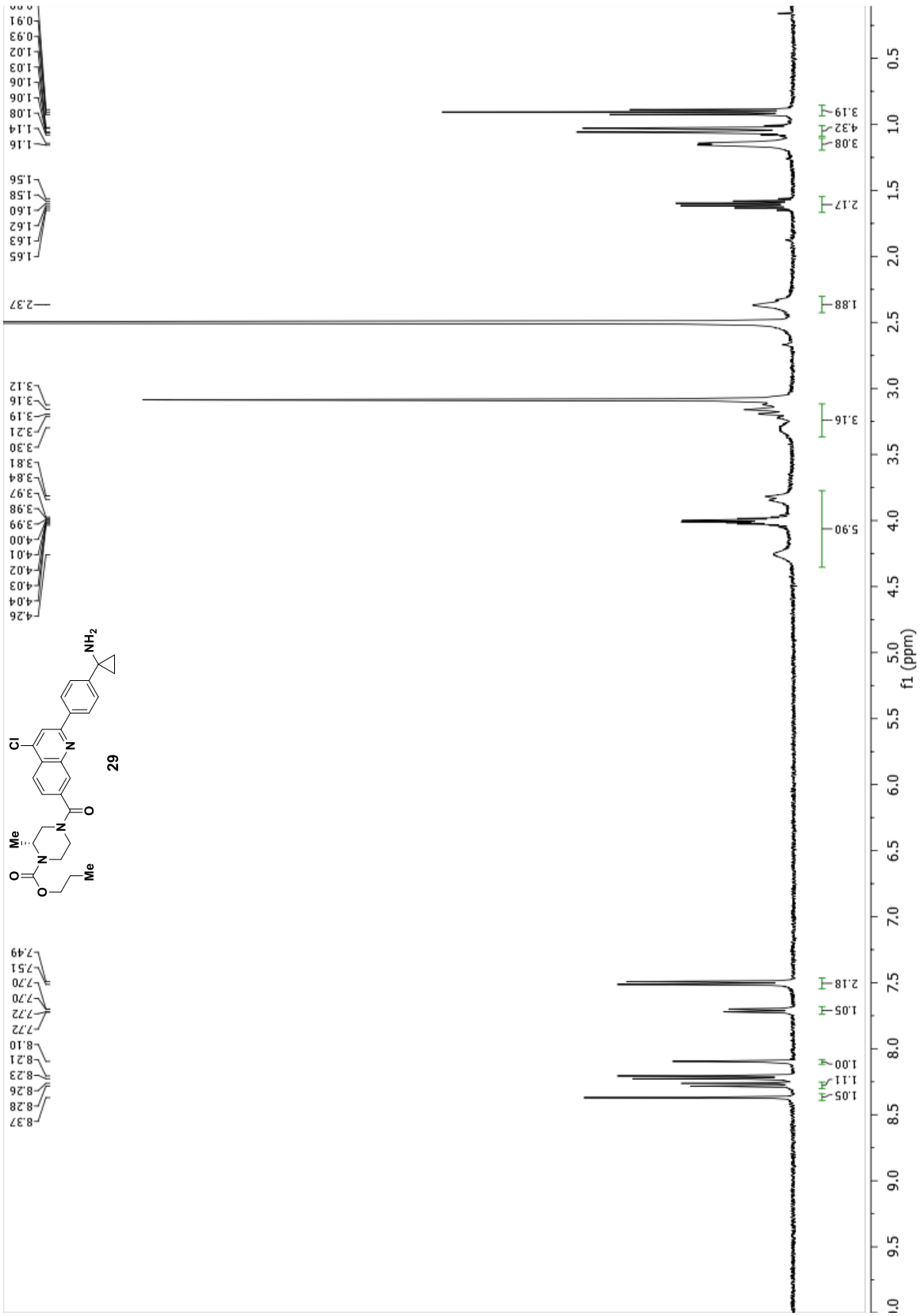


Figure S47. ^{13}C NMR (101 MHz, DMSO-d_6) of **28**.

Figure S48. ^{1}H NMR (400 MHz, 80 °C, DMSO-d_6) of **29**.



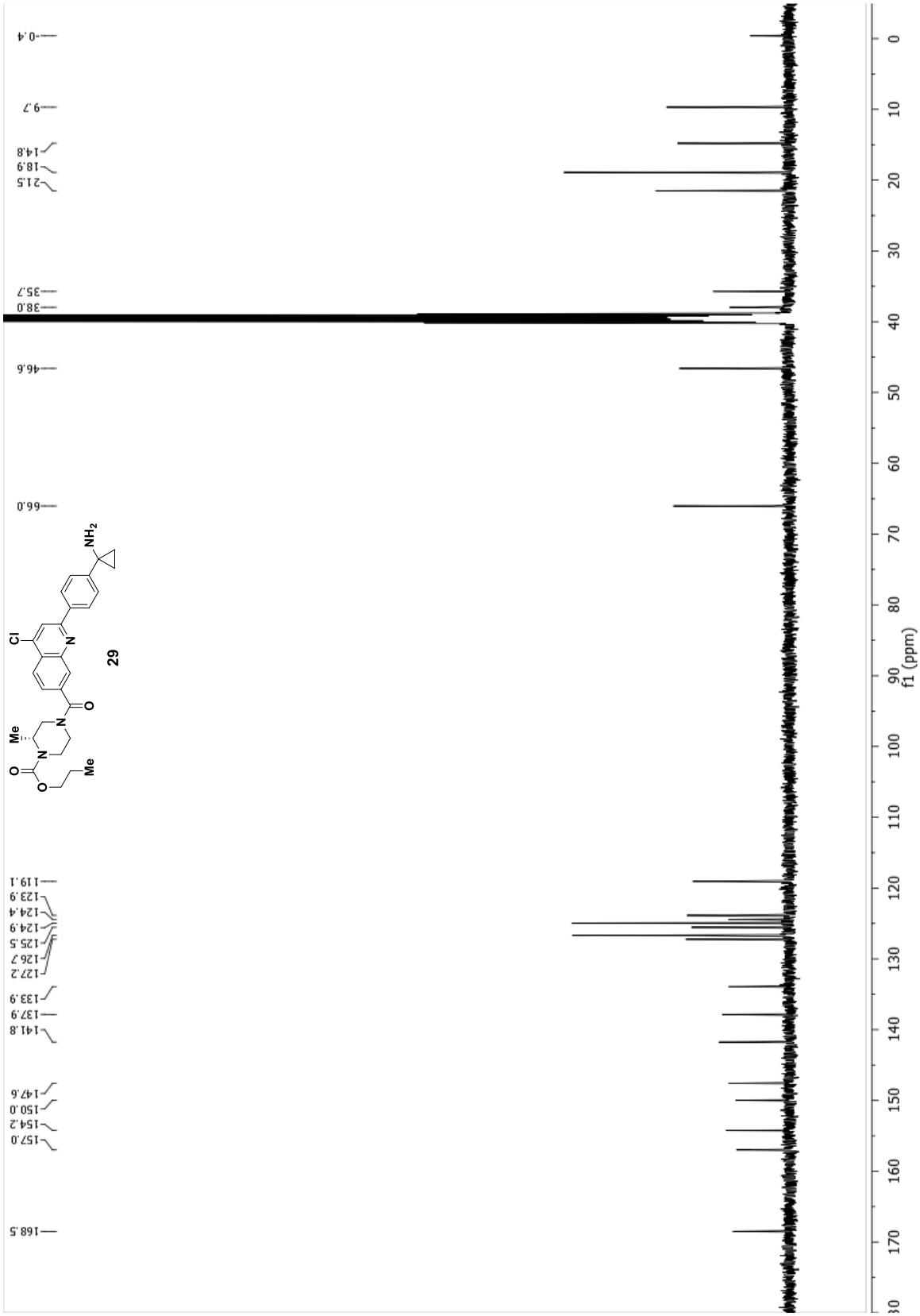


Figure S49. ^{13}C NMR (101 MHz, 80 °C, DMSO-d_6) of **29**.

Figure S50. ^{1}H NMR (400 MHz, 80 ºC, DMSO-d_6) of **30**.

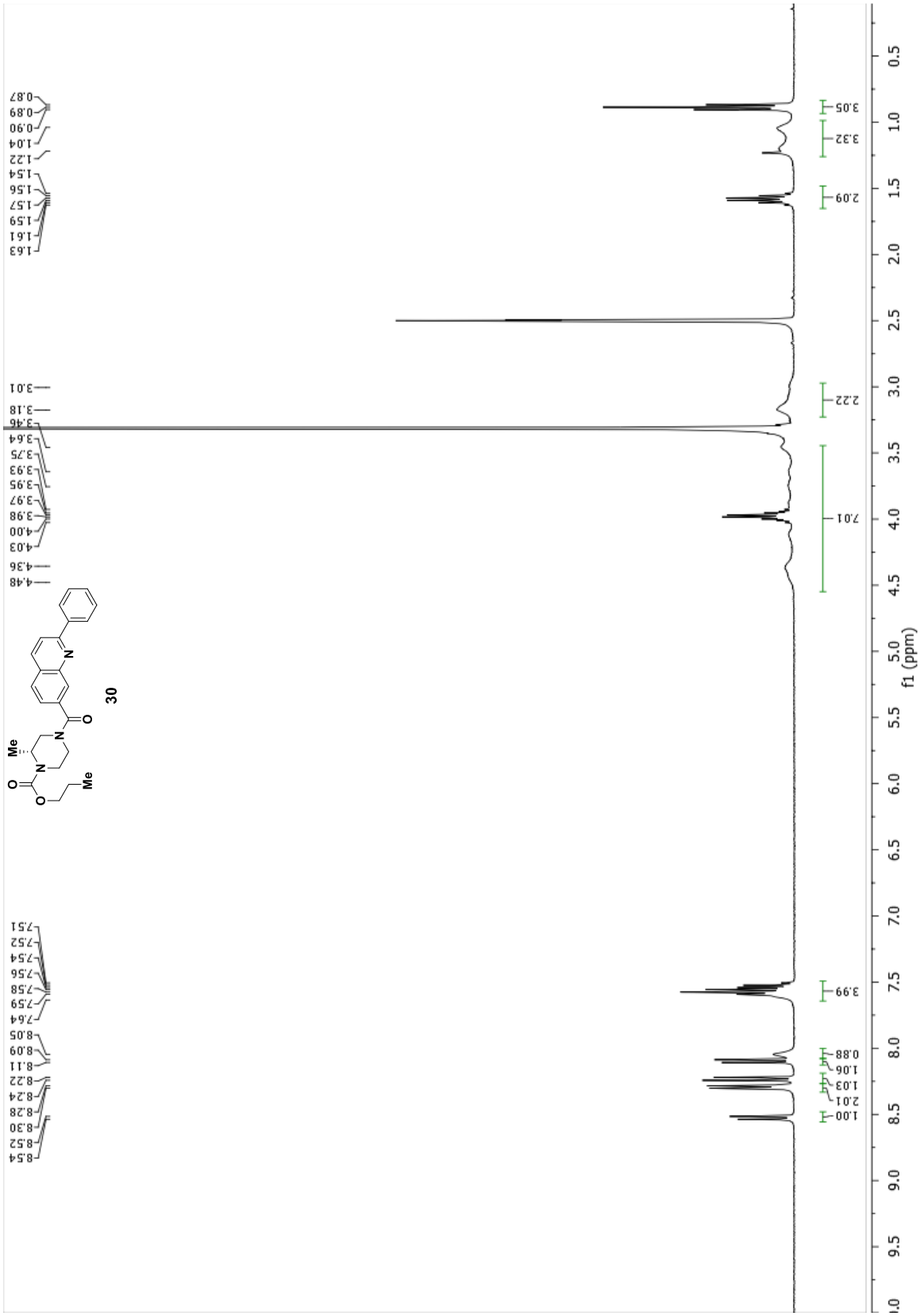


Figure S51. ^{13}C NMR (101 MHz, DMSO-d_6) of **30**.