

## Supporting Information

### Structure-based design of haloperidol analogues as inhibitors of acetyltransferase Eis from *Mycobacterium tuberculosis* to overcome kanamycin resistance

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#### Table of content

##### CHEMISTRY

Materials and instrumentations for chemistry S2-S3

Synthesis of intermediates A-C and compounds 1-35 S3-S17

##### BIOCHEMICAL, BIOLOGICAL, AND BIOPHYSICAL ASSAYS

Materials and instrumentations for biochemical and biological experiments S18

Bacterial strains S18-S19

Purification of EisC204A and crystallization of EisC204A-CoA in complex with compounds 1, 10, 30, 35, and DPD S19-S20

Diffraction data collection and structure determination and refinement of EisC204A-CoA in complex with compounds 1, 10, 30, 35, and DPD S20-S21

Table S1 S21

Inhibition kinetics S21-S22

Mode of inhibition S22

Table S2 S22

Selectivity of inhibitors towards Eis over other AACs S22-S23

Determination of MIC values of KAN against *Mycobacterium tuberculosis*

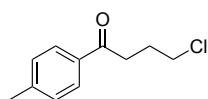
H37Rv and K204	S23
Generation of the <i>Mycobacterium tuberculosis</i> mc <sup>2</sup> 6230-K204 strain	S23-S24
Determination of MIC values of KAN against <i>Mtb</i> mc <sup>2</sup> 6230 and mc <sup>2</sup> 6230-K204	S24-S25
Determination of MIC values of compounds <b>1-35</b> against various bacterial strains	S25
Table S3	S26
Combination studies of haloperidol derivatives and anti-TB drugs by checkerboard assays	S26-S27
Table S4	S27
Table S5	S27
Table S6	S28
Table S7	S28
Table S8	S28
Table S9	S28
Table S10	S29
Measurements of cytotoxicity to mammalian cells	S29
Microsomal stability assays	S29
References	S30-S31
Figures S1-S89	S32-S76

## CHEMISTRY:

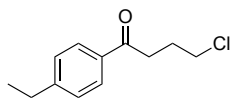
**Materials and instrumentations for chemistry.** All reagents were purchased from commercial sources and used without any further purification. TLC analyses were performed on silica gel plates (pre-coated on glass; 0.25 mm thickness with fluorescent indicator UV<sub>254</sub>) and were visualized by UV or charring in a KMnO<sub>4</sub> stain. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a 400 MHz NMR spectrometer (VARIAN INOVA) using CDCl<sub>3</sub>, CD<sub>3</sub>OD, or (CD<sub>3</sub>)<sub>2</sub>SO. Chemical shifts are reported in parts per million (ppm) and are referenced to residual solvent peaks. All reactions were carried out under nitrogen atmosphere and all yields reported represent isolated yields. Compounds **16-20** were synthesized as previously reported.<sup>1</sup> Known compounds were characterized by <sup>1</sup>H NMR and are in complete agreement with samples reported in the literature. All new compounds were characterized by <sup>1</sup>H as well as <sup>13</sup>C NMR and mass spectrometry. All compounds are ≥95% pure according to NMR spectra. Further confirmation of purity for the final

molecules was obtained by RP-HPLC, which was performed on an Agilent Technologies 1260 Infinity HPLC system by using the following general method 1 (for compounds **1-5**, **9-15**, and **21-33**): Flow rate = 1 mL/min;  $\lambda$  = 254 nm; column = Vydac 201SP<sup>TM</sup> C18, 250  $\times$  4.6 mm, 90A 5  $\mu$ m; Eluents: A = H<sub>2</sub>O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% B to 100% B over 20 min, holding at 100% B from 20-27 min, decreasing from 100% B to 5% B from 27-30 min. Prior to each injection, the HPLC column was equilibrated for 15 min with 5% B. For compound **35** general method 2 was used: Flow rate = 0.5 mL/min;  $\lambda$  = 254 nm; column = Vydac HPLC Denali C18, 250  $\times$  4.6 mm, 5  $\mu$ m; Eluents: A = H<sub>2</sub>O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% C to 100% B over 8 min, holding at 100% B from 8-22 min, decreasing from 100% B to 5% B from 22-27 min. Prior to each injection, the HPLC column was equilibrated for 10 min with 5% B. For compounds **6**, **7**, and **8** general method 3 was used: Flow rate = 0.5 mL/min;  $\lambda$  = 254 nm; column = Vydac HPLC Denali C18, 250  $\times$  4.6 mm, 5  $\mu$ m; Eluents: A = H<sub>2</sub>O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% B to 100% B over 8 min, holding at 100% B from 8-16 min, decreasing from 100% B to 5% B from 16-21 min. Prior to each injection, the HPLC column was equilibrated for 9 min with 5% B.

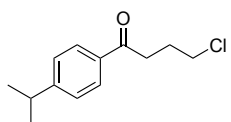
### Synthesis of intermediates A-C and compounds 1-35.



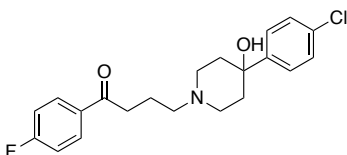
**Preparation of compound A (SGT1433).** To a solution of 4-chlorobutyl chloride (2.75 g, 19.5 mmol) in 1,2-dichloroethane (12 mL) at 0 °C, AlCl<sub>3</sub> (4.36 g, 28.3 mmol) was added. The reaction mixture was stirred at 0 °C for 15 min followed by the addition of toluene (1.00 g, 10.9 mmol). The reaction mixture was then stirred at room temperature for 12 h and progress of the reaction was monitored by TLC (1:19/EtOAc:Hexanes, R<sub>f</sub> 0.76). The reaction mixture was quenched with H<sub>2</sub>O (150 mL) and extracted with EtOAc (200 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, 1:19/EtOAc:Hexanes) to afford compound **A** (1.46 g, 68%) as a yellow liquid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S1)  $\delta$  7.86 (d, *J* = 8.4 Hz, 2H), 7.28-7.24 (m, 2H), 3.66 (t, *J* = 6.3 Hz, 2H), 3.14 (t, *J* = 7.0 Hz, 2H), 2.40 (s, 3H), 2.21 (p, *J* = 6.3 Hz, 2H).



**Preparation of compound B (SGT1432).** To a solution of 4-chlorobutyryl chloride (2.39 g, 16.9 mmol) in 1,2-dichloroethane (12 mL) at 0 °C, AlCl<sub>3</sub> (3.77 g, 28.3 mmol) was added. The reaction mixture was stirred at 0 °C for 15 min followed by the addition of ethylbenzene (1.00 g, 9.42 mmol). The reaction mixture was then stirred at room temperature for 12 h and progress of the reaction was monitored by TLC (1:19/EtOAc:Hexanes, R<sub>f</sub> 0.79). The reaction mixture was quenched with H<sub>2</sub>O (150 mL) and extracted with EtOAc (200 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, 1:19/EtOAc:Hexanes) to afford compound **B** (1.53 g, 77%) as a yellow liquid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S2) δ 7.89 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 3.66 (t, *J* = 8.2 Hz, 2H), 3.14 (t, *J* = 7.0 Hz, 2H), 2.70 (q, *J* = 7.6 Hz, 2H), 2.21 (p, *J* = 6.5 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H).

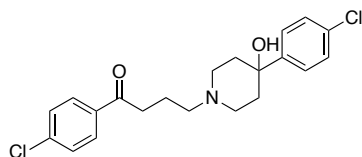


**Preparation of compound C (SGT1431).** To a solution of 4-chlorobutyryl chloride (2.11 g, 15.0 mmol) in 1,2-dichloroethane (12 mL) at 0 °C, AlCl<sub>3</sub> (3.33 g, 24.9 mmol) was added. The reaction mixture was stirred at 0 °C for 15 min followed by the addition of cumene (1.00 g, 8.32 mmol). The reaction mixture was then stirred at room temperature for 12 h and progress of the reaction was monitored by TLC (1:19/EtOAc:Hexanes, R<sub>f</sub> 0.82). The reaction mixture was quenched with H<sub>2</sub>O (150 mL) and extracted with EtOAc (200 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, 1:19/EtOAc:Hexanes) to afford compound **C** (1.16 g, 62%) as a yellow liquid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, Fig. S3) δ 7.92 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.3 Hz, 2H), 3.68 (t, *J* = 6.3 Hz, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.97 (septet, *J* = 6.9 Hz, 1H), 2.23 (p, *J* = 6.3 Hz, 2H), 1.27 (d, *J* = 7.0 Hz, 6H).

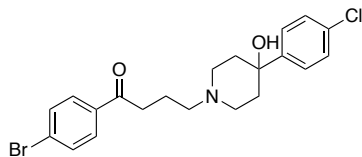


**General procedure for the amination reaction (e.g., synthesis of compound 1 (SGT537)).** Compound **1** was prepared following a previously published protocol for a similar molecule.<sup>2</sup> Sodium iodide (39 mg, 0.260 mmol) and sodium carbonate (50 mg, 0.472 mmol) were added to a stirred mixture of 4'-fluoro-4-chlorobutyrophenone (47 mg, 0.236 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (50 mg, 0.236 mmol) in MeCN (2 mL). The reaction mixture was refluxed for

12 h. The mixture was diluted with H<sub>2</sub>O, extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×10 mL), dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, MeOH:EtOAc/1:9, R<sub>f</sub> 0.19), to give compound **1** (27 mg, 30%) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S4) δ 7.99-7.96 (m, 2H), 7.38 (d, *J* = 6.8 Hz, 2H), 7.27 (dd, *J* = 8.6, 2.1 Hz, 2H), 7.11 (app. t, *J* = 8.5 Hz, 2H), 3.07-2.94 (m, 4H), 2.72 (t, *J* = 12.5 Hz, 2H), 2.67 (t, *J* = 6.0 Hz, 2H), 2.31-2.16 (m, 2H), 2.07 (p, *J* = 7.0 Hz, 2H), 1.78-1.70 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S5) δ 197.9, 167.1, 164.6, 146.2, 133.4, 133.1, 130.9, 130.8, 128.6, 126.2, 116.0, 115.7, 70.6, 57.5, 49.3, 37.4, 36.1, 20.7; LRMS *m/z* calcd for C<sub>21</sub>H<sub>24</sub>ClFNO<sub>2</sub> [M+H]<sup>+</sup>: 376.1; found 376.7. Purity of the compound was further confirmed by RP-HPLC by using method 1: *R*<sub>t</sub> = 7.62 min (100% pure; Fig. S6); Melting point: 144-146 °C.

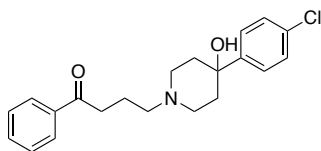


**Synthesis of compound 2 (SGT538).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (110 mg, 0.70 mmol), sodium carbonate (140 mg, 1.26 mmol), 4'-chloro-4-chlorobutyrophenone (140 mg, 0.63 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (140 mg, 0.63 mmol) in MeCN (6 mL) were used to afford compound **2** (38 mg, 15%, R<sub>f</sub> 0.18 in MeOH:EtOAc/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S7) δ 7.90 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 2H), 7.27 (d, *J* = 8.7 Hz, 2H), 2.97 (t, *J* = 6.9 Hz, 2H), 2.84-2.70 (m, 2H), 2.53-2.40 (m, 4H), 2.05-2.00 (m, 2H), 1.979 (p, *J* = 6.38 Hz, 2H) 1.69-1.62 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S8) δ 198.8, 147.0, 139.4, 135.7, 132.9, 129.7, 129.0, 128.5, 126.2, 71.2, 57.9, 49.4, 38.4, 36.4, 22.0; LRMS *m/z* calcd for C<sub>21</sub>H<sub>24</sub>Cl<sub>2</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 392.1; found 392.7. Purity of the compound was further confirmed by RP-HPLC by using method 1: *R*<sub>t</sub> = 8.34 min (97% pure; Fig. S9); Melting point: 146-148 °C.

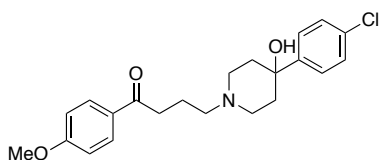


**Synthesis of compound 3 (SGT539).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (110 mg, 0.70 mmol), sodium carbonate (140 mg, 1.26 mmol), 4'-bromo-4-chlorobutyrophenone (170 mg, 0.63 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (140 mg, 0.63 mmol) in MeCN (6 mL) were used to afford compound **3** (42 mg, 12%, R<sub>f</sub> 0.18 in MeOH:EtOAc/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S10) δ 7.83 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.6 Hz,

2H), 3.01 (t,  $J = 6.8$  Hz, 2H), 2.97-2.87 (m, 2H), 2.70-2.51 (m, 4H), 2.20-2.10 (m, 2H), 2.06 (p,  $J = 7.1$  Hz, 2H), 1.78 (br s, 1H), 1.73-1.67 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $(\text{CD}_3)_2\text{SO}$ , Fig. S11)  $\delta$  199.0, 149.1, 136.3, 131.9, 130.9, 130.2, 127.8, 127.0, 126.8, 69.5, 57.2, 48.9, 37.5, 35.7, 21.9; LRMS  $m/z$  calcd for  $\text{C}_{21}\text{H}_{24}\text{ClBrNO}_2$   $[\text{M}+\text{H}]^+$ : 436.1; found 436.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 8.63$  min (98% pure; Fig. S12); Melting point: 164-166  $^\circ\text{C}$ .

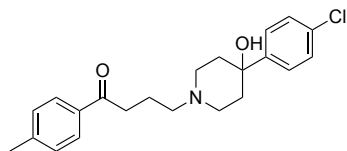


**Synthesis of compound 4 (SGT536).** Following the general procedure described for the synthesis of compound 1, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4-chlorobutyrophenone (43 mg, 0.236 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (50 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound 4 (14 mg, 17%,  $R_f$  0.12 in MeOH:EtOAc/5:95) as a light yellow solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ , Fig. S13)  $\delta$  8.02 (d,  $J = 7.0$  Hz, 2H), 7.60 (t,  $J = 7.4$  Hz, 1H), 7.51 (t,  $J = 7.6$  Hz, 2H), 7.42 (d,  $J = 8.6$  Hz, 2H), 7.31 (d,  $J = 8.6$  Hz, 2H), 3.08 (t,  $J = 6.9$  Hz, 2H), 2.87-2.80 (m, 2H), 2.62-2.48 (m, 4H), 2.07-1.95 (m, 4H), 1.73-1.64 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ , Fig. S14)  $\delta$  201.7, 149.1, 138.5, 134.2, 133.5, 129.7, 129.2, 129.1, 127.5, 71.4, 59.0, 50.4, 38.6, 37.1, 22.5; LRMS  $m/z$  calcd for  $\text{C}_{21}\text{H}_{25}\text{ClNO}_2$   $[\text{M}+\text{H}]^+$ : 358.2; found 358.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.60$  min (96% pure; Fig. S15); Melting point: 106-108  $^\circ\text{C}$ .

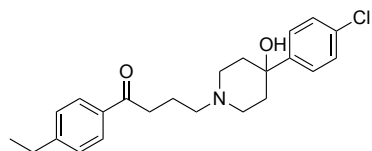


**Synthesis of compound 5 (SGT535).** Following the general procedure described for the synthesis of compound 1, sodium iodide (210 mg, 1.39 mmol), sodium carbonate (270 mg, 2.52 mmol), 4'-methoxy-4-chlorobutyrophenone (270 mg, 1.26 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (270 mg, 1.26 mmol) in MeCN (11 mL) were used to afford compound 5 (41 mg, 8%,  $R_f$  0.14 in MeOH:EtOAc/1:9) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , Fig. S16)  $\delta$  7.94 (d,  $J = 8.6$  Hz, 2H), 7.37 (d,  $J = 8.4$  Hz, 2H), 7.28 (d,  $J = 8.4$  Hz, 2H), 6.92 (d,  $J = 8.5$  Hz, 2H), 3.85 (s, 3H), 2.95 (t,  $J = 7.1$  Hz, 2H), 2.86-2.75 (m, 2H), 2.58-2.41 (m, 4H), 2.12-1.92 (m, 4H), 1.73-1.62 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , Fig. S17)  $\delta$  198.6, 163.5, 147.7, 132.9, 130.5, 130.4, 128.5, 126.2, 113.8, 71.1, 58.0, 55.6, 49.4, 38.3, 36.1, 21.9; LRMS  $m/z$  calcd for  $\text{C}_{22}\text{H}_{27}\text{ClNO}_3$   $[\text{M}+\text{H}]^+$ : 388.2; found 388.7. Purity of the compound was further confirmed by RP-

HPLC by using method 1:  $R_t = 7.94$  min (98% pure; Fig. S18).

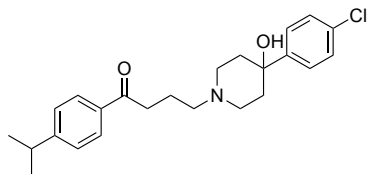


**Synthesis of compound 6 (SGT1430).** Sodium iodide (229 mg, 1.53 mmol) and sodium carbonate (269 mg, 2.54 mmol) were added to a stirred mixture of compound **A** (250 mg, 1.27 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (324 mg, 1.53 mmol) in MeCN (12 mL). The reaction mixture was refluxed for 24 h and progress of the reaction was monitored by TLC (1:19/MeOH:CH<sub>2</sub>Cl<sub>2</sub>,  $R_f$  0.21). The mixture was diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (150 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:19) to afford compound **6** (98 mg, 21%) as a yellow solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S19)  $\delta$  7.85 (d,  $J = 8.2$  Hz, 2H), 7.42 (d,  $J = 8.6$  Hz, 2H), 7.30 (d,  $J = 8.9$  Hz, 2H), 7.25 (d,  $J = 8.0$  Hz, 2H), 3.24-3.12 (m, 2H), 3.09 (t,  $J = 6.7$  Hz, 2H), 2.94-2.69 (m, 4H), 2.58-2.47 (m, 1H), 2.40 (s, 3H), 2.24-2.10 (m, 2H), 1.90-1.70 (m, 4H); <sup>13</sup>C NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>SO, Fig. S20)  $\delta$  198.8, 148.3, 143.3, 134.4, 131.0, 129.2, 128.0, 127.8, 126.7, 68.9, 56.4, 48.6, 36.5, 35.4, 21.1, 20.9; LRMS  $m/z$  calcd for C<sub>22</sub>H<sub>26</sub>ClNO<sub>2</sub> [M+H]<sup>+</sup>: 371.2; found 372.2. Purity of the compound was further confirmed by RP-HPLC by using method 3:  $R_t = 14.21$  min (98% pure; Fig. S21); Melting point: 140-142 °C.

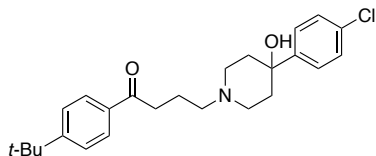


**Synthesis of compound 7 (SGT1429).** Sodium iodide (213 mg, 1.42 mmol) and sodium carbonate (252 mg, 2.38 mmol) were added to a stirred mixture of compound **B** (250 mg, 1.19 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (301 mg, 1.42 mmol) in MeCN (12 mL). The reaction mixture was refluxed for 24 h and progress of the reaction was monitored by TLC (1:19/MeOH:CH<sub>2</sub>Cl<sub>2</sub>,  $R_f$  0.19). The mixture was diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (150 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:19) to afford compound **7** (97 mg, 21%) as a yellow solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S22)  $\delta$  7.88 (d,  $J = 8.3$  Hz, 2H), 7.40 (d,  $J = 8.7$  Hz, 2H), 7.33-7.26 (m, 4H), 3.18-3.12 (m, 1H), 3.07 (t,  $J = 6.6$  Hz, 2H), 2.95-2.75 (m, 4H), 2.69 (q,  $J = 7.6$  Hz, 2H), 2.51-2.30 (m, 2H), 2.18-2.10 (m, 2H), 1.85-1.66 (m, 4H), 1.24 (t,  $J = 7.6$  Hz, 3H); <sup>13</sup>C NMR (100 MHz,

(CD<sub>3</sub>)<sub>2</sub>SO, Fig. S23)  $\delta$  198.8, 149.3, 148.3, 134.7, 131.0, 128.1, 128.0, 127.8, 126.7, 68.9, 56.5, 48.6, 36.6, 35.4, 28.1, 20.5, 15.2; LRMS  $m/z$  calcd for C<sub>23</sub>H<sub>28</sub>ClNO<sub>2</sub> [M+H]<sup>+</sup>: 385.2; found 386.2. Purity of the compound was further confirmed by RP-HPLC by using method 3:  $R_t$  = 14.51 min (97% pure; Fig. S24); Melting point: 126-128 °C.



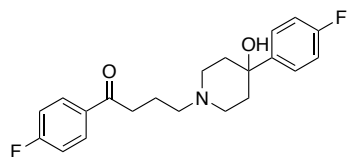
**Synthesis of compound 8 (SGT1428).** Sodium iodide (280 mg, 1.87 mmol) and sodium carbonate (331 mg, 3.12 mmol) were added to a stirred mixture of compound **C** (350 mg, 1.56 mmol) and 4-(4-chlorophenyl)-4-hydroxypiperidine (396 mg, 1.87 mmol) in MeCN (12 mL). The reaction mixture was refluxed for 24 h and progress of the reaction was monitored by TLC (1:19/ MeOH:CH<sub>2</sub>Cl<sub>2</sub>,  $R_f$  0.17). The mixture was diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (150 mL). The organic layer was washed with brine (30 mL), dried over MgSO<sub>4</sub>, removed under reduced pressure, and the residue was purified by flash column chromatography (SiO<sub>2</sub>, MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:19) to afford compound **8** (158 mg, 25%) as a yellow solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S25)  $\delta$  7.90 (d,  $J$  = 8.4 Hz, 2H), 7.38 (d,  $J$  = 8.6 Hz, 2H), 7.30 (d,  $J$  = 8.1 Hz, 2H), 7.29 (d,  $J$  = 8.7 Hz, 2H), 3.02 (t,  $J$  = 6.9 Hz, 2H), 2.95 (p,  $J$  = 6.9 Hz, 2H), 2.70-2.50 (m, 4H), 2.21-2.13 (m, 1H), 2.11-2.01 (m, 3H), 1.75-1.59 (m, 4H), 1.25 (d,  $J$  = 6.9 Hz, 6H); <sup>13</sup>C NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>SO, Fig. S26)  $\delta$  198.9, 153.7, 134.9, 130.8, 128.6, 128.1, 127.7, 126.7, 126.5, 69.1, 56.8, 48.7, 37.0, 35.4, 33.5, 23.5, 21.2; LRMS  $m/z$  calcd for C<sub>24</sub>H<sub>30</sub>ClNO<sub>2</sub> [M+H]<sup>+</sup>: 399.2; found 400.2. Purity of the compound was further confirmed by RP-HPLC by using method 3:  $R_t$  = 14.75 min (96% pure; Fig. S27); Melting point: 122-124 °C.



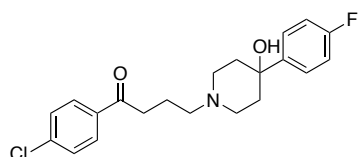
**Synthesis of compound 9 (SGT534).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (210 mg, 1.39 mmol), sodium carbonate (270 mg, 2.52 mmol), 4'- *tert*-butyl-4-chlorobutyrophenone (300 mg, 1.26 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (270 mg, 1.26 mmol) in MeCN (11 mL) were used to afford compound **9** (120 mg, 23%,  $R_f$  0.14 in MeOH:EtOAc/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S28)  $\delta$  7.90 (d,  $J$  = 7.0 Hz, 2H), 7.46 (d,  $J$  = 7.0 Hz, 2H), 7.37 (d,  $J$  = 8.2 Hz, 2H), 7.28 (d,  $J$  = 7.3 Hz, 2H), 3.05-2.95 (m, 2H), 2.90-2.75 (m, 2H), 2.65-2.30 (m, 4H), 2.20-1.85 (m, 4H), 1.70-1.62 (m, 2H), 1.32 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S29)  $\delta$  199.7, 156.7, 147.1, 134.8, 132.8,



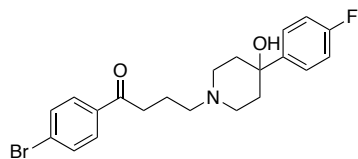
128.5, 128.2, 126.2, 125.6, 71.2, 58.1, 49.4, 38.4, 36.4, 35.21, 31.2, 22.1; LRMS  $m/z$  calcd for  $C_{25}H_{33}ClNO_2$   $[M+H]^+$ : 414.2; found 414.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 9.48$  min (100% pure; Fig. S30); Melting point: 144-146 °C.



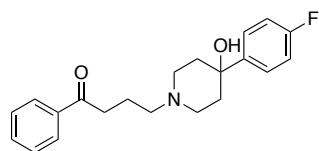
**Synthesis of compound 10 (SGT543).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4'-fluoro-4-chlorobutyrophenone (47 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **10** (11 mg, 13%,  $R_f$  0.14 in MeOH:EtOAc/1:9) as a white solid:  $^1H$  NMR (400 MHz,  $CDCl_3$ , Fig. S31)  $\delta$  8.01 (dd,  $J = 8.6, 5.4$  Hz, 2H), 7.50 (dd,  $J = 8.6, 5.3$  Hz, 2H), 7.14 (app. t,  $J = 8.4$  Hz, 2H), 7.05 (app. t,  $J = 8.4$  Hz, 2H), 3.54-3.47 (m, 2H), 3.37-3.30 (m, 2H), 3.28 (t,  $J = 6.6$  Hz, 2H), 3.17-3.08 (m, 2H), 2.97 (td,  $J = 14.2, 4.4$  Hz, 2H), 2.36 (p,  $J = 6.8$  Hz, 2H), 1.91 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , Fig. S32)  $\delta$  197.7, 167.2, 164.7, 163.3, 160.9, 133.2, 130.9, 130.8, 126.5, 126.4, 116.0, 115.8, 115.5, 115.2, 70.3, 57.3, 49.3, 37.0, 36.0, 20.2; LRMS  $m/z$  calcd for  $C_{21}H_{24}F_2NO_2$   $[M+H]^+$ : 360.2; found 360.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.06$  min (98% pure; Fig. S33); Melting point: 126-128 °C.



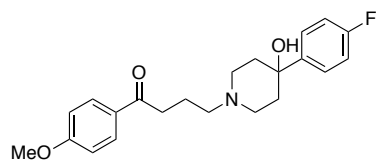
**Synthesis of compound 11 (SGT544).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4'-chloro-4-chlorobutyrophenone (51 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **11** (18 mg, 20%,  $R_f$  0.24 in MeOH:EtOAc/1:9) as a white solid:  $^1H$  NMR (400 MHz,  $CDCl_3$  Fig. S34)  $\delta$  7.88 (d,  $J = 8.4$  Hz, 2H), 7.43-7.39 (m, 4H), 6.98 (app. t,  $J = 8.4$  Hz, 2H), 3.05 (t,  $J = 6.8$  Hz, 4H), 2.83 (t,  $J = 10.8$  Hz, 2H), 2.74 (m, 2H), 2.33 (m, 2H), 2.11 (p,  $J = 6.8$  Hz, 2H), 1.78 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$  Fig. S35)  $\delta$  198.1, 163.3, 160.8, 143.1, 139.8, 135.2, 129.6, 129.1, 126.5, 126.4, 115.4, 115.2, 70.3, 57.3, 49.3, 37.1, 36.0, 20.3; LRMS  $m/z$  calcd for  $C_{21}H_{24}ClFNO_2$   $[M+H]^+$ : 376.1; found 376.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.82$  min (98% pure; Fig. S36).



**Synthesis of compound 12 (SGT545).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4'-bromo-4-chlorobutyrophenone (62 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **12** (16 mg, 16%,  $R_f$  0.21 in MeOH:EtOAc/1:9) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$  Fig. S37)  $\delta$  7.82 (d,  $J = 8.6$  Hz, 2H), 7.58 (d,  $J = 8.6$  Hz, 2H), 7.39 (dd,  $J = 8.6, 5.4$  Hz, 2H), 6.99 (app. t,  $J = 8.6$  Hz, 2H), 3.00 (t,  $J = 6.9$  Hz, 2H), 2.93 (m, 2H), 2.69-2.57 (m, 4H), 2.17 (t,  $J = 11.2$  Hz, 2H), 2.05 (p,  $J = 6.8$  Hz, 2H), 1.90 (br s, 1H), 1.73 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$  Fig. S38)  $\delta$  198.6, 163.2, 160.8, 143.7, 135.8, 132.0, 129.8, 128.3, 126.45, 126.37, 115.3, 115.1, 70.7, 57.6, 49.4, 37.8, 36.2, 21.1; LRMS  $m/z$  calcd for  $\text{C}_{21}\text{H}_{24}\text{FBrNO}_2$   $[\text{M}+\text{H}]^+$ : 420.1; found 420.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.96$  min (99% pure; Fig. S39); Melting point: 128-130  $^\circ\text{C}$ .

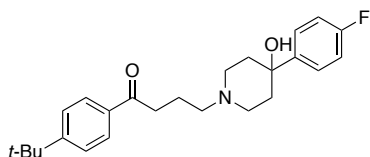


**Synthesis of compound 13 (SGT542).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4-chlorobutyrophenone (43 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **13** (12 mg, 15%,  $R_f$  0.23 in MeOH:EtOAc/1:9) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$  Fig. S40)  $\delta$  7.94 (d,  $J = 7.6$  Hz, 2H), 7.55 (t,  $J = 7.3, 2.0$  Hz, 1H), 7.46 (d,  $J = 7.6$  Hz, 2H), 7.44-7.38 (m, 2H), 6.99 (app. t,  $J = 8.8$  Hz, 2H), 3.06 (t,  $J = 6.9$  Hz, 2H), 3.04-2.99 (m, 2H), 2.78 (t,  $J = 12.1$  Hz, 2H), 2.72 (t,  $J = 7.5$  Hz, 2H), 2.28 (t,  $J = 11.4$  Hz, 2H), 2.10 (p,  $J = 7.3$  Hz, 2H), 1.78 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$  Fig. S41)  $\delta$  199.5, 163.3, 160.8, 143.6, 136.9, 133.3, 128.8, 128.2, 126.5, 126.4, 115.4, 115.2, 70.4, 57.5, 49.3, 37.4, 36.1, 20.6; LRMS  $m/z$  calcd for  $\text{C}_{21}\text{H}_{25}\text{FNO}_2$   $[\text{M}+\text{H}]^+$ : 342.2; found 342.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 6.88$  min (98% pure; Fig. S42); Melting point: 112-114  $^\circ\text{C}$ .

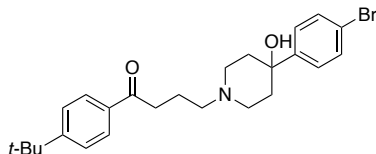


**Synthesis of compound 14 (SGT541).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472

mmol), 4'-methoxy-4-chlorobutyrophenone (50 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **14** (6 mg, 7%,  $R_f$  0.19 in MeOH:EtOAc/1:9) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , Fig. S43)  $\delta$  7.93 (d,  $J = 8.6$  Hz, 2H), 7.44 (dd,  $J = 7.7, 5.2$  Hz, 2H), 7.00 (app. t,  $J = 8.7$  Hz, 2H), 6.92 (d,  $J = 8.5$  Hz, 2H), 3.85 (s, 3H), 3.12-3.06 (m, 2H), 3.04 (t,  $J = 6.8$  Hz, 2H), 2.92-2.70 (m, 4H), 2.47-2.32 (m, 2H), 2.12 (p,  $J = 7.6$  Hz, 2H), 1.80 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , Fig. S44)  $\delta$  197.6, 163.6, 163.2, 160.8, 142.3, 130.3, 129.6, 126.34, 126.26, 115.4, 115.1, 113.8, 70.0, 57.1, 55.5, 49.0, 36.5, 35.3, 19.6; LRMS  $m/z$  calcd for  $\text{C}_{22}\text{H}_{27}\text{FNO}_3$   $[\text{M}+\text{H}]^+$ : 372.2; found 372.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.44$  min (100% pure; Fig. S45); Melting point: 114-116  $^\circ\text{C}$ .

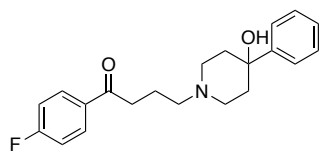


**Synthesis of compound 15 (SGT540).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4'-tert-butyl-4-chlorobutyrophenone (56 mg, 0.236 mmol), and 4-(4-fluorophenyl)-4-hydroxypiperidine (46 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **15** (22 mg, 23%,  $R_f$  0.11 in MeOH:EtOAc/5:95) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ , Fig. S46)  $\delta$  7.96 (d,  $J = 8.6$  Hz, 2H), 7.55 (d,  $J = 8.6$  Hz, 2H), 7.45 (dd,  $J = 8.6, 5.6$  Hz, 2H), 7.03 (app. t,  $J = 8.8$  Hz, 2H), 3.06 (t,  $J = 6.9$  Hz, 2H), 2.87 (m, 2H), 2.66-2.54 (m, 4H), 2.05-1.94 (m, 4H), 1.70 (m, 2H), 1.34 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ , Fig. S47)  $\delta$  201.5, 164.3, 161.9, 158.1, 146.1, 135.9, 129.2, 127.7, 127.6, 126.7, 115.7, 115.5, 71.2, 58.9, 50.4, 38.6, 37.0, 36.0, 31.5, 22.5; LRMS  $m/z$  calcd for  $\text{C}_{25}\text{H}_{33}\text{FNO}_2$   $[\text{M}+\text{H}]^+$ : 398.2; found 398.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 9.12$  min (98% pure; Fig. S48); Melting point: 134-136  $^\circ\text{C}$ .

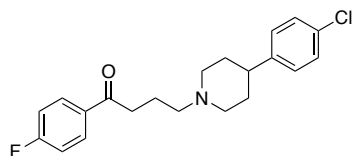


**Synthesis of compound 21 (SGT546).** Following the general procedure described for the synthesis of compound **1**, sodium iodide (39 mg, 0.260 mmol), sodium carbonate (50 mg, 0.472 mmol), 4'-tert-butyl-4-chlorobutyrophenone (56 mg, 0.236 mmol), and 4-(4-bromophenyl)-4-hydroxypiperidine (60 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **21** (30 mg, 28%,  $R_f$  0.30 in MeOH:EtOAc/1:9) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , Fig. S49)  $\delta$

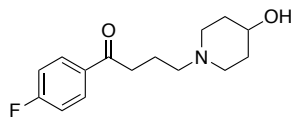
7.90 (d,  $J = 8.2$  Hz, 2H), 7.46 (d,  $J = 8.0$  Hz, 2H), 7.42 (d,  $J = 8.4$  Hz, 2H), 7.30 (d,  $J = 8.2$  Hz, 2H), 2.98 (t,  $J = 6.9$  Hz, 2H), 2.84 (m, 2H), 2.54-2.45 (m, 4H), 2.06-1.96 (m, 2H), 2.00 (p,  $J = 6.8$  Hz, 2H), 1.81 (very br s, 1H), 1.66 (m, 2H), 1.32 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , Fig. S50)  $\delta$  199.6, 156.8, 147.4, 134.7, 131.5, 128.2, 126.6, 125.6, 121.0, 71.1, 58.0, 49.4, 38.1, 36.2, 35.2, 31.2, 21.8; LRMS  $m/z$  calcd for  $\text{C}_{25}\text{H}_{33}\text{BrNO}_2$   $[\text{M}+\text{H}]^+$ : 458.2; found 458.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 9.84$  min (98% pure; Fig. S51); Melting point: 168-170 °C.



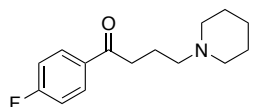
**Synthesis of compound 22 (SGT564).** Following the general procedure described for the synthesis of compound 1,  $\text{KIO}_3$  (190 mg, 0.90 mmol), potassium carbonate (240 mg, 1.74 mmol), 4'-fluoro-4-chlorobutyrophenone (190 mg, 0.92 mmol), and 4-phenylpiperidin-4-ol (100 mg, 0.56 mmol) in toluene (10 mL) were used to afford compound **22** (51 mg, 27%,  $R_f$  0.20 in  $\text{MeOH}:\text{CH}_2\text{Cl}_2/5:95$ ) as a yellow solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ , which matches lit.,<sup>3</sup> Fig. S52)  $\delta$  8.13-8.06 (m, 2H), 7.46 (d,  $J = 7.1$  Hz, 2H), 7.32 (t,  $J = 7.1$  Hz, 2H), 7.26-7.17 (m, 3H), 2.92-2.83 (m, 2H), 2.62 (t,  $J = 11.9$  Hz, 2H), 2.58 (t,  $J = 8.0$  Hz, 2H), 2.12-1.96 (m, 4H), 1.76-1.68 (m, 2H), 1.34-1.25 (m, 3H). Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 6.44$  min (100% pure; Fig. S53).



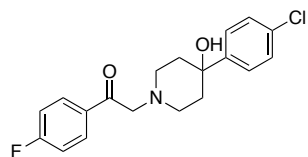
**Synthesis of compound 23 (SGT565).** Compound **27** (70 mg, 0.20 mmol) and Pd (20 mg, 10% on activated charcoal) were added to deoxygenated absolute EtOH (10 mL). The hydrogen was applied *via* a balloon. The reaction mixture was stirred at room temperature for 2 h, and then filtered through celite®. The filtrate was collected and the solvent was evaporated under reduced pressure to yield compound **23** (35 mg, 50% yield) as a white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , which matches lit.,<sup>4</sup> Fig. S54)  $\delta$  8.00 (dd,  $J = 8.6, 5.5$  Hz, 2H), 7.28 (app. t,  $J = 8.0$  Hz, 2H), 7.19 (d,  $J = 7.2$  Hz, 2H), 7.12 (app. t,  $J = 8.5$  Hz, 2H), 3.14-3.03 (m, 2H), 3.00 (t,  $J = 7.0$  Hz, 2H), 2.54-2.42 (m, 2H), 2.14-2.03 (m, 1H), 2.05-1.95 (m, 2H), 1.85-1.75 (m, 3H), 1.68-1.52 (m, 3H). Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.71$  min (96% pure; Fig. S55); Melting point: 94-96 °C.



**Synthesis of compound 24 (SGT566).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (650 mg, 3.94 mmol), potassium carbonate (820 mg, 5.91 mmol), 4'-fluoro-4-chlorobutyrophenone (590 mg, 2.94 mmol), and 4-hydroxypiperidine (200 mg, 1.97 mmol) in toluene (10 mL) were used to afford the known compound **24** (72 mg, 14%,  $R_f$  0.20 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:9) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, which matches lit.,<sup>5</sup> Fig. S56)  $\delta$  7.98 (dd,  $J$  = 8.8, 5.6 Hz, 2H), 7.11 (app. t,  $J$  = 8.7 Hz, 2H), 3.72-3.62 (m, 1H), 2.96 (t,  $J$  = 7.1 Hz, 2H), 2.78-2.70 (m, 2H), 2.39 (t,  $J$  = 7.2 Hz, 2H), 2.18-2.05 (m, 2H), 1.92 (p,  $J$  = 7.2 Hz, 2H), 1.89-1.77 (m, 2H), 1.61 (br s, 1H), 1.55-1.45 (m, 2H). Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t$  = 4.69 min (100% pure; Fig. S57).

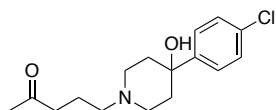


**Synthesis of compound 25 (SGT567).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (960 mg, 5.80 mmol), potassium carbonate (1.22 g, 8.81 mmol), 4'-fluoro-4-chlorobutyrophenone (880 mg, 4.39 mmol), and piperidine (240 mg, 2.87 mmol) in toluene (15 mL) were used to afford the known compound **25** (120 mg, 17%,  $R_f$  0.15 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/5:95) as a yellow oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, which matches lit.,<sup>5</sup> Fig. S58)  $\delta$  7.98 (dd,  $J$  = 8.8, 5.6 Hz, 2H), 7.10 (app. t,  $J$  = 8.6 Hz, 2H), 2.95 (t,  $J$  = 7.2 Hz, 2H), 2.35 (t,  $J$  = 7.2 Hz, 6H), 1.92 (p,  $J$  = 7.2 Hz, 2H), 1.56-1.48 (m, 4H), 1.43-1.35 (m, 2H). Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t$  = 6.99 min (100% pure; Fig. S59).

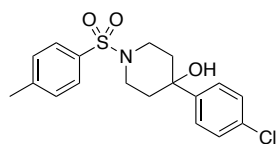


**Synthesis of compound 26 (SGT568).** Following the general procedure described for the synthesis of compound **1**, potassium carbonate (50 mg, 0.36 mmol), 4'-fluoro-2-chloroacetophenone (40 mg, 0.23 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (50 mg, 0.23 mmol) in EtOH (10 mL) were used to afford compound **26** (67 mg, 100%,  $R_f$  0.33 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S60)  $\delta$  8.05 (dd,  $J$  = 7.6, 5.6 Hz, 2H), 7.43 (d,  $J$  = 8.54 Hz, 2H), 7.30 (d,  $J$  = 7.2 Hz, 2H), 7.12 (app. t,  $J$  = 8.5 Hz, 2H), 3.84 (s, 2H), 2.93-2.84 (m, 2H), 2.62 (t,  $J$  = 11.8 Hz, 2H), 2.21 (td,  $J$  = 13.2, 4.5 Hz, 2H), 1.79-1.65 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S61)  $\delta$  195.1, 167.2, 164.7, 146.9, 133.0, 132.4, 131.0, 130.9, 128.6, 126.2, 116.0, 115.7, 70.8, 64.7, 49.9, 38.4; LRMS  $m/z$  calcd for C<sub>19</sub>H<sub>20</sub>ClFNO<sub>2</sub> [M+H]<sup>+</sup>: 348.1; found 348.7. Purity of the compound was

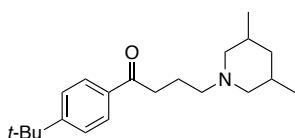
further confirmed by RP-HPLC by using method 1:  $R_t = 6.85$  min (95% pure; Fig. S62); Melting point: 140-142 °C.



**Synthesis of compound 27 (SGT569).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (550 mg, 3.33 mmol), potassium carbonate (690 mg, 5.00 mmol), 5-chloropentan-2-one (85% technical grade, 200 mg, 1.41 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (700 mg, 3.31 mmol) in toluene (20 mL) were used to afford compound **27** (44 mg, 11%,  $R_f$  0.10 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/5:95) as a light yellow solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S63)  $\delta$  7.42 (d,  $J = 8.5$  Hz, 2H), 7.30 (d,  $J = 8.5$  Hz, 2H), 2.90-2.78 (m, 2H), 2.49 (t,  $J = 7.2$  Hz, 2H), 2.46-2.37 (m, 4H), 2.22-2.07 (m, 5H), 1.83 (p,  $J = 7.2$  Hz, 2H), 1.76-1.67 (m, 2H), 1.60 (br s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S64)  $\delta$  208.7, 146.7, 133.0, 128.6, 126.2, 71.0, 57.7, 49.4, 41.5, 38.1, 30.3, 20.9; LRMS  $m/z$  calcd for C<sub>16</sub>H<sub>23</sub>ClNO<sub>2</sub> [M+H]<sup>+</sup>: 296.1; found 296.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 6.28$  min (100% pure; Fig. S65); Melting point: 90-92 °C.

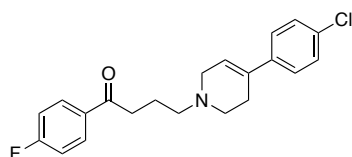


**Synthesis of compound 28 (SGT570).** Following the general procedure described for the synthesis of compound **1**, sodium carbonate (50 mg, 0.472 mmol), *p*-TsCl (45 mg, 0.236 mmol), and 4-(4-chlorophenyl)-4-hydroxypiperidine (50 mg, 0.236 mmol) in MeCN (2 mL) were used to afford compound **28** (52 mg, 60%,  $R_f$  0.83 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, Fig. S66)  $\delta$  7.70 (d,  $J = 8.0$  Hz, 2H), 7.44 (d,  $J = 8.0$  Hz, 2H), 7.41 (d,  $J = 8.6$  Hz, 2H), 7.31 (d,  $J = 8.4$  Hz, 2H), 3.68-3.63 (m, 2H), 2.75 (t,  $J = 12.1$  Hz, 2H), 2.46 (s, 3H), 2.08 (td,  $J = 13.4, 4.6$  Hz, 2H), 1.77-1.68 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S67)  $\delta$  146.1, 143.8, 133.4, 129.9, 128.80, 128.77, 127.8, 126.1, 70.5, 42.4, 37.8, 21.7; LRMS  $m/z$  calcd for C<sub>18</sub>H<sub>21</sub>ClNO<sub>3</sub>S [M+H]<sup>+</sup>: 366.1; found 366.7. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 9.89$  min (99% pure; Fig. S68).

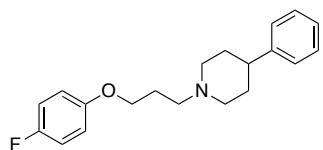


**Synthesis of compound 29 (SGT571).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (580 mg, 3.52 mmol), potassium carbonate (730 mg, 5.30 mmol), 1-(4-(*tert*-

butyl)phenyl)-3-chloropropan-1-one (630 mg, 2.65 mmol), and 3,5-dimethylpiperidine (200 mg, 1.76 mmol) in toluene (20 mL) were used to afford compound **29** (68 mg, 24%,  $R_f$  0.45 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/5:95) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S69) δ 7.88 (d,  $J$  = 8.2 Hz, 2H), 7.45 (d,  $J$  = 8.5 Hz, 2H), 3.16-2.98 (m, 4H), 2.68 (t,  $J$  = 7.8 Hz, 2H), 2.11 (p,  $J$  = 7.8 Hz, 2H), 2.06-1.85 (m, 3H), 1.82-1.68 (m, 3H), 1.31 (s, 9H), 0.87 (d,  $J$  = 6.6 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S70) δ 199.2, 157.1, 134.3, 128.1, 125.7, 60.3, 57.6, 41.3, 36.1, 35.3, 31.2, 30.0, 20.3, 19.4; LRMS  $m/z$  calcd for C<sub>21</sub>H<sub>34</sub>NO [M+H]<sup>+</sup>: 316.3; found 316.9. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t$  = 8.84 min (100% pure; Fig. S71).

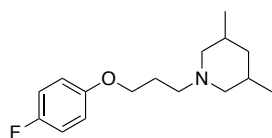


**Synthesis of compound 30 (SGT572).** Compound **1** (380 mg, 1 mmol) was dissolved in AcOH (10 mL) and HCl (2 mL). The reaction mixture was heated at reflux for 24 h, and then allowed to cool down to room temperature. KOH was added gradually until pH 8. The organic material was extracted with two portions of EtOAc (30 mL). The organic solvents were collected, dried using anhydrous MgSO<sub>4</sub>, and evaporated under reduced pressure. The obtained solid was purified by flash column chromatography (SiO<sub>2</sub>, MeOH:CH<sub>2</sub>Cl<sub>2</sub>/3:97,  $R_f$  0.44) to yield compound **30** (300 μg, 84% yield) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, which matches lit.,<sup>6</sup> Fig. S72) δ 7.98 (dd,  $J$  = 8.5, 5.7 Hz, 2H), 7.31-7.25 (m, 4H), 7.09 (app. t,  $J$  = 8.5 Hz, 2H), 6.03 (m, 1H), 3.18-3.10 (m, 2H), 3.01 (t,  $J$  = 7.2 Hz, 2H), 2.74-2.65 (m, 2H), 2.54 (t,  $J$  = 7.4 Hz, 2H), 2.51-2.44 (m, 2H), 2.00 (p,  $J$  = 7.2 Hz, 2H). Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t$  = 8.57 min (100% pure; Fig. S73); Melting point: 88-90 °C.

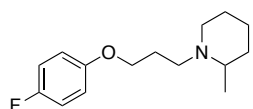


**Synthesis of compound 31 (SGT573).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (120 mg, 0.72 mmol), potassium carbonate (260 mg, 1.83 mmol), 3-(4-fluorophenoxy)propyl chloride (170 mg, 0.91 mmol), and 4-phenylpiperidine (150 mg, 0.91 mmol) in THF (10 mL) were used to afford compound **31** (130 mg, 45%,  $R_f$  0.59 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:9) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S74) δ 7.29 (app. t,  $J$  = 7.4 Hz, 2H), 7.25-7.15 (m, 3H), 6.95 (t,  $J$  = 8.1 Hz, 2H), 6.86-6.76 (m, 2H), 3.98 (t,  $J$  = 6.2 Hz, 2H), 3.15-3.05 (m, 2H), 2.59 (t,  $J$  = 7.5 Hz, 2H), 2.52 (p,  $J$  = 8.0 Hz, 1H), 2.17-2.07 (m, 2H), 2.02 (p,  $J$  = 7.2 Hz, 2H), 1.90-1.75 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S75) δ 158.5, 156.2, 155.2,

146.2, 128.6, 127.0, 126.4, 116.0, 115.8, 115.64, 115.56, 67.1, 55.7, 54.5, 42.7, 33.3, 26.9; LRMS  $m/z$  calcd for  $C_{20}H_{25}FNO$   $[M+H]^+$ : 314.2; found 314.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.71$  min (99% pure; Fig. S76); Melting point: 132-134 °C.

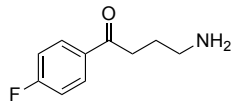


**Synthesis of compound 32 (SGT574).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (30 mg, 0.18 mmol), potassium carbonate (210 mg, 1.54 mmol), 3-(4-fluorophenoxy)propyl chloride (150 mg, 0.77 mmol), and 3,5-dimethylpiperidine (90 mg, 0.77 mmol) in THF (10 mL) were used to afford compound **32** (22 mg, 10%,  $R_f$  0.56 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:9) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S77)  $\delta$  6.93 (app. t,  $J = 8.6$  Hz, 2H), 6.84-6.74 (m, 2H), 3.94 (t,  $J = 6.6$  Hz, 2H), 2.89-2.77 (m, 2H), 2.47 (t,  $J = 7.6$  Hz, 2H), 1.96 (p,  $J = 7.2$  Hz, 2H), 1.69 (d,  $J = 9.6$  Hz, 4H), 1.44 (t,  $J = 10.9$  Hz, 2H), 0.84 (d,  $J = 6.4$  Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S78)  $\delta$  158.5, 156.1, 155.3, 116.0, 115.7, 115.63, 115.55, 67.2, 61.8, 55.6, 42.3, 31.2, 26.9, 19.8; LRMS  $m/z$  calcd for  $C_{16}H_{25}FNO$   $[M+H]^+$ : 266.2; found 266.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.29$  min (100% pure; Fig. S79).

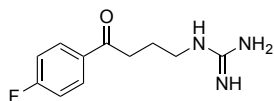


**Synthesis of compound 33 (SGT575).** Following the general procedure described for the synthesis of compound **1**, potassium iodide (30 mg, 0.18 mmol), potassium carbonate (210 mg, 1.54 mmol), 3-(4-fluorophenoxy)propyl chloride (150 mg, 0.77 mmol), and 2-methylpiperidine (80 mg, 0.77 mmol) in THF (10 mL) were used to afford compound **33** (12 mg, 6%,  $R_f$  0.30 in MeOH:CH<sub>2</sub>Cl<sub>2</sub>/1:9) as a white solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Fig. S80)  $\delta$  6.93 (app. t,  $J = 7.6$  Hz, 2H), 6.84-6.76 (m, 2H), 4.00-3.85 (m, 2H), 2.94-2.80 (m, 2H), 2.58-2.44 (m, 1H), 2.38-2.25 (m, 1H), 2.19 (t,  $J = 10.9$  Hz, 1H), 1.92 (p,  $J = 6.6$  Hz, 2H), 1.72-1.50 (m, 4H), 1.38-1.19 (m, 2H), 1.07 (d,  $J = 3.8$  Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S81)  $\delta$  158.5, 156.1, 155.3, 116.0, 115.8, 115.6, 115.5, 67.2, 56.2, 52.3, 50.7, 34.6, 26.1, 25.6, 24.0, 19.1; LRMS  $m/z$  calcd for  $C_{15}H_{23}FNO$   $[M+H]^+$ : 252.2; found 252.8. Purity of the compound was further confirmed by RP-HPLC by using method 1:  $R_t = 7.61$  min (98% pure; Fig. S82).





**Synthesis of compound 34 (SGT1416).** To a solution of 4-chloro-4'-fluorobutyrophenone (2.00 g, 9.97 mmol) in DMF (10 mL), sodium azide (3.24 g, 49.9 mmol) was added. The reaction mixture was then stirred at 60 °C for 12 h, and progress of the reaction was monitored by TLC (1:19/EtOAc:Hexanes,  $R_f$  0.74). The reaction mixture was quenched with H<sub>2</sub>O (150 mL), extracted with EtOAc (150 mL), washed successively with H<sub>2</sub>O (200 mL) and brine (20 mL), and then dried over MgSO<sub>4</sub>. The organic layer was removed under reduced pressure to afford the intermediate azide (1.95 g, 94%) as a red liquid. The resulting crude product was then dissolved in THF (8 mL), and 1 M PMe<sub>3</sub> in THF (15.1 mL) and H<sub>2</sub>O (2 mL) were added. The reaction mixture was stirred at 50 °C for 1 h, and progress of the reaction was monitored by TLC (1:9/EtOAc:Hexanes,  $R_f$  0.67). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography (SiO<sub>2</sub>, 1:19/MeOH:CH<sub>2</sub>Cl<sub>2</sub>) to afford the compound **34** (1.13 g, 66%) as a yellow solid: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, Fig. S83) δ 7.89-7.83 (m, 2H), 7.21-7.13 (m, 2H), 4.01 (tt,  $J_1 = 7.4$  Hz,  $J_2 = 2.1$  Hz, 2H), 3.03 (tt,  $J_1 = 8.0$  Hz,  $J_2 = 2.1$  Hz, 2H), 2.08 (p,  $J = 7.4$  Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, Fig. S84) δ 172.2, 165.4, 162.9, 129.7, 129.6, 115.6, 115.4, 61.6, 35.0, 22.9. LRMS  $m/z$  calcd for C<sub>10</sub>H<sub>12</sub>FNO [M+H]<sup>+</sup>: 181.1; found 182.1.



**Synthesis of compound 35 (SGT1264).** To a solution of compound **34** (120 mg, 0.66 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine (309 mg, 0.79 mmol) and Et<sub>3</sub>N (0.11 mL, 0.79 mmol) were added. The reaction mixture was stirred at room temperature for 48 h, and progress of the reaction was monitored by TLC (2:3/EtOAc:Hexanes,  $R_f$  0.48). The organic layer was removed under reduced pressure, the resulting residue was then dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and trifluoroacetic acid (1 mL) was added. The reaction mixture was stirred at room temperature for 1 h, and progress of the reaction was monitored by TLC (1:9/MeOH:CH<sub>2</sub>Cl<sub>2</sub>,  $R_f$  0.26). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography (SiO<sub>2</sub>, 1:9/MeOH:CH<sub>2</sub>Cl<sub>2</sub>) to afford compound **35** (48 mg, 76%) as a white solid: <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO, Fig. S85) δ 8.08-8.01 (m, 2H), 7.64 (t,  $J = 5.8$  Hz, 1H), 7.42-7.33 (m, 2H), 7.25-6.90 (very br s, 3H), 3.16 (q,  $J = 7.1$  Hz, 2H), 3.08 (t,  $J = 7.1$  Hz, 2H), 1.82 (p,  $J = 7.1$  Hz, 2H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD, Fig. S86) δ 199.8, 168.6, 166.1, 158.8, 132.1, 132.0, 116.8, 116.6, 41.8, 36.0, 24.2. LRMS  $m/z$  calcd for C<sub>11</sub>H<sub>14</sub>FN<sub>3</sub>O [M+H]<sup>+</sup>: 223.1; found 224.1. Purity of the

compound was further confirmed by RP-HPLC by using method 2:  $R_t = 13.22$  min (98% pure; Fig. S87); Melting point: 72-74 °C.

### **BIOCHEMICAL, BIOLOGICAL, AND BIOPHYSICAL ASSAYS:**

**Materials and instrumentations for biochemical and biological experiments.** UV-Vis assays were performed using a multimode SpectraMax M5 plate reader from Molecular Devices (Sunnyvale, CA) and 96-well plates from Greiner (Monroe, NC, USA). Reagents for Eis inhibition assays such as 5',5'-dithiobis-(2-nitrobenzoic acid) DTNB, Tween® 80, kanamycin A (KAN), neomycin B (NEO), acetyl-CoA (AcCoA), and chlorhexidine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Albumin-dextrose-catalase (ADC) was purchased from BD Biosciences (San Jose, CA, USA). Eis was screened at the Center for Chemical Genomics (CCG, University of Michigan) against ~2,500 compounds from a BioFocus NCC library (446 molecules) and a MicroSource MS2000 library (2,000 molecules). Stock compounds for HTS were dissolved in DMSO. The human embryonic kidney cell line HEK-293 (ATCC CRL-1573) was bought from ATCC (Manassas, VA, USA). The human lung carcinoma epithelial cell line A549 (ATCC CCL-185) and the murine macrophage cell line J774A.1 (ATCC TIB-67) were received from Drs. David K. Orren and David J. Feola (University of Kentucky, Lexington, KY, USA), respectively. A549 and HEK-293 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (ATCC, Manassas, VA, USA) with 10% fetal bovine serum (FBS) (ATCC, Manassas, VA, USA) and 1% penicillin/streptomycin (ATCC, Manassas, VA, USA) at 37 °C with 5% CO<sub>2</sub>. J774A.1 cells were grown under the same conditions, except that the medium used was a different type of DMEM (catalog # 30-2002, ATCC, Manassas, VA, USA). A549 and HEK-293 cells were dislodged from the tissue culture petri dish by trypsinization with 0.05%-trypsin-0.53 mM EDTA (ATCC, Manassas, VA, USA) for subculturing. J774A.1 cells were sub dislodged from the tissue culture petri dish by mechanical scraping for subculturing. The cells' confluence was observed by using a Nikon Eclipse TS100 microscope (Minato, Tokyo, Japan). Resazurin was purchased from Sigma Aldrich (Milwaukee, WI, USA). Fluorescence of the resazurin product, resorufin, was detected with a SpectraMax M5 plate reader.

**Bacterial strains.** Bacterial strains were obtained from several sources: *Mycobacterium smegmatis* mc<sup>2</sup>155 (strain A) was a gift from Prof. Sabine Ehrt (Weill Cornell Medical College,

NY, USA). *Mycobacterium abscessus* ATCC 19977 (strain *B*), *Mycobacterium intracellulare* ATCC 13950 (strain *C*), *Mycobacterium avium* ATCC 25921 (strain *D*), and *Mycobacterium tuberculosis* H37Ra ATCC NRS22 (strain *F*) were purchased from the American Type Tissue Collection. *Mycobacterium bovis* BCG ATCC 35734 (strain *E*) was obtained from Dr. Anthony Baughn (University of Minnesota, MN, USA). *Mycobacterium tuberculosis* mc<sup>2</sup>6230 (strain *G*) was a gift from Prof. William Jacobs Jr. (Albert Einstein College of Medicine, NY, USA). *Staphylococcus epidermidis* ATCC 12228 (strain *H*), *Staphylococcus aureus* ATCC 25923 (strain *I*), *Enterobacter cloacae* ATCC 13047 (strain *N*), and *Pseudomonas aeruginosa* ATCC 27853 (strain *R*) were obtained from Prof. Dev Arya (Clemson University, SC, USA). *Listeria monocytogenes* ATCC 19115 (strain *L*), *Escherichia coli* MC1061 (strain *M*), *Acinetobacter baumannii* ATCC 19606 (strain *O*), *Klebsiella pneumoniae* ATCC 27736 (strain *P*), and *Salmonella enterica* ATCC14028 (strain *Q*) were gathered from Prof. Paul Hergenrother (University of Illinois at Urbana-Champaign, IL, USA). *Bacillus anthracis* Sterne F32 (strain *J*) was a gift from Prof. Phil Hannah (University of Michigan, MI, USA). Vancomycin resistant enterococci (VRE) (strain *K*) was obtained from Prof. David Sherman (University of Michigan, MI, USA).

**Purification of EisC204A and crystallization of EisC204A-CoA with compounds 1, 10, 30, 35, and DPD.** Overexpression of EisC204A in *E. coli* BL21 (DE3) was carried out as described previously.<sup>7</sup> *E. coli* BL21 (DE3) cells were transformed with EisC204A-pET28a plasmid and plated on LB agar containing 50 µg/mL of KAN. A single colony was inoculated in 5 mL of LB medium supplemented with 50 µg/mL of KAN and shaken for 4 h in a 37 °C incubator. The small-scale culture was then transferred to 1 L of LB media with 50 µg/mL of KAN. The cells were grown at 37 °C until an attenuation (OD<sub>600nm</sub>) of ~0.6 was reached. The cultures were then moved to 16 °C for 1.5 h. Protein expression was induced with the addition of a final concentration of 0.5 mM IPTG, and cells were shaken for 16 hours at 16 °C. Bacteria were harvested by centrifugation at 5,000 rpm for 10 min. The cell pellet was resuspended in a lysis buffer consisting of 40 mM Tris-HCl pH 8, 300 mM NaCl, 10% glycerol and 2 mM β-mercaptoethanol. Cells were lysed via sonication and the cell debris was removed by centrifugation (16,000 rpm, 30 min). The clarified lysate was loaded onto a 5 mL Ni-IMAC HisTrap FF column (GE Healthcare), pre-equilibrated with lysis buffer. Contaminants were removed by washing with 100 mL of lysis buffer containing

20 mM imidazole. His-tagged EisC204A protein was then eluted out with 10 mL of lysis buffer supplemented with 500 mM imidazole. The protein was further purified by loading onto size exclusion S-200 column (GE Healthcare), which was pre-equilibrated in buffer consisting of 40 mM Tris pH 8, 100 mM NaCl, 2 mM  $\beta$ -mercaptoethanol. Protein purity was assessed via SDS-PAGE and fractions containing EisC204A were pooled together and concentrated to 10 mg/mL using Amicon Ultra (10,000 MWCO) centrifugal filter device (Millipore).

Crystals of EisC204A for complex formation with compounds **1**, **10**, **30**, **35**, and DPD were grown by vapor diffusion method in 2  $\mu$ L hanging drops at 22 °C. Each drop contained 1  $\mu$ L of EisC204A (10 mg/mL), KAN (10 mM), and CoA (8 mM) mixed with 1  $\mu$ L of the reservoir solution (100 mM Tris-HCl pH 8.5, 9.5-12.5% *w/v* PEG 8000, and 400-500 mM  $(\text{NH}_4)_2\text{SO}_4$ ). The drops were equilibrated against 1 mL of the reservoir solution, which yielded crystals in 2-3 weeks. The  $(\text{NH}_4)_2\text{SO}_4$ , CoA, and KAN were then removed by gradual transfer of crystals into a transfer solution lacking these components and incubated for 10 min. For crystals that were used in complex formation with haloperidol, **10**, and **30**, 100 mM Tris-HCl pH 8.5 and 10% *w/v* PEG 8000 was used. The crystals that were used in complex formation with inhibitor **35**, 100 mM Tris-HCl pH 8.5 and 12.5% *w/v* PEG 8000 was used and those for droperidol complex formation a 100 mM Tris-HCl pH 8.5 and 9.5% *w/v* PEG 8000 transfer solution was used. The crystals were then transferred to the cryoprotectant solution, which is same as transfer solution with an additional 20% *v/v* glycerol and incubated for another 10 min. The crystals were then incubated in the same solution containing additional 1 mM of inhibitor haloperidol (**SGT537**), **10** (**SGT543**), **30** (**SGT572**), **35** (**SGT1264**), or DPD for 20 min, and then frozen by rapid immersion in liquid nitrogen.

**Diffraction data collection and structure determination and refinement of EisC204A-CoA in complex with compounds 1, 10, 30, 35, and DPD.** All X-ray diffraction data were collected at 100 K using a synchrotron beamline 22-ID at the Advanced Photon Source in the Argonne National Laboratory (Argonne, IL). The data were indexed, integrated and scaled using HKL2000.<sup>8</sup> The crystals had a trigonal R32 symmetry, identical to our previously reported Eis-inhibitor complex (PDB ID: 6B3T), which served as search model in structure determination, after removal of its bound ligand. The crystal structures were determined by molecular replacement

method using PHASER.<sup>9</sup> All five Eis-inhibitor complex structure contained one Eis monomer per asymmetric unit. Strong electron density maps were observed that helped building of Eis bound inhibitors by iterative adjustment and refinement using COOT<sup>10</sup> and REFMAC,<sup>11</sup> respectively. The crystal structure coordinates of Eis-haloperidol, Eis-10, Eis-30, Eis-35, and Eis-droperidol were deposited in the Protein Data Bank (PDB) with an accession numbers 6X10, 6X6I, 6X7A, 6X6Y, and 6X6G, respectively. Associated information including data collection and structure refinement statistics can be found in Table S1.

**Table S1.** X-ray diffraction data collection and structure refinement statistics for Eis-haloperidol scaffold inhibitor (1, 10, 30, 35, and DPD) complexes.

Eis-inhibitor complexes	Eis-HPD (1) (SGT537)	Eis-10 (SGT543)	Eis-30 (SGT572)	Eis-35 (SGT1264)	Eis-DPD
PDB ID	6X10	6X6I	6X7A	6X6Y	6X6G
<b>Data collection</b>					
Space group	R32	R32	R32	R32	R32
Number of protomers per asymmetric unit	1	1	1	1	1
Unit cell dimensions					
<i>a, b, c</i> (Å)	175.2, 175.2, 123.4	175.0, 175.0, 124.1	175.1, 175.1, 123.7	175.5, 175.5, 124.8	175.0, 175.0, 124.5
$\alpha, \beta, \gamma$ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	50.00-2.02 (2.07-2.02)	50.00-1.90 (1.93-1.90)	50.00-2.08 (2.12-2.08) <sup>a</sup>	50.00-2.50 (2.54-2.50) <sup>a</sup>	50.00-2.15 (2.19-2.15)
<i>R</i> <sub>merge</sub>	0.11 (0.72)	0.10 (0.96)	0.09 (0.77)	0.10 (0.65)	0.13 (0.86)
<i>CC</i> <sub>1/2</sub>	0.97 (0.95)	0.99 (0.83)	1.00 (0.85)	1.00 (0.78)	0.99 (0.79)
<i>I</i> / $\sigma$ <i>I</i>	14.5 (2.1)	24.3 (2.0)	20.9 (2.2)	15.4 (2.0)	19.0 (2.0)
Completeness (%)	94.3 (94.0)	98.7 (100.0)	99.4 (100.0)	99.9 (100.0)	99.3 (100.0)
Redundancy	4.0 (3.3)	7.5 (6.8)	6.4 (6.3)	6.4 (6.1)	6.3 (6.2)
Number of unique reflections	44763	56009	43424	25879	39772
<b>Structure refinement statistics</b>					
Resolution (Å)	37.26-2.03	37.43-1.90	40.00-2.08	39.97-2.50	40.00-2.15
<i>R</i> (%)	17.5	17.4	17.1	17.4	17.3
<i>R</i> <sub>free</sub> (%)	20.1	20.0	19.2	21.3	20.6
R.m.s. deviations					
Bond lengths (Å)	0.005	0.004	0.003	0.004	0.004
Bond angles (°)	1.337	1.283	1.250	1.326	1.312
Ramachandran plot statistics <sup>b</sup>					
% of residues in favored region	98.7	98.7	99.2	98.0	98.5
% of residues in allowed region	1.3	1.3	0.8	2.0	1.5
% of residues in outlier region	0	0	0	0	0

<sup>a</sup> Numbers in parentheses indicate the values in the highest resolution shell.

<sup>b</sup> Indicates Rampage<sup>12</sup> statistics.

**Inhibition kinetics.** IC<sub>50</sub> values were determined in 96-well plates by using a UV-Vis assay taking measurements every 30 s for 20 min. Compounds **1-35** and DPD were dissolved in Tris-HCl (50 mM, pH 8.0 adjusted at room temperature containing 10% v/v DMSO) (100 μL) and a 5-fold dilution was performed. A mixture (50 μL) of Eis (1 μM), KAN (400 μM), and Tris-HCl (50 mM, pH 8.0 adjusted at room temperature) was added to the compounds **1-35** and DPD solutions and incubated for 10 min, to allow for competitive binding. Reactions were initiated by addition (50 μL) of AcCoA (2 mM), DTNB (2 mM), and Tris-HCl (50 mM, pH 8.0 adjusted at room temperature). Initial rates (first 2-5 min of reaction) were used to determine the IC<sub>50</sub> values. All assays were performed at least in triplicate. Data were fit to a Hill-plot fit using SigmaPlot 14.0 software and IC<sub>50</sub> values were calculated. All IC<sub>50</sub> values are listed in Table 1 and example IC<sub>50</sub> curves are presented in Figs. S88-89.

**Mode of inhibition.** Using the same assay as that used to calculate the IC<sub>50</sub> values determination, the inhibition kinetics were determined by performing Michaelis-Menten kinetics analysis in quadruplicate using various concentrations of inhibitor (0-1 μM) and using Equation 1 with the resulting  $V_{max}$  and  $K_m$  values. Representative Michaelis-Menten curves (Fig. 6A-C) and double reciprocal plots (Fig. 6D-F) are presented in Fig. 6 and  $K_i$  values are presented in Table S2.

Equation 1: 
$$v = \frac{V_{max}[S]}{[S] + K_m \left(1 + \frac{[I]}{K_i}\right)}$$

**Table S2.** Mode of inhibition data.

Cpd #	[I] (μM)	$V_{max}$ (μM/s)	$K_{m,KAN}$ (μM)	$K_{i,KAN}$ (μM)
<b>1</b>	0	1.93 ± 0.07	221 ± 21	0.39 ± 0.02
<b>1</b>	0.25	1.79 ± 0.09	329 ± 40	
<b>1</b>	0.5	1.87 ± 0.11	543 ± 68	
<b>1</b>	1	1.47 ± 0.06	425 ± 39	
<b>10</b>	0	2.22 ± 0.12	211 ± 32	0.91 ± 0.22
<b>10</b>	0.25	2.42 ± 0.15	255 ± 41	
<b>10</b>	0.5	2.53 ± 0.81	298 ± 24	
<b>10</b>	1	1.73 ± 0.24	414 ± 129	0.24 ± 0.02
<b>16</b>	0	2.00 ± 0.12	246 ± 38	
<b>16</b>	0.25	1.89 ± 0.19	341 ± 82	
<b>16</b>	0.5	1.72 ± 0.14	577 ± 92	
<b>16</b>	1	0.51 ± 0.12	257 ± 129	

**Selectivity of inhibitors towards Eis over other AACs.** To determine if the inhibitors were Eis specific or general to AACs, we verified the specificity of five inhibitors, **1**, **2**, **3**, **5**, and **9**, with three regio-specific AACs: AAC(6')-Ie from the bifunctional AAC(6')-Ie/APH(2'')-Ia,<sup>13</sup> AAC(3)-

IV,<sup>13</sup> and AAC(2')-Ic.<sup>14</sup> Similar conditions to those described above were used. AAC(6')-Ie (0.5  $\mu$ M) and AAC(3)-IV (0.125  $\mu$ M) were tested in MES buffer (50 mM, pH 6.6 adjusted at room temperature), and AAC(2')-Ic (0.125  $\mu$ M) was tested in phosphate buffer (50 mM, pH 7.0 adjusted at room temperature) using NEO (100  $\mu$ M) as the substrate. AcCoA (150  $\mu$ M) was used to initiate the reactions. Both AAC(2')-Ic and AAC(3)-IV were incubated at 25 °C, while AAC(6')-Ie was incubated at 37 °C. All compounds were tested in triplicate and found to be inactive at 200  $\mu$ M against all enzymes tested.

#### **Determination of MIC values of KAN against *Mycobacterium tuberculosis* H37Rv and K204.**

The compounds were tested at 100  $\mu$ M. The assays were performed in 96-well dishes as previously reported.<sup>15</sup> Briefly, the *Mtb* strains were cultured in Middlebrook 7H9 supplemented with ADC (10%), Tween® 80 (0.05%), and glycerol (0.4%) at 37 °C until somewhat turbid, then diluted in fresh 7H9 to attenuation at 600 nM of 0.2, diluted again 1:25 in fresh 7H9 in a 50 mL polypropylene tube containing glass beads, vortexed for 30 s, and set to rest for 10 min. Compounds were diluted to 200  $\mu$ M in 7H9 and 100  $\mu$ L of these solutions was added to test wells along with 90  $\mu$ L of prepared bacteria cultures. Plates were incubated at 37 °C in a humid environment for 24 h. KAN was diluted to 20x the desired final concentration in water and then 10  $\mu$ L of this solution was added to test wells. H37Rv was tested at 2.5 and 1.25  $\mu$ g/mL KAN, and K204 was tested at 10, 5, 2.5, and 1.25  $\mu$ g/mL KAN. The plates were incubated for 6 days at 37 °C. To analyze growth inhibition, AlamarBlue® was diluted 1:1 in 10% Tween® 80 and 40  $\mu$ L of this solution was added to each test well. The plates were then incubated at 37 °C, and the color of the wells was observed at 24 h and 48 h. AlamarBlue® color changes from indigo blue to pink in the presence of bacterial growth. The MIC<sub>KAN</sub> was defined as the lowest concentration of KAN that resulted in no change in color. Compounds were tested at least twice in duplicate. The controls for this study were: uninoculated 7H9, inoculated 7H9 only, inoculated 7H9 + DMSO only, and compound and inoculated 7H9 only. To prevent drying out of the plates, 200  $\mu$ L of sterile water was added to perimeter wells.

**Generation of the *Mycobacterium tuberculosis* mc<sup>2</sup>6230-K204 strain.** Construction of the homologous recombination plasmid. The clinically observed C-14T mutation of the *eis* promoter

was generated using a standard overlapping extension PCR. Primers 5'-GTGGGGGATCCCCGCTTGCGGGGA-3' (forward) and 5'-GATCTTAAGCTTGAACCGGCCCATC-3' (reverse) were used to amplify the 2-kbp region of interest of the *Mycobacterium tuberculosis* (*Mtb*) mc<sup>2</sup>6230 genome. The following primers were used to generate the desired mutation: 5'-CCGCGGCATATGCTACAGTCGGATTCT-3' and 5'-AGAATCCGACTGTAGCATATGCCGCGG-3'. The mutated segment of the genomic DNA was cloned between the *Bam*HI and *Hind*III sites of the p2NIL plasmid (Addgene). The generated plasmid was then digested with *Pac*I and ligated with pGOAL19 cut with the same endonuclease. The ligation reaction was transformed into chemically competent *E. coli* TOP10 cells. Colonies were streaked and re-streaked 4 times on Luria-Bertani agar plates containing KAN (50 µg/mL) and X-Gal (50 µg/mL) until only blue colonies were observed. The DNA was isolated and used to generate the *Mtb* mc<sup>2</sup>6230-K204 strain, as follows.

*Mtb* mc<sup>2</sup>6230 electrocompetent cells<sup>16</sup> (200 µL) were placed in an electroporation cuvette with 1 µg of the isolated recombination plasmid. A single pulse at 2.5 kV, 25 µF and 1000 Ω (room temperature) was used on the mixture.<sup>16</sup> Cells were chilled on ice before and after electroshock. The cells were rested for 1 min prior to being transferred to 5 mL of the standard growth medium: 7H9 with 10% OADC, 0.5% glycerol, 0.05% tyloxapol, 0.2% casamino acids, and 24 µg/mL pantothenate. After overnight incubation at 37 °C (~20 h), the cells were collected and resuspended in 1 mL of medium. The resuspended cells were plated on 7H10 agar supplemented with 10% OADC, 0.5% glycerol, 0.2% casamino acids, 24 µg/mL pantothenate, 20 µg/mL KAN, 100 µg/mL hygromycin, and 50 µg/mL X-Gal and grown at 37 °C. Any blue colonies that grew were streaked on non-selective plates (37 °C). The colonies were grown in 5 mL of liquid medium at 37 °C. The genomic DNA was isolated and used as the template in PCR amplifying the fragment of interest using the forward and reverse primers from the generation of the homologous recombination plasmid. Sequencing was done on the PCR products with primers 5'-CCGGCGTGTGAGCCG-3' and 5'-CCGGTTCGGCTACGG-3' to confirm the sequence.

**Determination of MIC values of KAN against *Mtb* mc<sup>2</sup>6230 and mc<sup>2</sup>6230-K204.** MIC determination was performed using the standard microdilution method in a 96-well plate. KAN was two-fold serially diluted in the presence or absence of Eis inhibitors in the standard growth



medium: 7H9 with 10% OADC, 0.5% glycerol, 0.05% tyloxapol, 0.2% casamino acids, and 24 µg/mL pantothenate, in a 100 µL mix. *Mtb* mc<sup>2</sup>6230 and mc<sup>2</sup>6230-K204 bacteria were grown at 37 °C to a density of 0.5, measured by a densitometer and compared to the McFarland standard, in the standard growth medium. A 1:100 dilution (100 µL) of the bacterial culture was added to the KAN (10-1.25 µg/mL)/compound (100 µM) mixture. The treated cultures were incubated for one week at 37 °C before staining with 5 µL of 2.5 mg/mL resazurin. The staining was complete in two days. The assay was performed in duplicate. The MIC<sub>KAN</sub> values are given in Table 1.

#### **Determination of MIC values of compounds 1-35 against various bacterial strains.**

Antibacterial activity was determined using the standard double-dilution method. All non-mycobacterial strains (*H-R*) and *Mycobacterium smegmatis* mc<sup>2</sup>155 (strain *A*) were grown in Mueller-Hinton broth. *Mycobacterium abscessus* ATCC 19977 (strain *B*) was grown in 7H9 broth supplemented with Tween80® (0.05%) and glycerol (0.4%). *M. tuberculosis* mc<sup>2</sup>6230 (strain *G*) was grown in 7H9 broth supplemented with tyloxapol (0.05%), glycerol (0.5%), OADC (10%), casaminoacids (0.2%), and pantothenate (24 µg/mL). All other mycobacteria (strains *C-F*) were grown in 7H9 broth supplemented with Tween80® (0.05%), glycerol (0.4%), and ADC (10%). For non-mycobacteria, cultures were grown to an attenuation of ~0.4 (OD<sub>600nm</sub>) and diluted 1:1000 and added to a 96-well plate. MIC were determined after overnight growth (~10-16 h) at 37 °C and determined by visual inspection or by the addition of 50 µL of 1 mg/mL MTT). The mycobacterial strains were grown on agar plates corresponding to the broth (*e.g.*, *M. smegmatis* on Mueller-Hinton agar, *M. abscessus* on 7H9 agar supplemented with Tween80® (0.05%) and glycerol (0.4%), and all other mycobacteria on 7H10 supplemented with Tween80® (0.05%), glycerol (0.4%), and OADC (10%)). Cells were transferred from the plates to the corresponding liquid medium until the attenuation was equal to that of a 0.5 McFarland standard. The culture was then diluted 1:100 and added to the compounds dissolved in the appropriate media. Plates were incubated at 37 °C until growth was observed in the wells containing no compound (3 days to 3 weeks, strain dependent). To positively assess the growth inhibition 5 µL of a 2.5 mg/mL solution of resazurin was added to each well and incubated overnight at 37 °C. These MIC data against mycobacteria and non-mycobacteria were determined in duplicate and are presented in Tables 2 and S3.

**Table S3.** MIC values ( $\mu\text{M}$ ) of haloperidol (**1**) and its derivatives **2-35** and DPD against non-mycobacteria.

Cpd #	Gram-positive				Gram-negative						
	<i>H</i>	<i>I</i>	<i>J</i>	<i>K</i>	<i>L</i>	<i>M</i>	<i>N</i>	<i>O</i>	<i>P</i>	<i>Q</i>	<i>R</i>
<b>7</b>	> 62.5	62.5	62.5	62.5	NT	31.3	62.5	62.5	> 62.5	> 62.5	> 62.5
<b>8</b>	> 62.5	31.3	31.3	62.5	NT	15.6	15.6	> 62.5	> 62.5	> 62.5	> 62.5
<b>9</b>	15.6	31.3	> 62.5	> 62.5	31.3	3.9	> 62.5	> 62.5	31.3	> 62.5	> 62.5
<b>15</b>	> 62.5	> 62.5	62.5	62.5	> 62.5	31.3	> 62.5	> 62.5	62.5	> 62.5	> 62.5
<b>21</b>	31.3	> 62.5	31.3	> 62.5	> 62.5	3.9	> 62.5	> 62.5	> 62.5	> 62.5	> 62.5
AMK	0.5	7.8	31.3	31.3	1	0.5	0.5	7.8	2	7.8	2

*H* = *S. epidermidis* ATCC 12228, *I* = *S. aureus* ATCC 25923, *J* = *B. anthracis* 34F2 Sterne, *K* = VRE, *L* = *L. monocytogenes* ATCC 19115, *M* = *E. coli* MC1061, *N* = *E. cloacae* ATCC 13047, *O* = *A. baumannii* ATCC 19606, *P* = *K. pneumoniae* ATCC 27736, *Q* = *S. enterica* ATCC 14028, *R* = *P. aeruginosa* ATCC 27853.

Note: NT = not tested.

### Combination studies of haloperidol derivatives and anti-TB drugs by checkerboard assays.

To assess the synergistic effect of our compounds, we employed the standard checkerboard assay as previously described<sup>17, 18</sup> with slight variations. A panel of 19 antibiotics including traditional anti-TB drugs (isoniazid, INH; pyrazinamide, PZA; ethambutol, EMB; and rifampin, RIF), aminoglycosides (amikacin, AMK; kanamycin, KAN; streptomycin, STR; and spectinomycin, SPC), azoles (fluconazole, FLC; itraconazole, ITC; ketoconazole, KTC; posaconazole, POS; and voriconazole, VRC), macrolides (azithromycin, AZM and clarithromycin, CLR), and others (*p*-aminosalicylate; clofazimine, CLO; novobiocin; and tetracycline, TET) was tested with haloperidol at 1/8<sup>th</sup> its MIC concentration for *M. smegmatis* mc<sup>2</sup>155 (strain *A*, Table S4). Any antibiotic that showed a 4-fold reduction in the observed MIC value was then tested in combination with an additional nine haloperidol derivatives (**1**, **2**, **9**, **10**, **11**, **15-17**, and **21**) (Table S5). The commercially available antibiotics were serially diluted (two-fold dilutions) in 96-well plates by column, while the haloperidol derivatives **1**, **2**, **9-11**, **15-17**, and **21**, were diluted (two-fold dilutions) by rows. The bacteria were prepared as described above for MIC value determination. The observed results for the two compounds in combo were then used to calculate the fractional inhibitory concentration index (FICI) using Equation 2. The combinational effect of the two tested compounds were considered synergistic (abbreviated SYN) if  $FICI \leq 0.5$ , additive (abbreviated ADD) if  $0.5 < FICI \leq 4$ , and antagonistic if  $FICI > 4$  (Tables S5-S10). The best three compounds (**2**, **11**, and **17**) and the most corresponding synergistic antibiotics, CLR, CLO, and SPT were then tested against other mycobacteria including: *M. abscessus* ATCC 19977 (strain *B*, Table S6), *M. intracellulare* ATCC 13950 (strain *C*, Table S7), *M. avium* ATCC 25921 (strain *D*, Table S8), *M. bovis* BCG ATCC 35734 (strain *E*, Table S9), *M. tuberculosis* H37Ra ATCC NRS22 (strain *F*, Table S10, SPT only). All combination tests were completed in at least duplicate independent trials.

$$\text{Equation 2: } \text{FIC} = \frac{\text{MIC of antibiotic}_{\text{combo}}}{\text{MIC of antibiotic}_{\text{alone}}} + \frac{\text{MIC of our compound}_{\text{combo}}}{\text{MIC of our compound}_{\text{alone}}}$$

**Table S4.** Initial combination test of *M. smegmatis* mc<sup>2</sup>155 (strain *A*) with antibiotics and haloperidol (**1**) at 8 µg/mL.

Antibiotic class	Antibiotic	MIC alone (µg/mL)	MIC combo (µg/mL)
Anti-TB drugs	INH	8	32
	EMB	0.5	2
	PZA	> 128	128
	RIF	16	32
Aminoglycosides	AMK	< 0.25	1
	KAN	0.5	2
	SPT	64	4
	STR	0.5	2
Macrolides	AZM	8	4
	CLR	1	0.25
Azoles	FLC	> 128	> 128
	ITC	> 128	> 128
	KTC	32	32
	POS	> 128	> 128
	VRC	> 128	32
Others	<i>p</i> -aminosalicylate	> 128	> 128
	CLO	0.25	0.25
	Novobiocin	8	32
	TET	0.5	0.125

**Table S5.** Combinatorial results of treating *M. smegmatis* mc<sup>2</sup>155 (strain *A*) with haloperidol derivatives with 3 anti-TB agents.

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone		MIC combo		FICI	Interp.
					(µg/mL)	Antibiotic	(µg/mL)	Cpd		
CLR	1 (HPD)	F	OH	<i>p</i> -Cl-Ph	0.25	64	0.125	2	0.53	ADD
	2	Cl	OH	<i>p</i> -Cl-Ph	0.25	32	0.063	8	0.5	SYN
	9	<i>t</i> -Bu	OH	<i>p</i> -Cl-Ph	0.25	8	0.125	0.25	0.53	ADD
	10	F	OH	<i>p</i> -F-Ph	0.25	> 64	0.124	4	0.53	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.25	> 64	0.063	32	0.5	SYN
	15	<i>t</i> -Bu	OH	<i>p</i> -F-Ph	0.25	16	0.125	16	0.53	ADD
	16 (BPD)	F	OH	<i>p</i> -Br-Ph	0.25	> 64	0.063	8	0.38	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	0.25	64	0.063	8	0.38	SYN
	21	<i>t</i> -Bu	OH	<i>p</i> -Br-Ph	0.25	8	0.125	0.25	0.53	ADD
CLO	1 (HPD)	F	OH	<i>p</i> -Cl-Ph	0.5	64	0.063	16	0.38	SYN
	2	Cl	OH	<i>p</i> -Cl-Ph	0.5	64	0.063	8	0.25	SYN
	9	<i>t</i> -Bu	OH	<i>p</i> -Cl-Ph	0.5	8	0.063	1	0.25	SYN
	10	F	OH	<i>p</i> -F-Ph	0.5	> 64	0.125	32	0.5	SYN
	11	Cl	OH	<i>p</i> -F-Ph	0.5	> 64	0.031	32	0.31	SYN
	15	<i>t</i> -Bu	OH	<i>p</i> -F-Ph	0.5	16	0.063	4	0.38	SYN
	16 (BPD)	F	OH	<i>p</i> -Br-Ph	0.5	> 64	0.063	32	0.38	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	0.5	32	0.063	8	0.38	SYN
	21	<i>t</i> -Bu	OH	<i>p</i> -Br-Ph	0.5	8	0.063	8	0.25	SYN
SPT	1 (HPD)	F	OH	<i>p</i> -Cl-Ph	32	> 64	2	2	0.08	SYN
	2	Cl	OH	<i>p</i> -Cl-Ph	32	64	4	2	0.16	SYN
	9	<i>t</i> -Bu	OH	<i>p</i> -Cl-Ph	32	8	4	1	0.25	SYN
	10	F	OH	<i>p</i> -F-Ph	32	> 64	2	8	0.13	SYN
	11	Cl	OH	<i>p</i> -F-Ph	32	64	2	2	0.09	SYN
	15	<i>t</i> -Bu	OH	<i>p</i> -F-Ph	32	32	4	8	0.38	SYN
	16 (BPD)	F	OH	<i>p</i> -Br-Ph	32	> 64	4	2	0.14	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	32	32	4	1	0.16	SYN
	21	<i>t</i> -Bu	OH	<i>p</i> -Br-Ph	32	8	4	1	0.25	SYN

**Abbreviations:** CLR = clarithromycin; CLO = clofazimine; SPT = spectinomycin; BPD = bromperidol; HPD = haloperidol.

**Table S6.** Combinatorial results of treating *M. abscessus* ATCC 19977 (strain B) with haloperidol derivatives with three anti-TB agents.

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone (µg/mL)		MIC combo (µg/mL)		FICI	Interp.
					Antibiotic	Cpd	Antibiotic	Cpd		
CLR	2	Cl	OH	<i>p</i> -Cl-Ph	0.039	> 64	0.020	4	0.54	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.039	32	0.020	8	0.76	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	NT	NT	NT	NT	NT	NT
CLO	2	Cl	OH	<i>p</i> -Cl-Ph	0.125	> 64	0.032	16	0.38	SYN
	11	Cl	OH	<i>p</i> -F-Ph	0.125	64	0.032	4	0.38	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	0.125	64	0.016	16	0.38	SYN
SPT	2	Cl	OH	<i>p</i> -Cl-Ph	4	> 64	2	8	0.56	ADD
	11	Cl	OH	<i>p</i> -F-Ph	4	64	0.5	16	0.38	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	4	> 64	1	16	0.38	SYN

**Abbreviations:** CLR = clarithromycin; CLO = clofazimine; SPT = spectinomycin.  
**Note:** NT = not tested.

**Table S7.** Combinatorial results of treating *M. intracellulare* ATCC 13950 (strain C) with haloperidol derivative with three anti-TB agents.

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone (µg/mL)		MIC combo (µg/mL)		FICI	Interp.
					Antibiotic	Cpd	Antibiotic	Cpd		
CLR	2	Cl	OH	<i>p</i> -Cl-Ph	0.03	64	0.03	64	2	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.03	64	0.03	64	2	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	0.03	> 64	0.03	>64	2	ADD
CLO	2	Cl	OH	<i>p</i> -Cl-Ph	0.125	8	0.125	8	2	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.125	32	0.063	8	0.75	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	0.125	8	0.063	4	1	ADD
SPT	2	Cl	OH	<i>p</i> -Cl-Ph	2	64	2	64	2	ADD
	11	Cl	OH	<i>p</i> -F-Ph	2	64	2	64	2	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	2	> 64	1	>64	1.5	ADD

**Abbreviations:** CLR = clarithromycin; CLO = clofazimine; SPT = spectinomycin.

**Table S8.** Combinatorial results of treating *M. avium* ATCC 25921 (strain D) with haloperidol derivatives with three anti-TB agents.

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone (µg/mL)		MIC combo (µg/mL)		FICI	Interp.
					Antibiotic	Cpd	Antibiotic	Cpd		
CLR	2	Cl	OH	<i>p</i> -Cl-Ph	0.03	8	0.015	4	1	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.03	64	0.002	32	1	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	0.03	16	0.03	16	1	ADD
CLO	2	Cl	OH	<i>p</i> -Cl-Ph	1	> 64	0.25	> 64	1.25	ADD
	11	Cl	OH	<i>p</i> -F-Ph	1	64	0.25	32	0.75	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	1	> 64	0.5	8	0.625	ADD
SPT	2	Cl	OH	<i>p</i> -Cl-Ph	4	8	2	4	1	ADD
	11	Cl	OH	<i>p</i> -F-Ph	4	64	1	32	0.75	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	4	16	2	16	1.5	ADD

**Abbreviations:** CLR = clarithromycin; CLO = clofazimine; SPT = spectinomycin.

**Table S9.** Combinatorial results of treating *M. bovis* BCG ATCC 35734 (strain E) with haloperidol derivatives with three anti-TB agents.

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone (µg/mL)		MIC combo (µg/mL)		FICI	Interp.
					Antibiotic	Cpd	Antibiotic	Cpd		
CLR	2	Cl	OH	<i>p</i> -Cl-Ph	0.008	8	0.008	8	2	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.008	32	0.004	16	1	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	0.008	32	0.004	4	0.625	ADD
CLO	2	Cl	OH	<i>p</i> -Cl-Ph	0.008	8	0.008	32	2	ADD
	11	Cl	OH	<i>p</i> -F-Ph	0.008	16	0.008	16	2	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	0.008	16	0.005	8	1	ADD
SPT	2	Cl	OH	<i>p</i> -Cl-Ph	4	16	0.25	2	0.15	SYN
	11	Cl	OH	<i>p</i> -F-Ph	4	> 64	0.5	32	0.25	SYN
	17	Cl	OH	<i>p</i> -Br-Ph	2	> 16	0.125	2	0.13	SYN

**Abbreviations:** CLR = clarithromycin; CLO = clofazimine; SPT = spectinomycin.

**Table S10.** Combinatorial results of treating *M. tuberculosis* H37Ra ATCC NRS22 (strain *F*) with haloperidol derivatives with spectinomycin (SPT).

Antibiotic	Cpd #	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MIC alone	MIC combo		FICI	Interp.	
					( $\mu\text{g/mL}$ ) Antibiotic	Cpd	( $\mu\text{g/mL}$ ) Antibiotic			Cpd
SPT	2	Cl	OH	<i>p</i> -Cl-Ph	4	16	0.25	8	0.56	ADD
	11	Cl	OH	<i>p</i> -F-Ph	4	64	1	32	0.75	ADD
	17	Cl	OH	<i>p</i> -Br-Ph	4	32	0.125	8	0.56	ADD

**Measurements of cytotoxicity to mammalian cells.** Cells from three cell lines, human lung carcinoma (A549), human embryonic kidney (HEK-293), and mouse macrophage (J774A.1), were used in the cytotoxicity assays. The HEK-293 cell line was purchased from the ATCC, the BEAS-2B cell line was obtained from Prof. David K. Orren (University of Kentucky, Lexington, KY), and the J774A.1 cell line was obtained from Dr. David J. Feola (University of Kentucky, Lexington, KY). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; catalog # VWRL0100, VWR, Chicago, IL) supplemented with 10% fetal bovine serum (FBS; from ATCC) and 1% penicillin/streptomycin (from ATCC) at 37 °C with 5% CO<sub>2</sub>.

A resazurin cell viability assay was used to determine the toxicity of the compounds. The cells were counted using a hemocytometer and plated on 96-well plates. HEK-293 and J774A.1 cells were plated at  $1 \times 10^4$  cells/mL, while A549 cells were plated at  $5 \times 10^3$  cells/mL. After incubation for 24 h, the cells were treated with a compound (**1**, **2**, **3**, **5**, **9**, or **21**). 1% Triton X was used as a positive control. The compounds were tested at concentrations ranging from 0.78 to 200  $\mu\text{M}$ , with final concentration of DMSO at 0.5%. Resazurin dye was applied 24 h later and results read after 6 h of incubation. In instances where > 100% cell survival was observed, we displayed the data as 100% cell survival in Fig. 7. All assays were done in quadruplicate.

**Microsomal stability assays.** Compounds were incubated at final concentrations 2-4  $\mu\text{M}$  with 0.5 mg/mL of human or mouse liver microsomes (Xenotech) in 100 mM phosphate buffer at pH 7.4, 3 mM MgCl<sub>2</sub>, 2 mM NADPH at 37 °C with shaking. As internal standards, we used diclofenac, imipramine, and labetalol. Acetonitrile was added to the mixture at time 0 and after 30 min to quench the reaction. The samples were analyzed using LC-MS/MS (Agilent-SciEx). Multiquant was used to scale the measurements to the internal standards. The metabolized fraction of a compound was calculated from the ratio of the areas under the curve for the sample quenched at 30 min compared to that quenched at time 0. These assays were performed in two replicates.

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Figures S1-S89

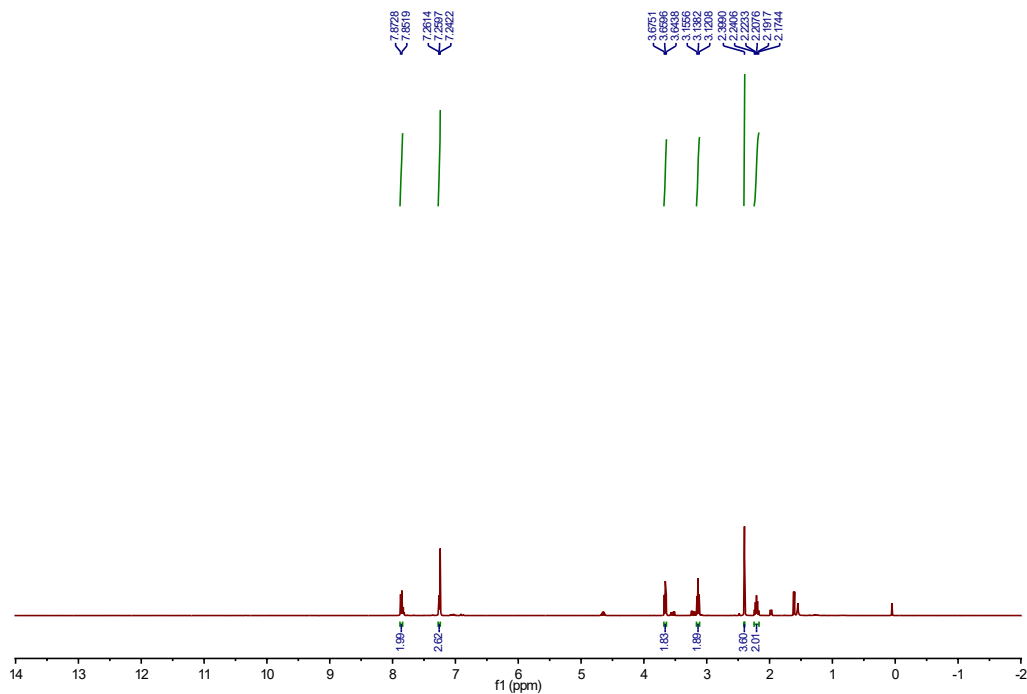


Fig. S1:  $^1\text{H}$  NMR spectrum for compound **A** (SGT1433) in  $\text{CDCl}_3$ .

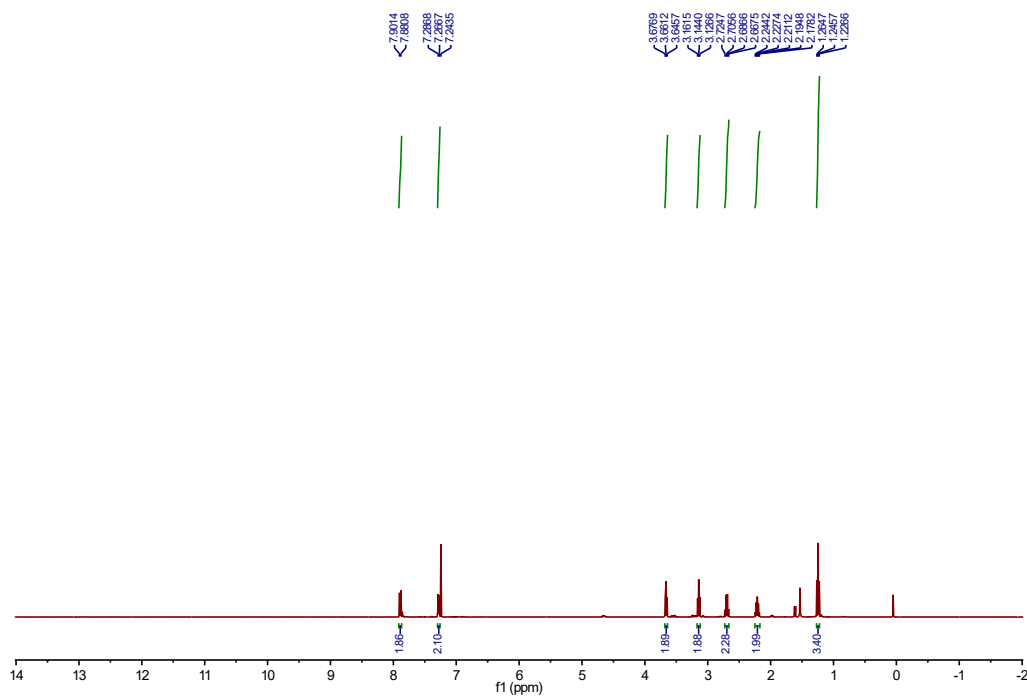


Fig. S2:  $^1\text{H}$  NMR spectrum for compound **B** (SGT1432) in  $\text{CDCl}_3$ .



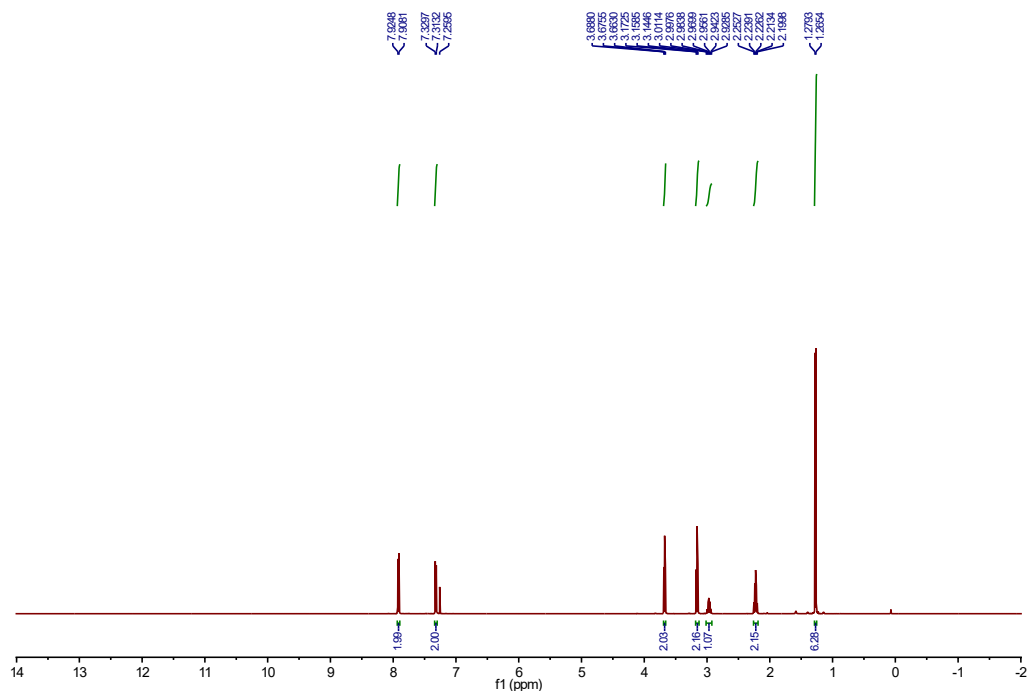


Fig. S3:  $^1\text{H}$  NMR spectrum for compound **C** (SGT1431) in  $\text{CDCl}_3$ .

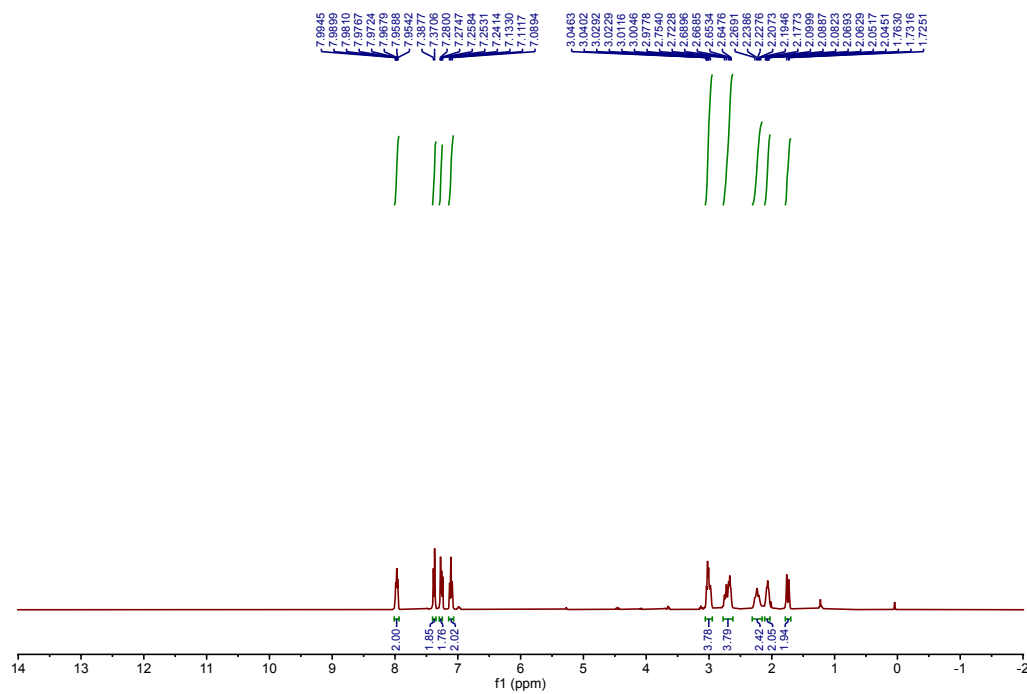
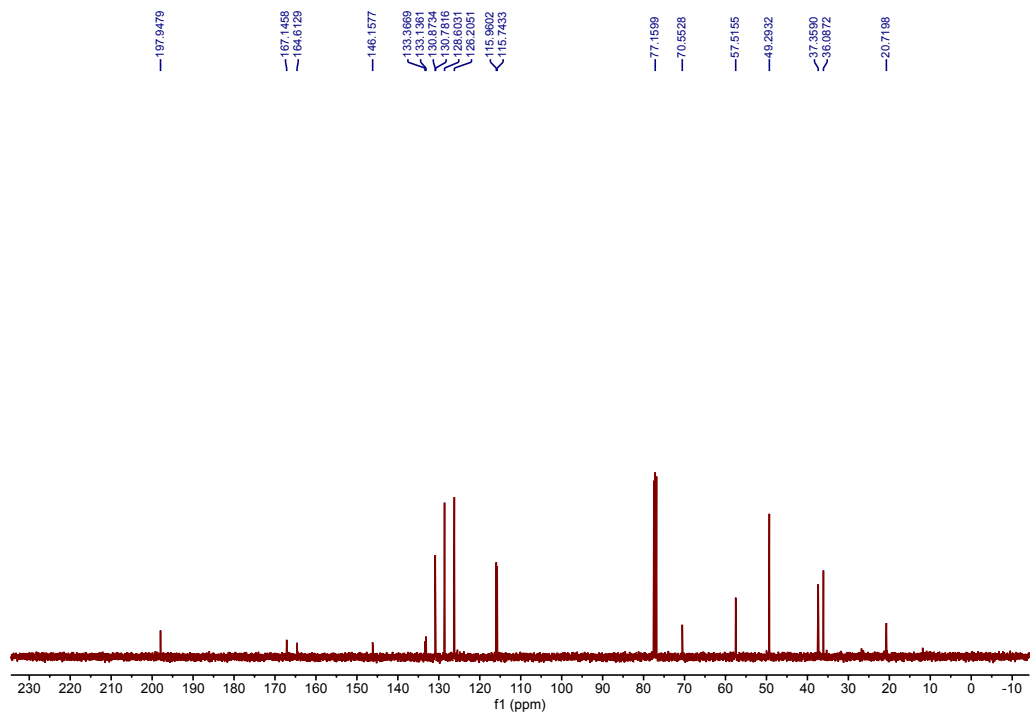
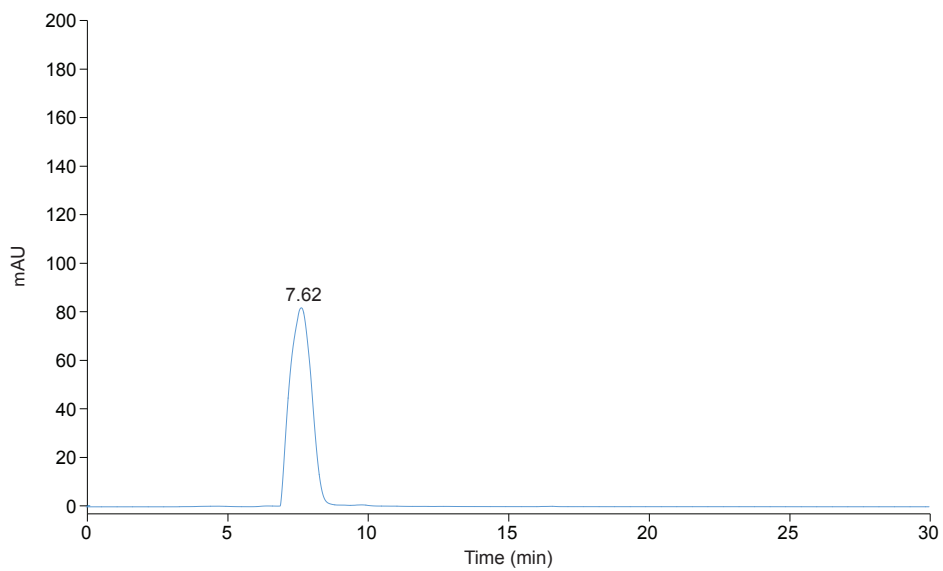


Fig. S4:  $^1\text{H}$  NMR spectrum for compound **1** (SGT537) in  $\text{CDCl}_3$ .



**Fig. S5:**  $^{13}\text{C}$  NMR spectrum for compound **1** (SGT537) in  $\text{CDCl}_3$ .



**Fig. S6:** HPLC trace for compound **1** (SGT537).  $R_t = 7.62$  min.

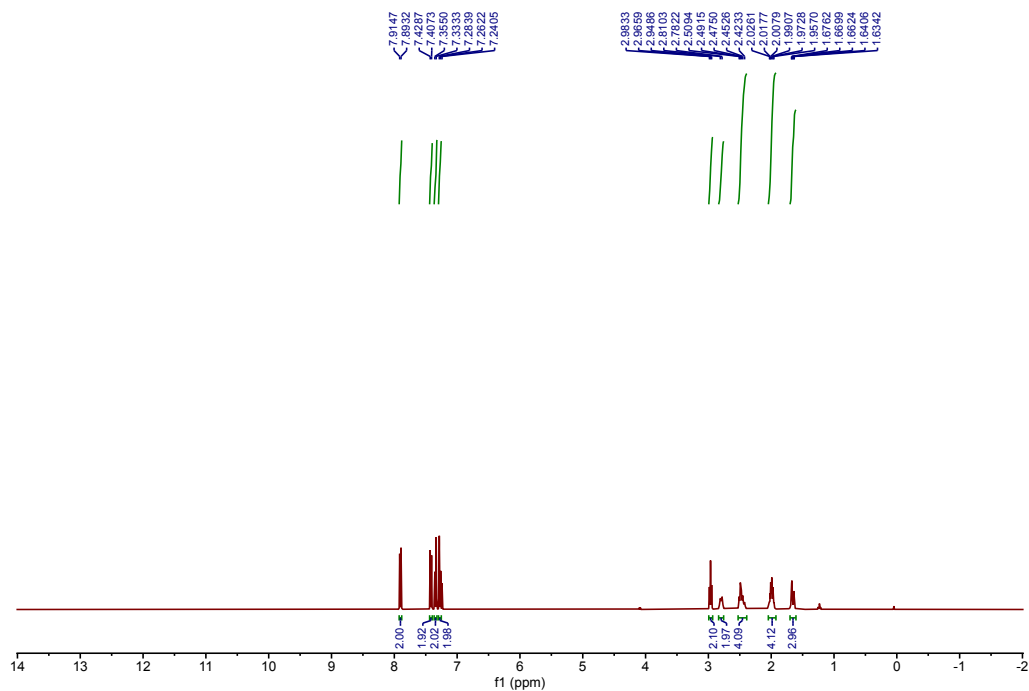


Fig. S7:  $^1\text{H}$  NMR spectrum for compound **2** (SGT538) in  $\text{CDCl}_3$ .

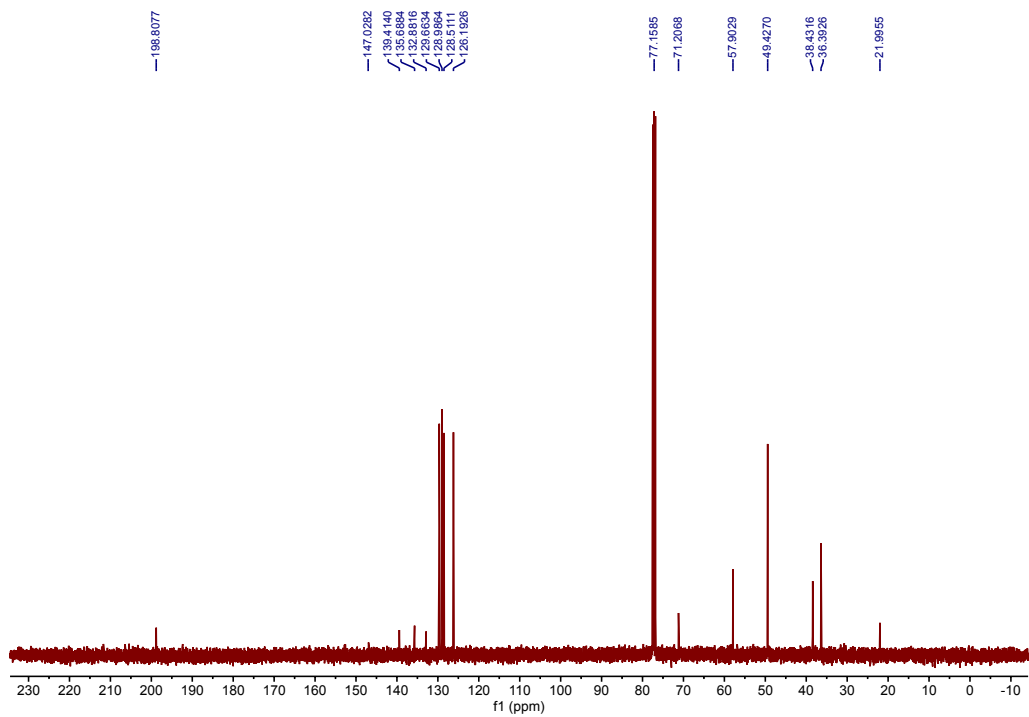
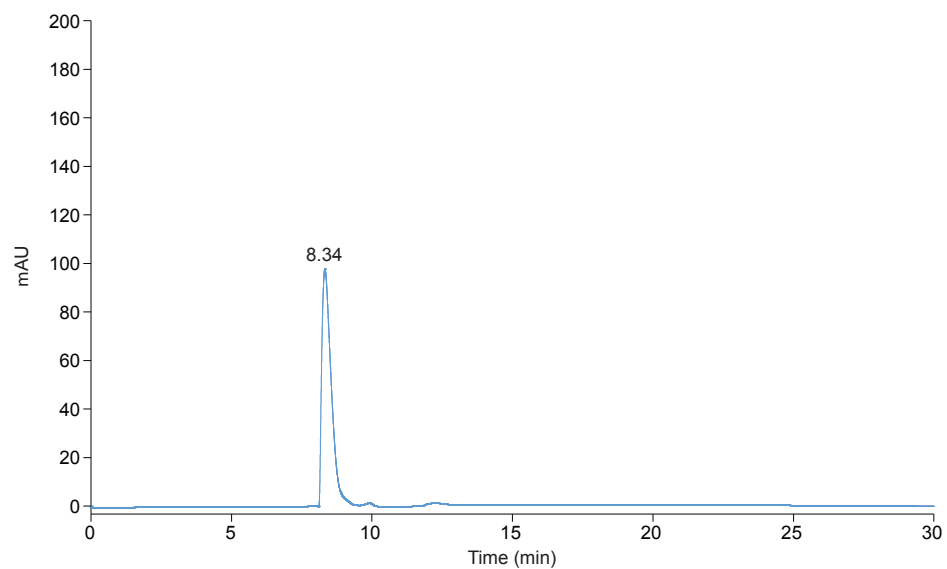
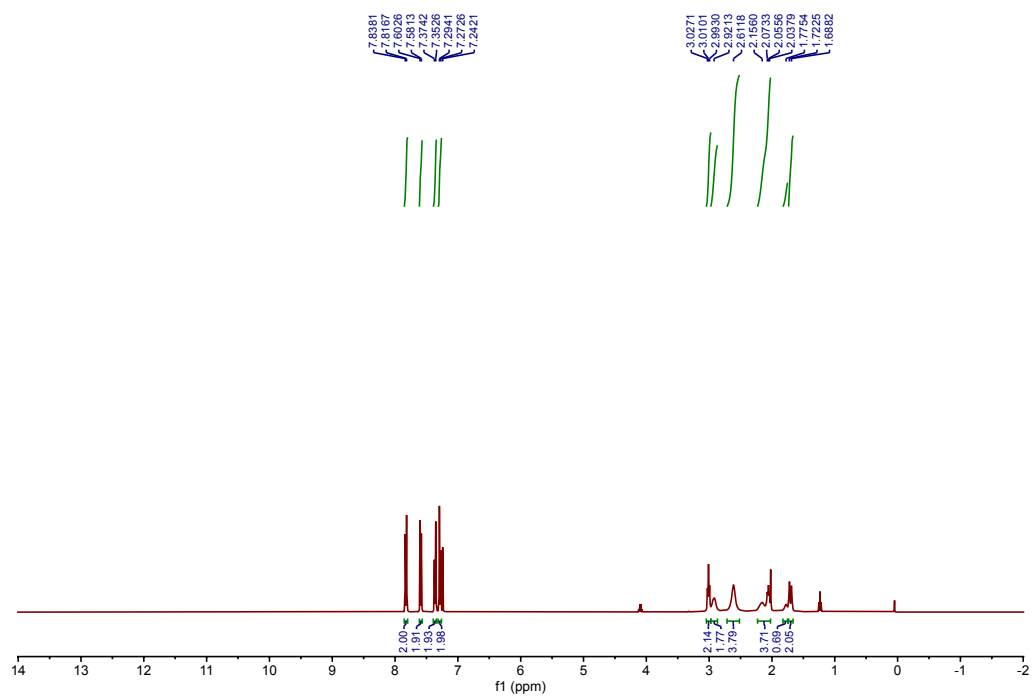


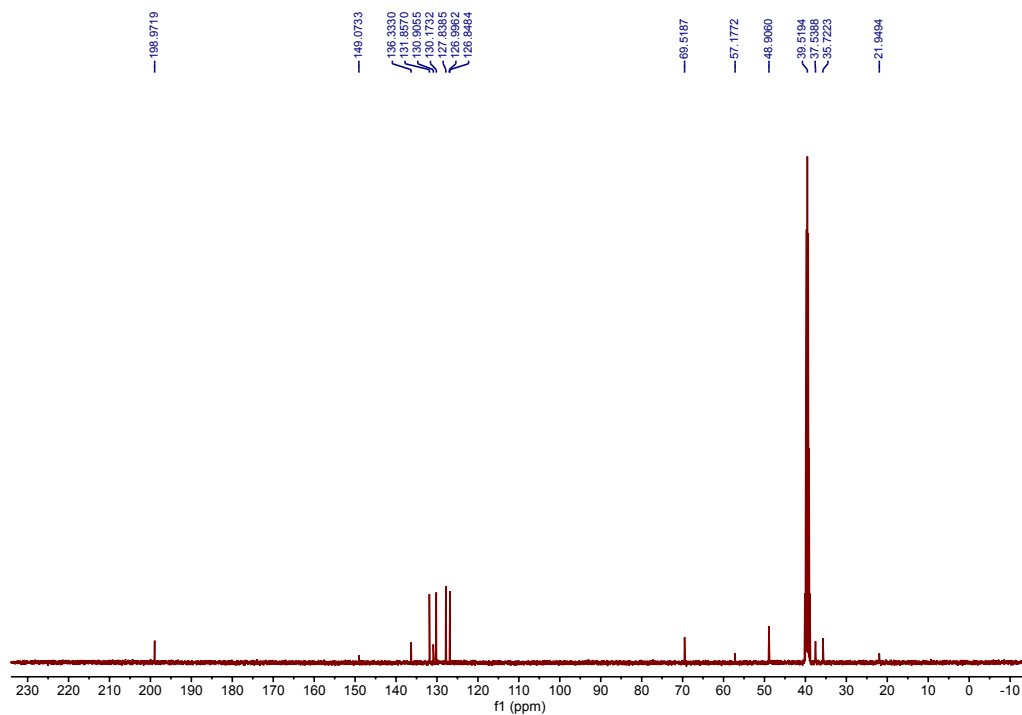
Fig. S8:  $^{13}\text{C}$  NMR spectrum for compound **2** (SGT538) in  $\text{CDCl}_3$ .



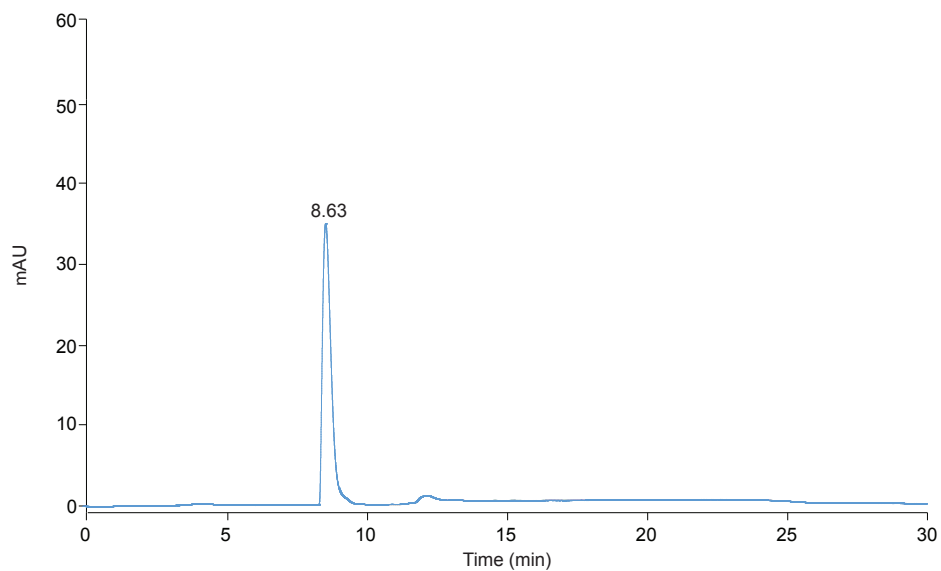
**Fig. S9:** HPLC trace for compound **2** (SGT538).  $R_t = 8.34$  min.



**Fig. S10:**  $^1\text{H}$  NMR spectrum for compound **3** (SGT539) in  $\text{CDCl}_3$ .



**Fig. S11:**  $^{13}\text{C}$  NMR spectrum for compound **3** (SGT539) in  $(\text{CD}_3)_2\text{SO}$ .



**Fig. S12:** HPLC trace for compound **3** (SGT539).  $R_t = 8.63$  min.

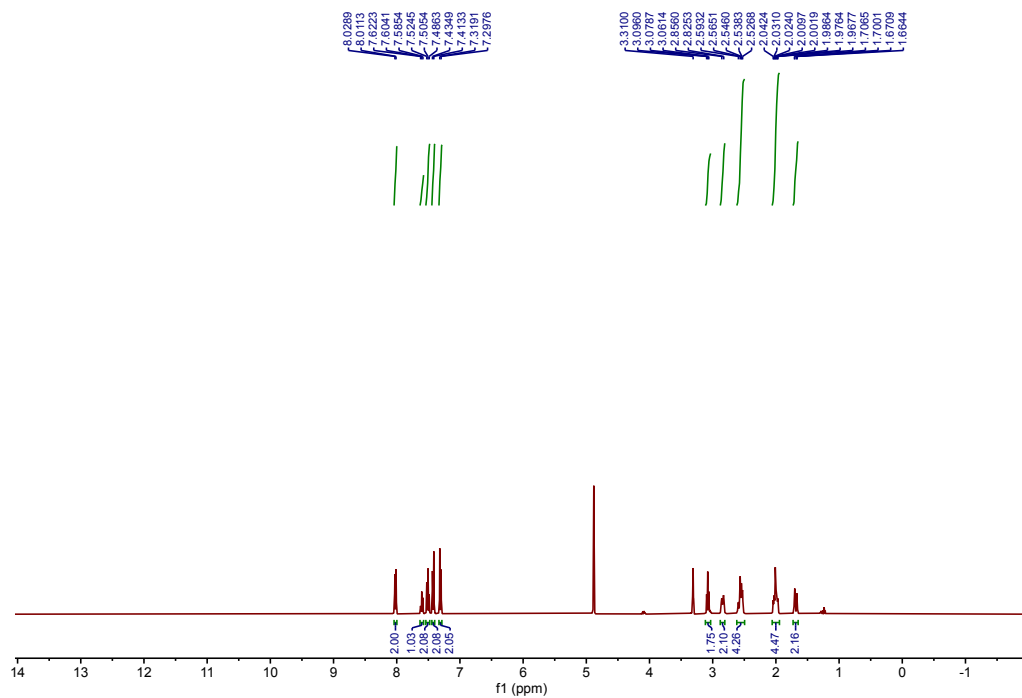


Fig. S13:  $^1\text{H}$  NMR spectrum for compound **4** (SGT536) in  $\text{CD}_3\text{OD}$ .

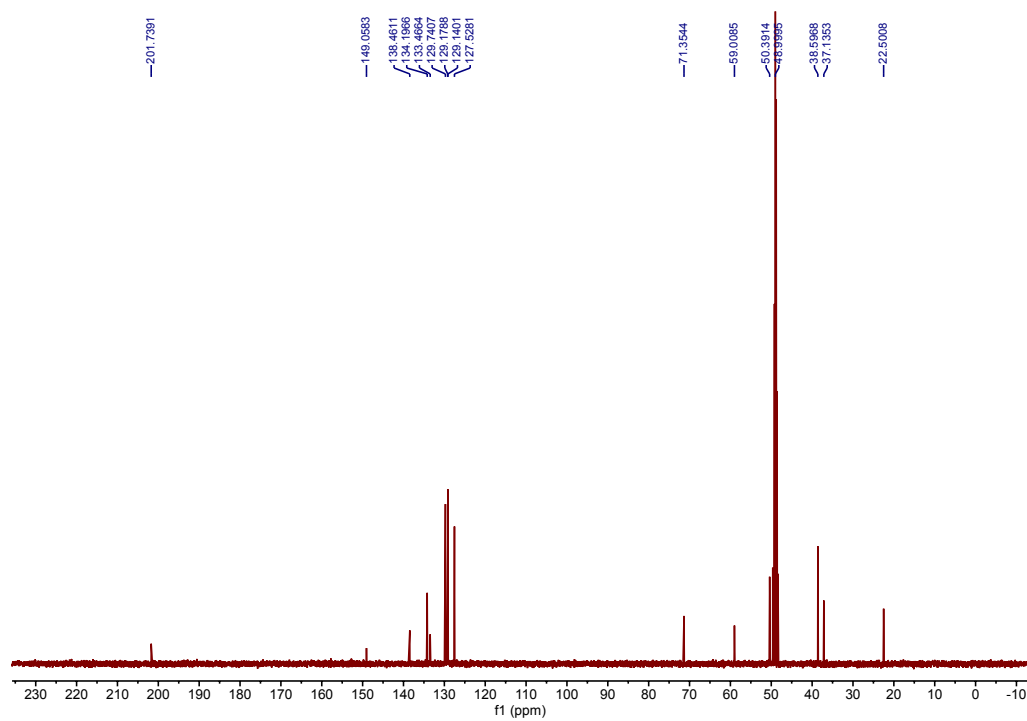
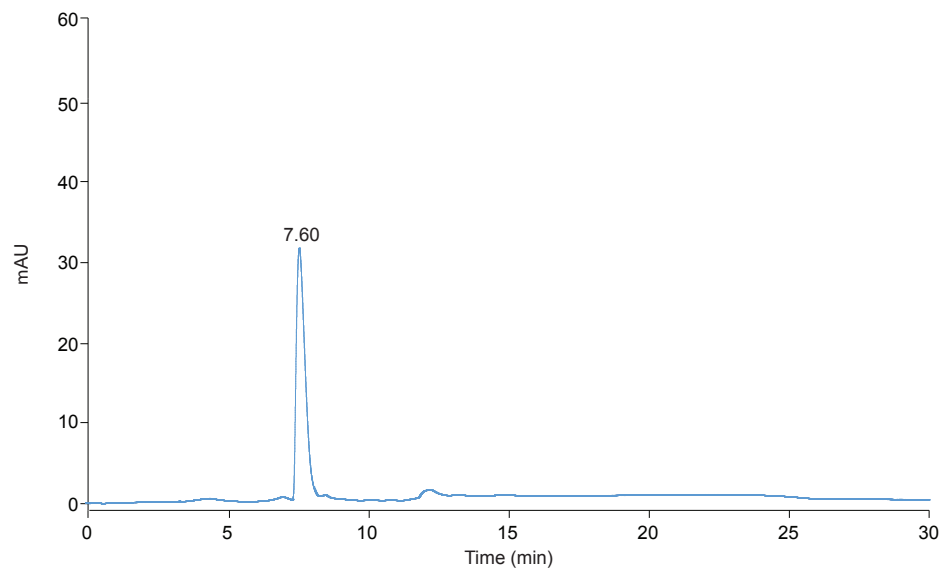
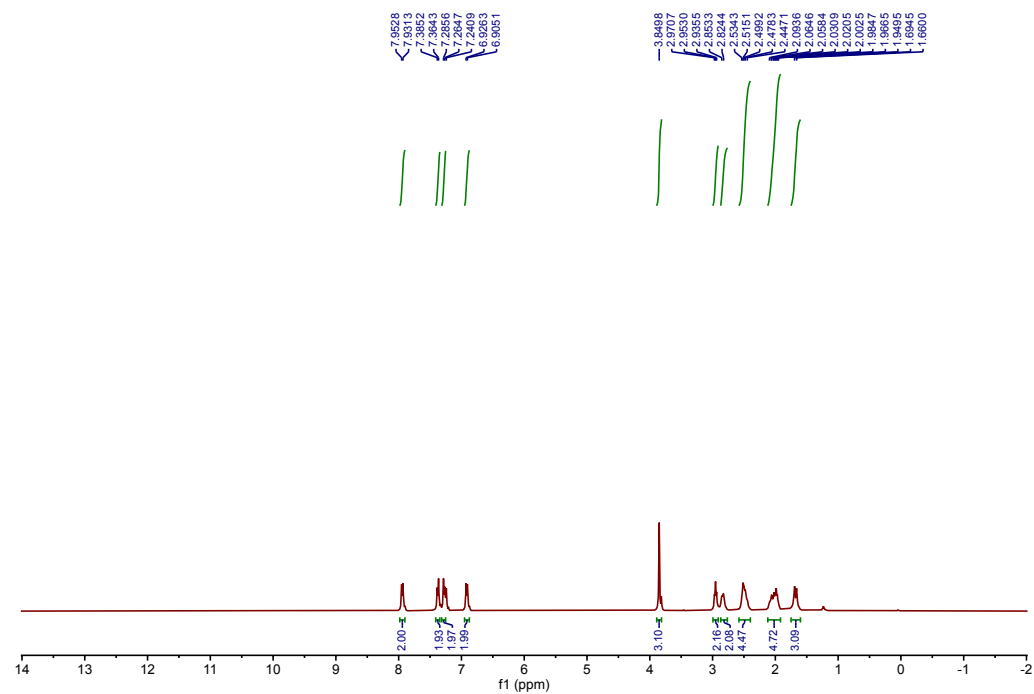


Fig. S14:  $^{13}\text{C}$  NMR spectrum for compound **4** (SGT536) in  $\text{CD}_3\text{OD}$ .



**Fig. S15:** HPLC trace for compound **4** (SGT536).  $R_t = 7.60$  min.



**Fig. S16:** <sup>1</sup>H NMR spectrum for compound **5** (SGT535) in CDCl<sub>3</sub>.

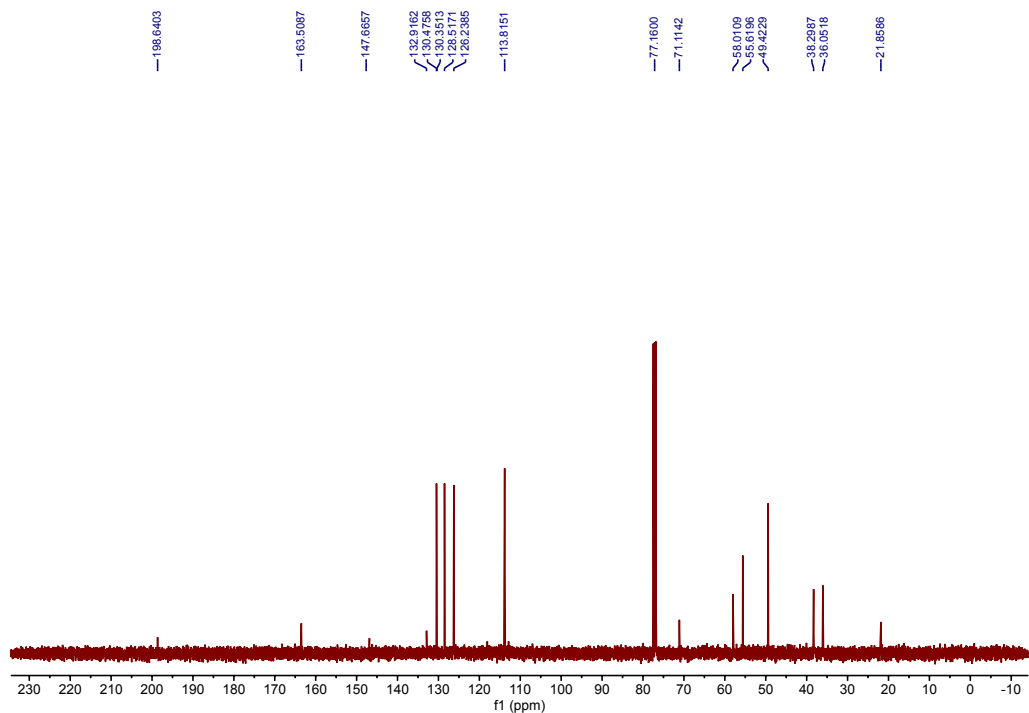


Fig. S17:  $^{13}\text{C}$  NMR spectrum for compound **5** (SGT535) in  $\text{CDCl}_3$ .

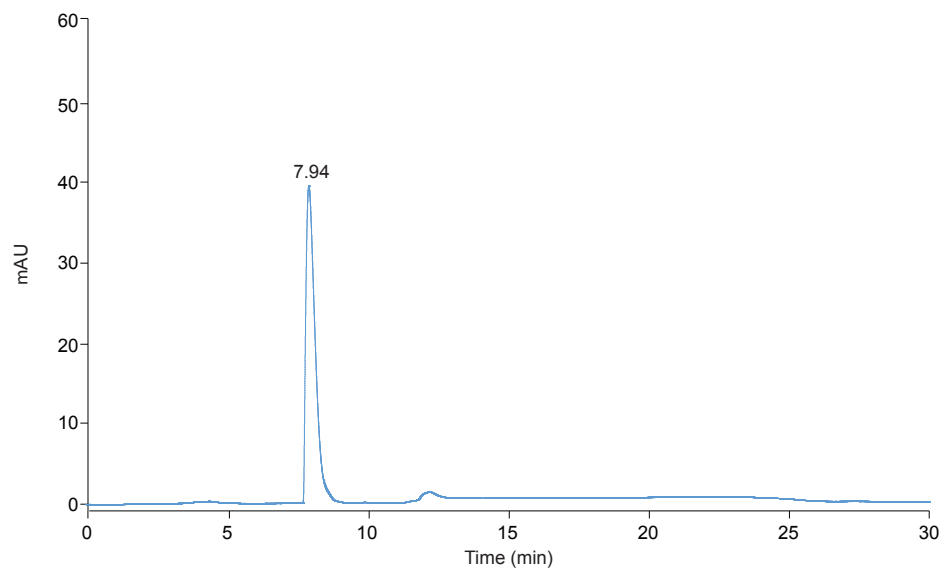


Fig. S18: HPLC trace for compound **5** (SGT535).  $R_t = 7.94$  min.



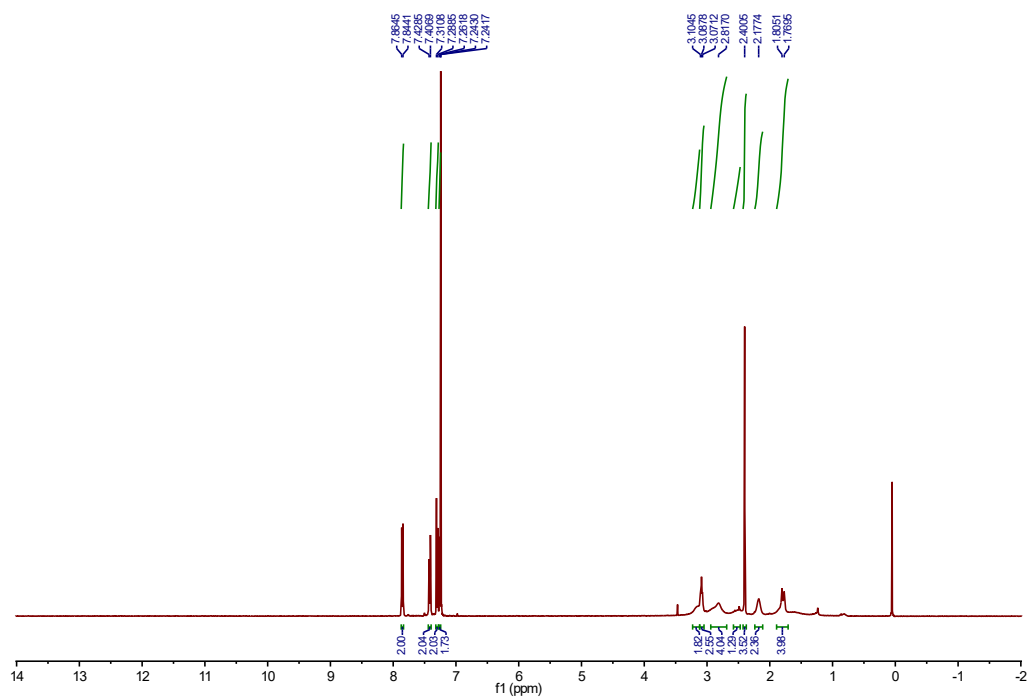


Fig. S19:  $^1\text{H}$  NMR spectrum for compound **6** (SGT1430) in  $\text{CDCl}_3$ .

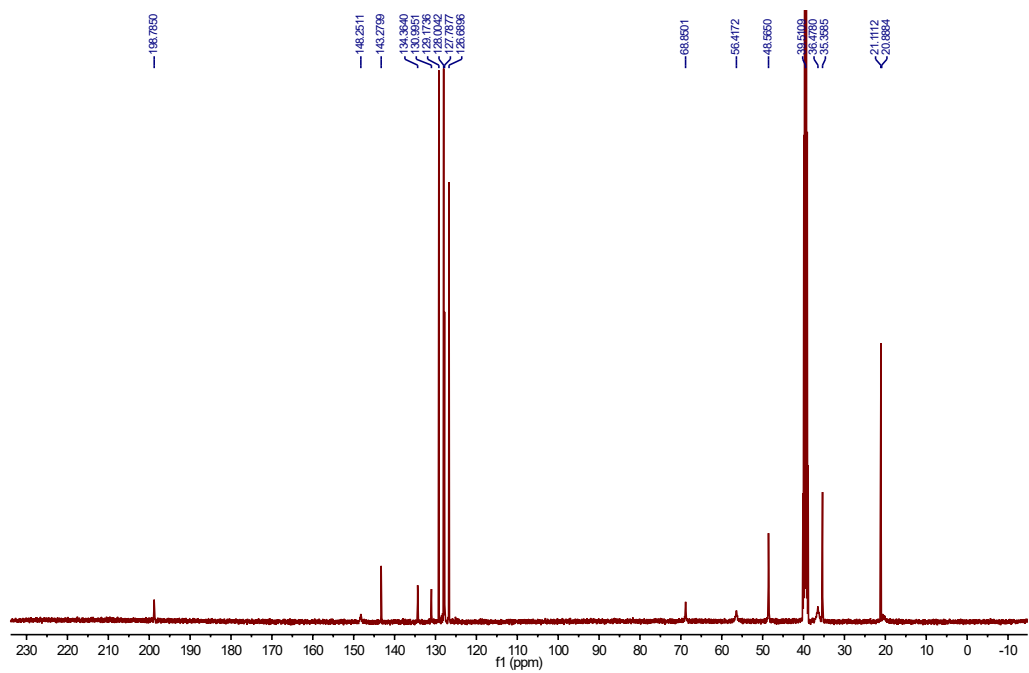
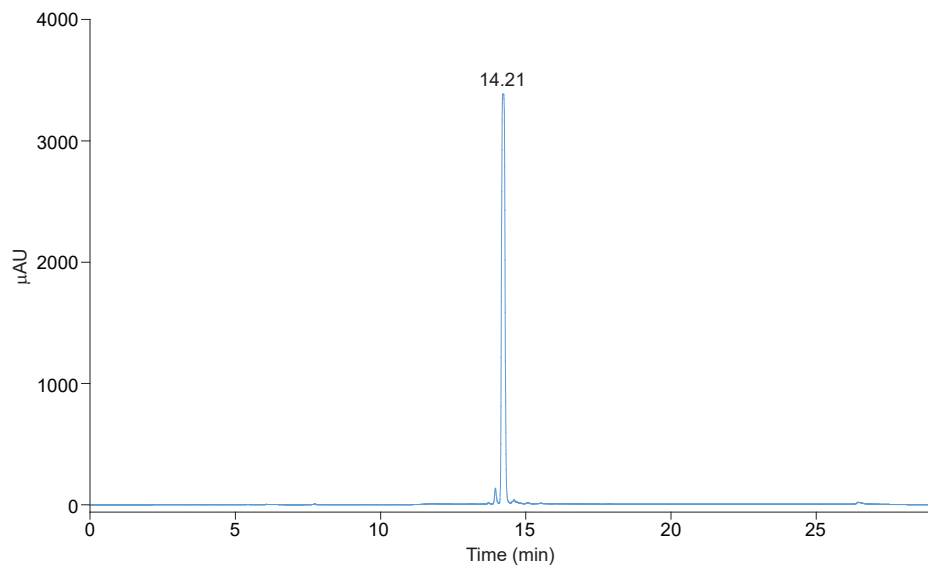
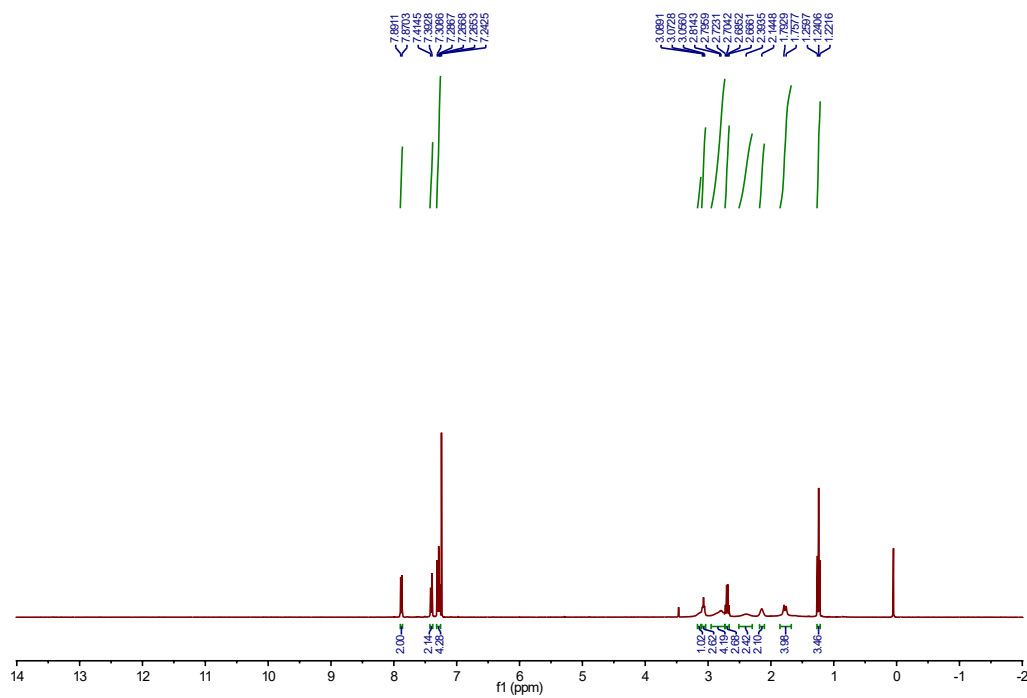


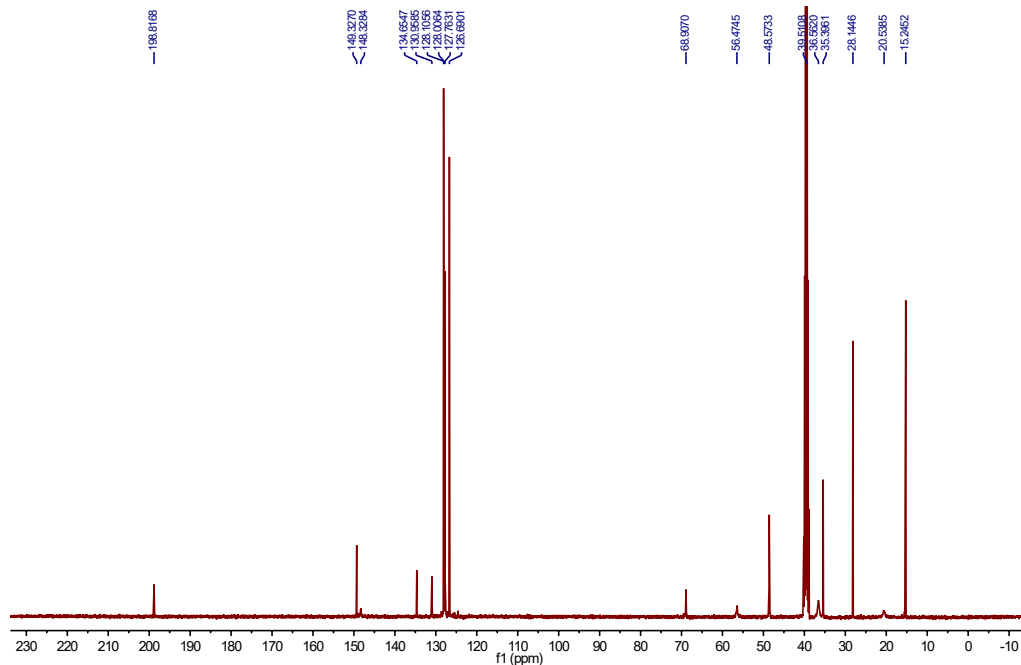
Fig. S20:  $^{13}\text{C}$  NMR spectrum for compound **6** (SGT1430) in  $(\text{CD}_3)_2\text{SO}$ .



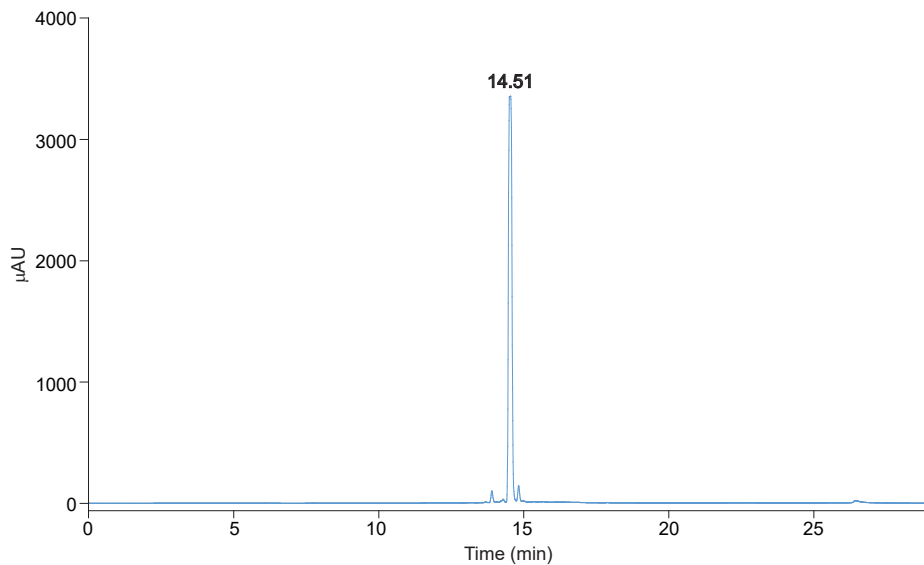
**Fig. S21:** HPLC trace for compound **6** (SGT1430).  $R_t = 14.21$  min.



**Fig. S22:**  $^1\text{H}$  NMR spectrum for compound **7** (SGT1429) in  $\text{CDCl}_3$ .



**Fig. S23:**  $^{13}\text{C}$  NMR spectrum for compound **7** (SGT1429) in  $(\text{CD}_3)_2\text{SO}$ .



**Fig. S24:** HPLC trace for compound **7** (SGT1429).  $R_t = 14.51$  min.

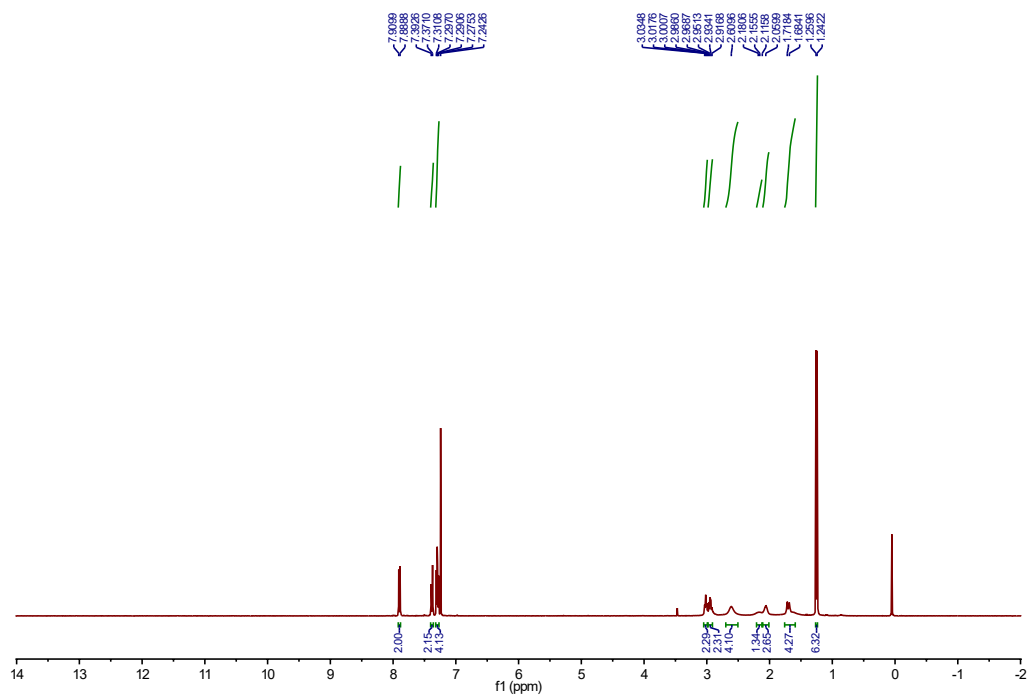


Fig. S25:  $^1\text{H}$  NMR spectrum for compound **8** (SGT1428) in  $\text{CDCl}_3$ .

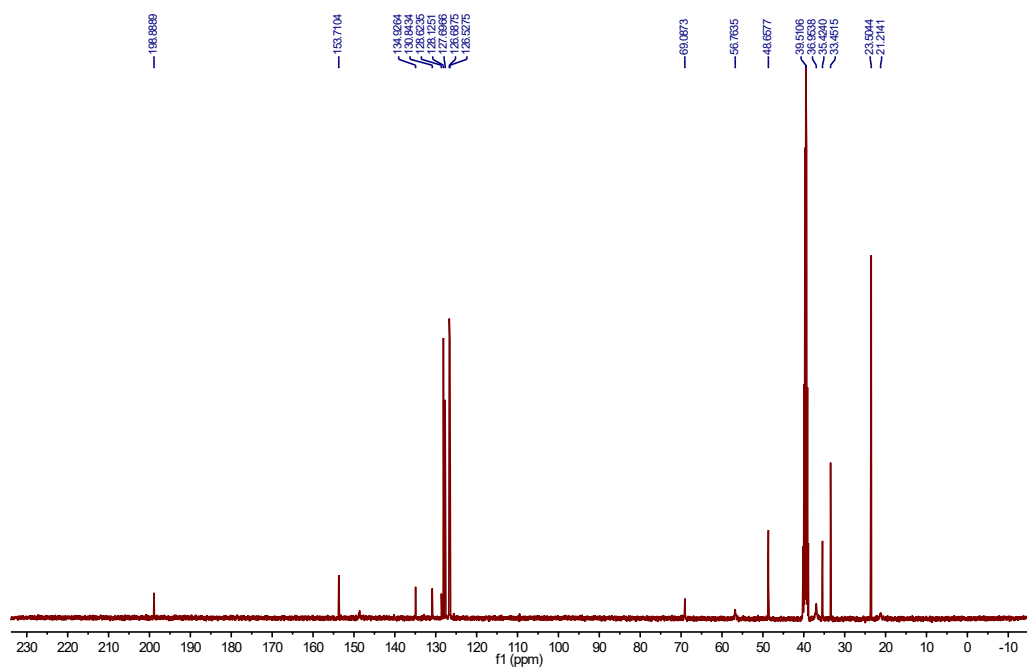


Fig. S26:  $^{13}\text{C}$  NMR spectrum for compound **8** (SGT1428) in  $(\text{CD}_3)_2\text{SO}$ .

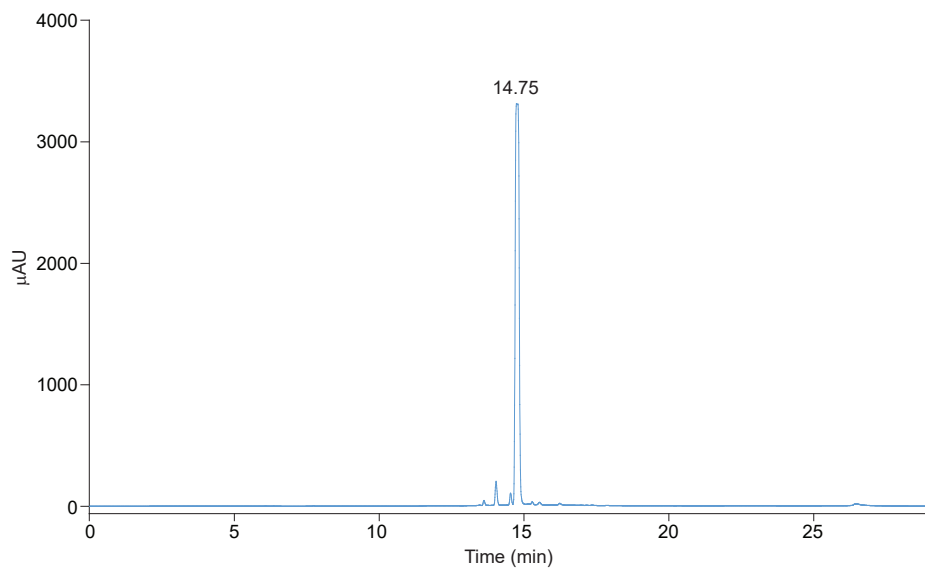


Fig. S27: HPLC trace for compound **8** (SGT1428).  $R_t = 14.75$  min.

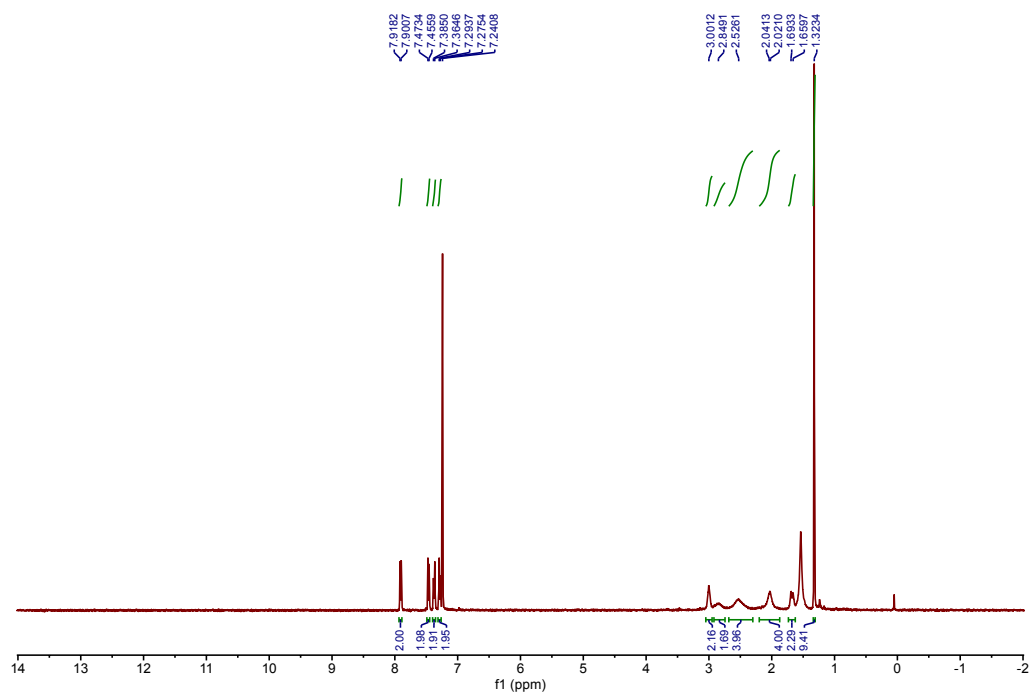
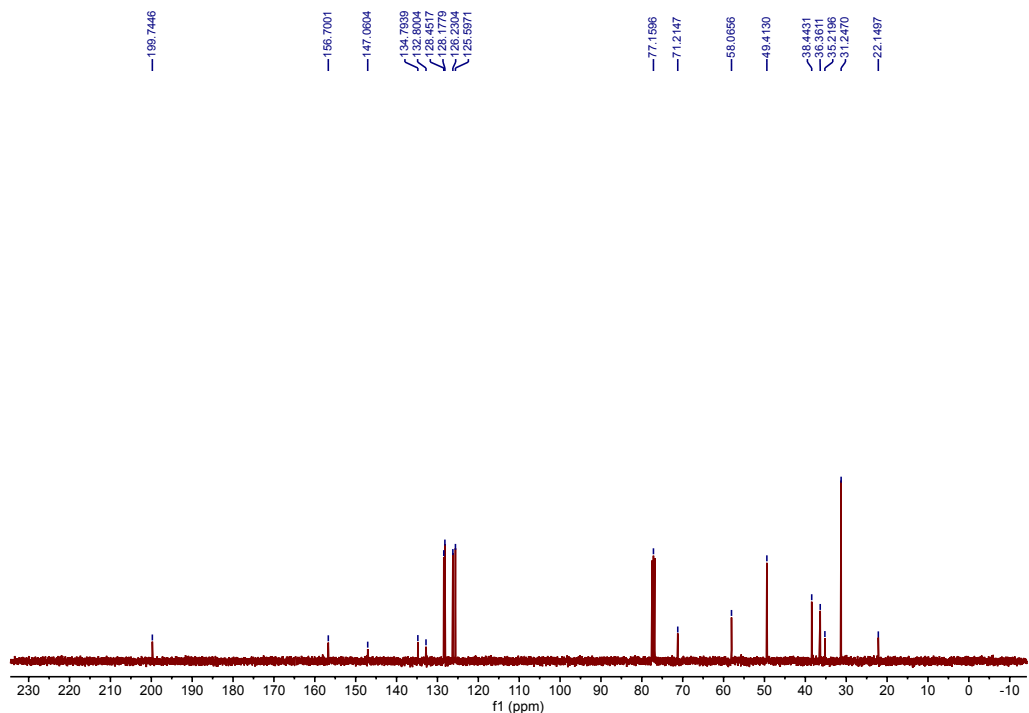
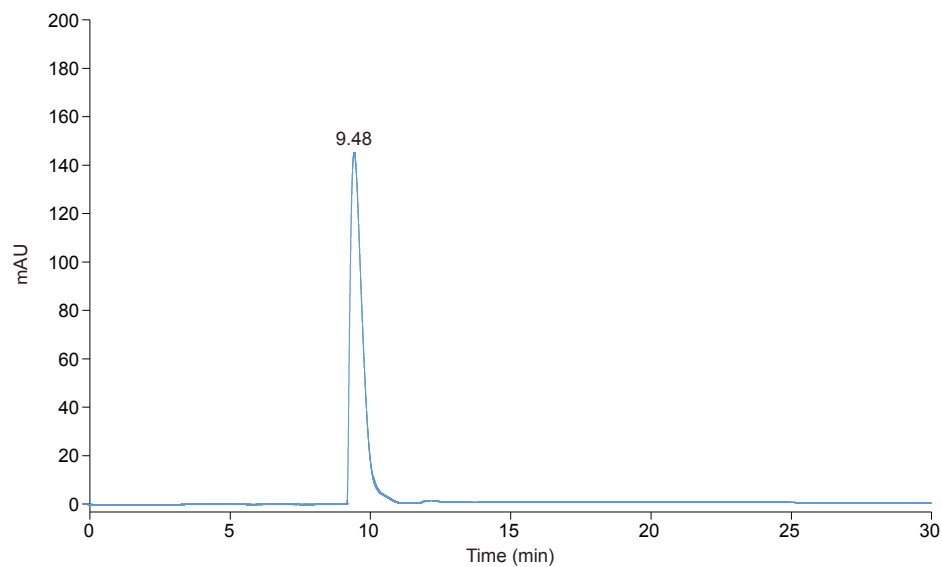


Fig. S28:  $^1\text{H}$  NMR spectrum for compound **9** (SGT534) in  $\text{CDCl}_3$ .



**Fig. S29:**  $^{13}\text{C}$  NMR spectrum for compound **9** (SGT534) in  $\text{CDCl}_3$ .



**Fig. S30:** HPLC trace for compound **9** (SGT534).  $R_t = 9.48$  min.

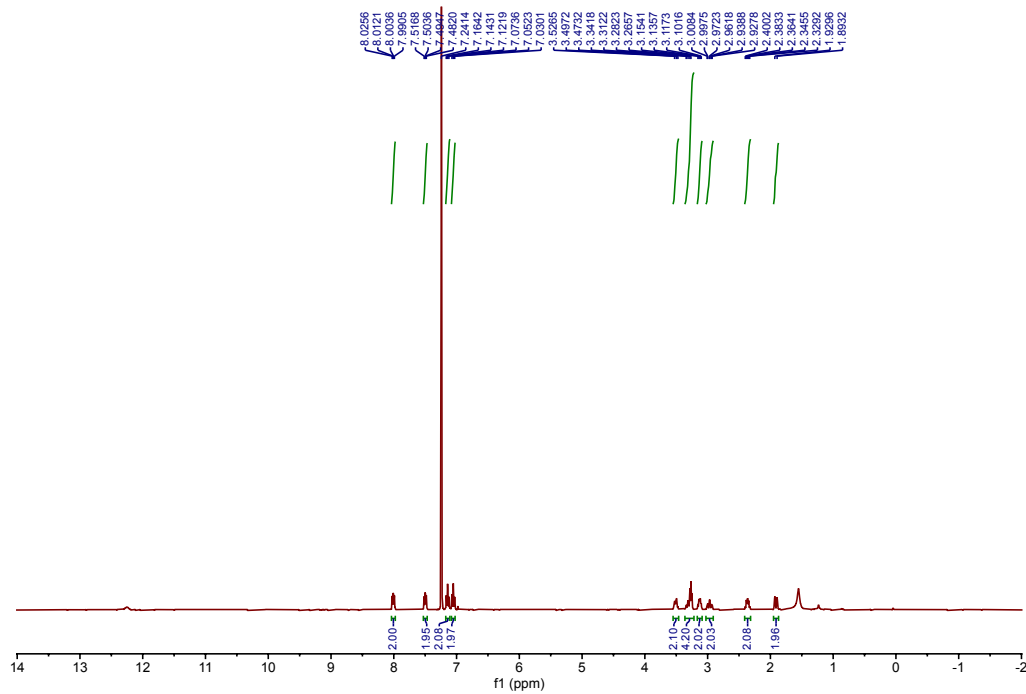


Fig. S31:  $^1\text{H}$  NMR spectrum for compound **10** (SGT543) in  $\text{CDCl}_3$ .

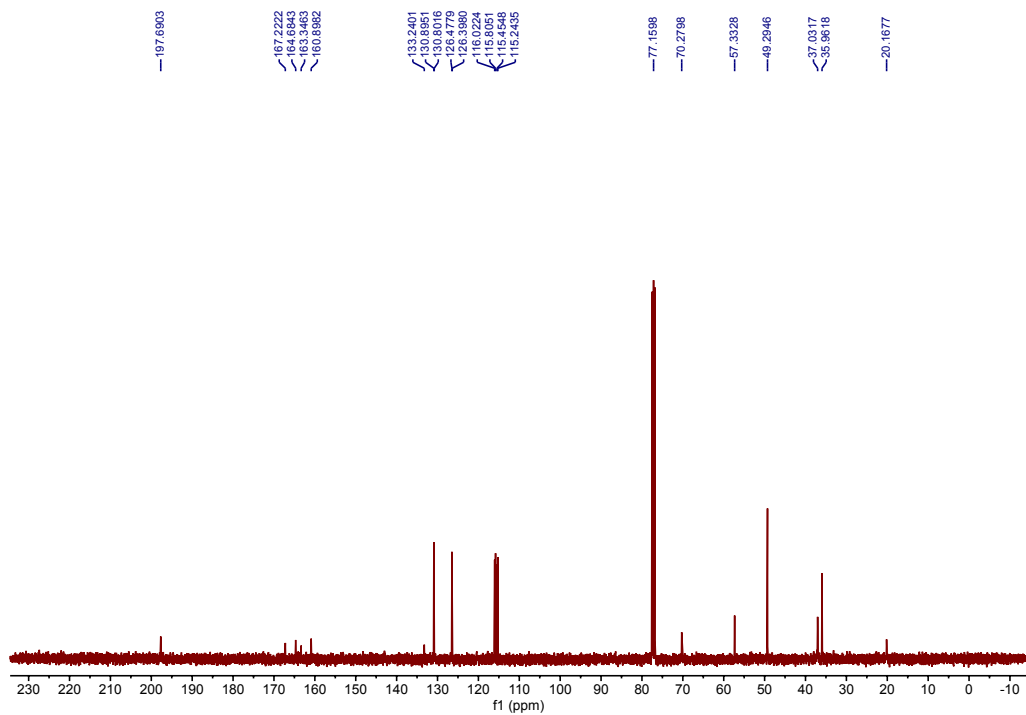
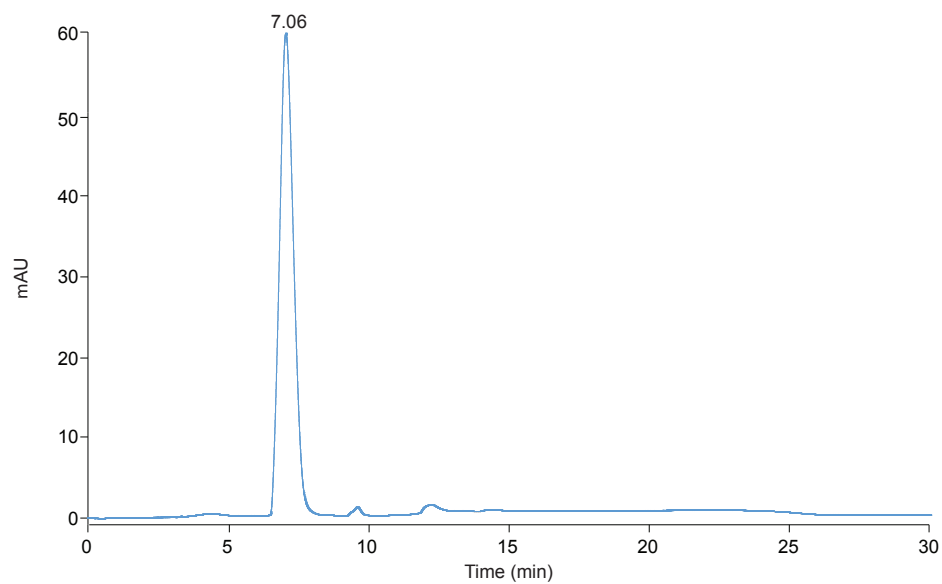
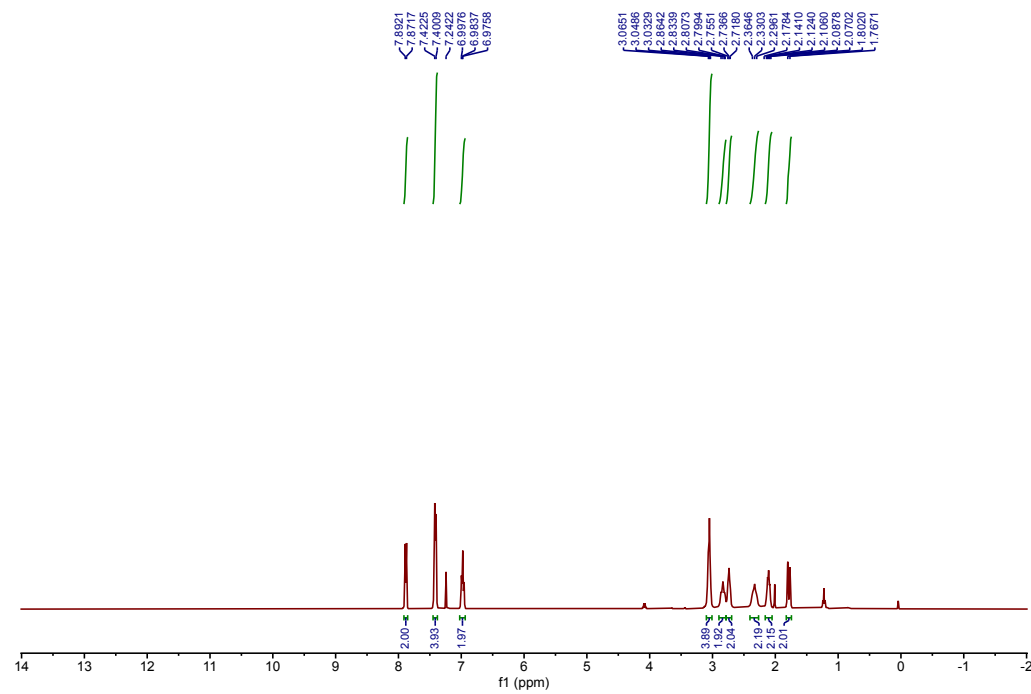


Fig. S32:  $^{13}\text{C}$  NMR spectrum for compound **10** (SGT543) in  $\text{CDCl}_3$ .



**Fig. S33:** HPLC trace for compound **10 (SGT543)**.  $R_t = 7.06$  min.



**Fig. S34:** <sup>1</sup>H NMR spectrum for compound **11 (SGT544)** in CDCl<sub>3</sub>.



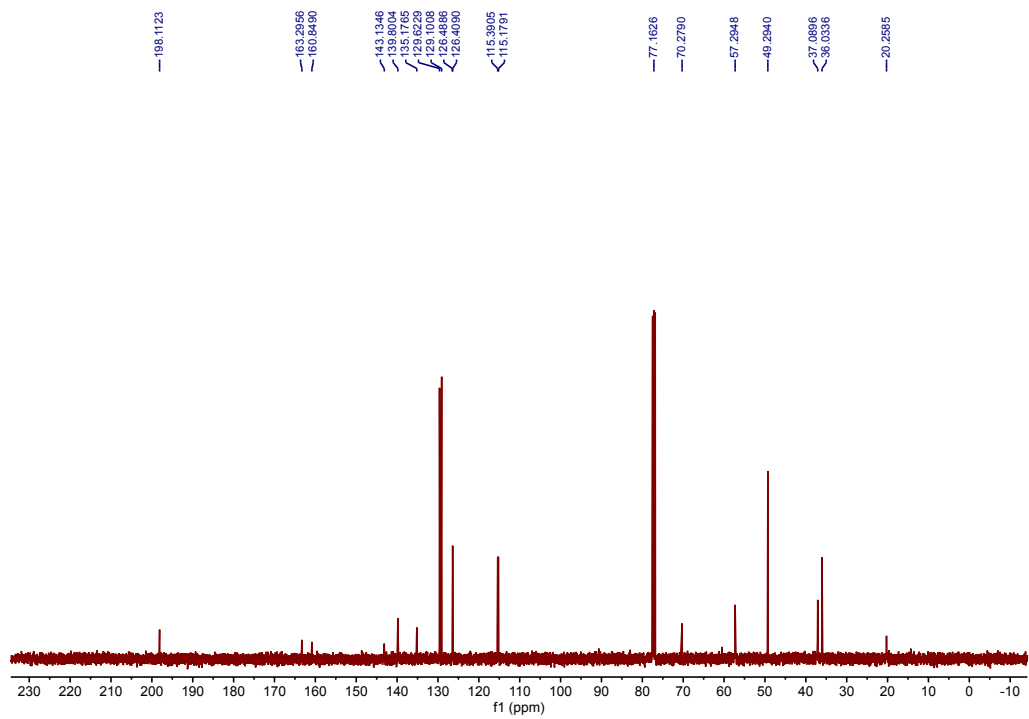


Fig. S35:  $^{13}\text{C}$  NMR spectrum for compound **11** (SGT544) in  $\text{CDCl}_3$ .

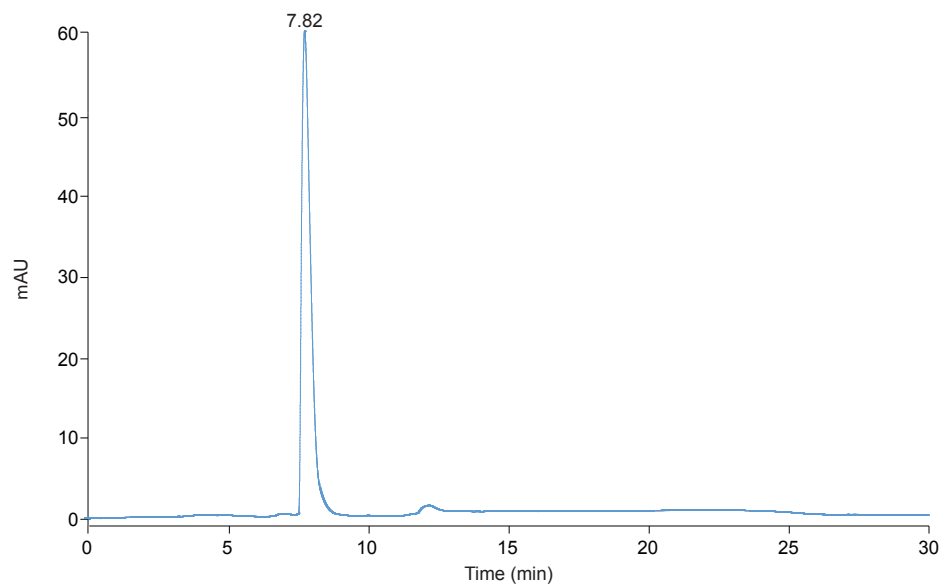


Fig. S36: HPLC trace for compound **11** (SGT544).  $R_t = 7.82$  min.

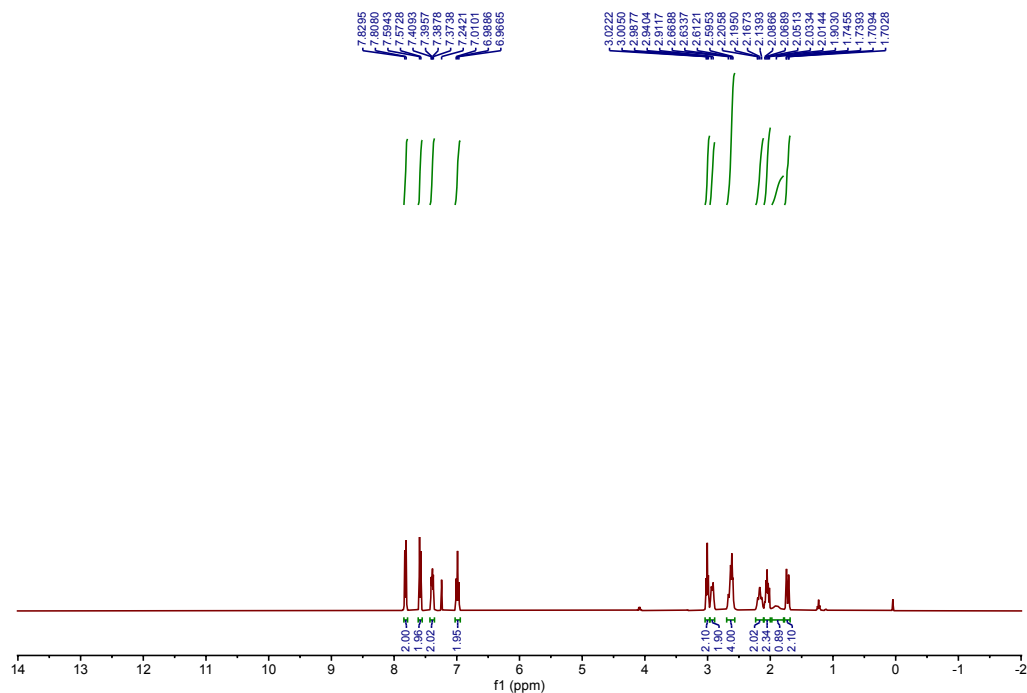


Fig. S37:  $^1\text{H}$  NMR spectrum for compound **12** (SGT545) in  $\text{CDCl}_3$ .

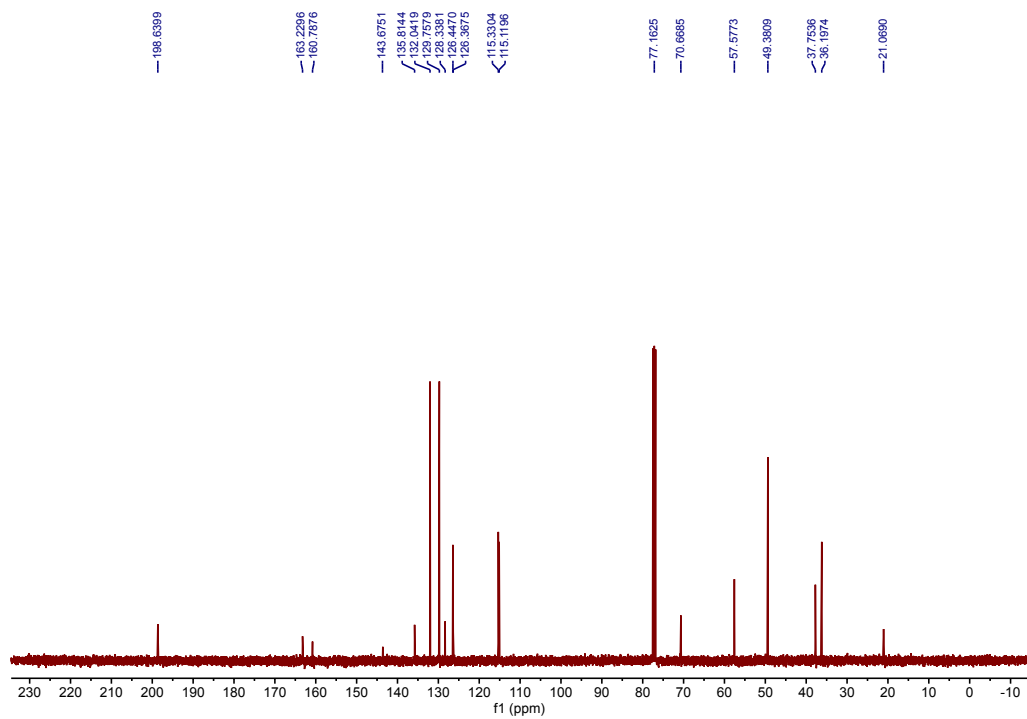
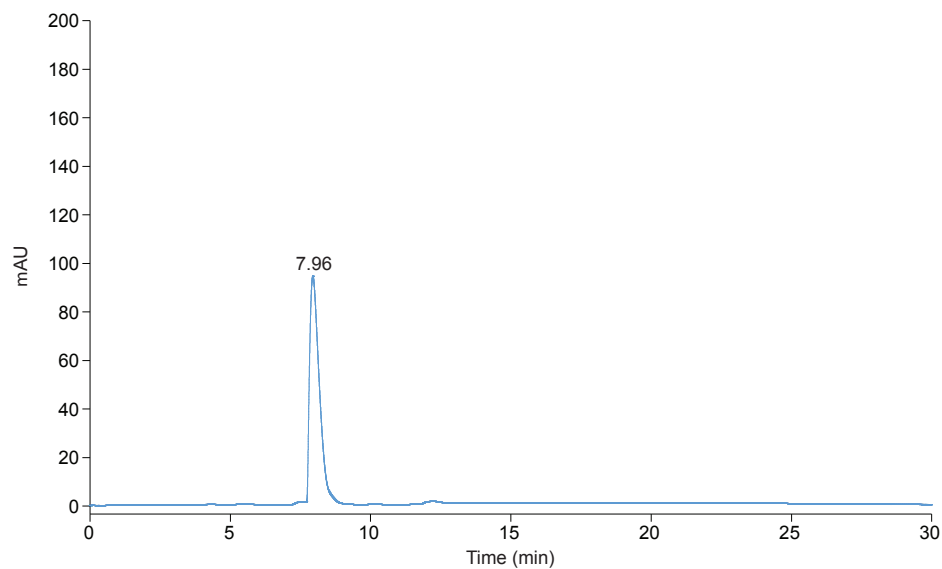
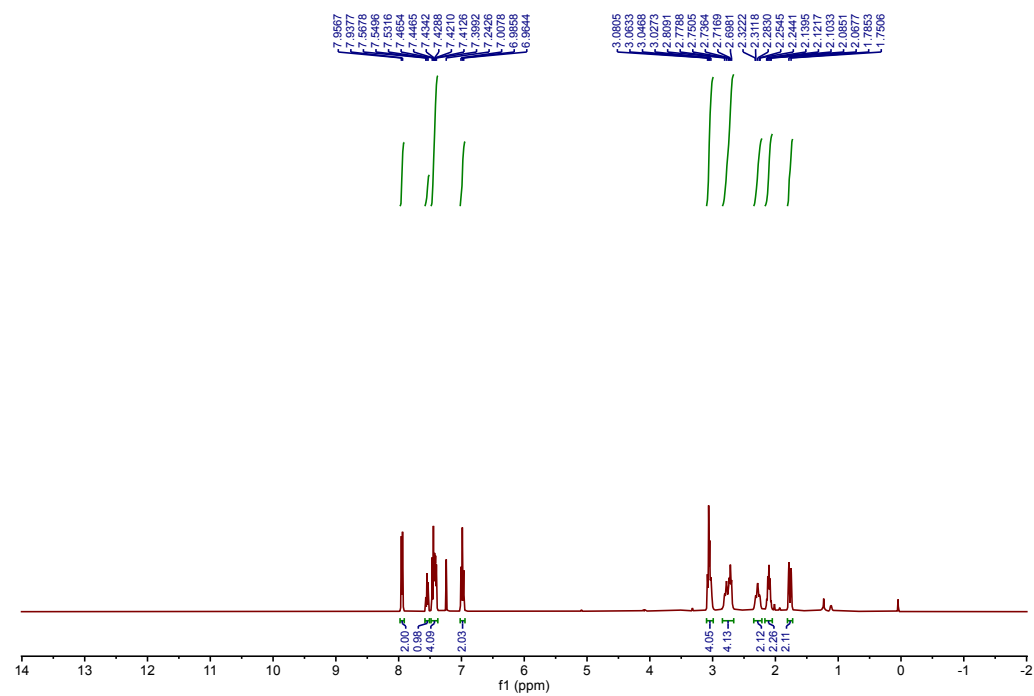


Fig. S38:  $^{13}\text{C}$  NMR spectrum for compound **12** (SGT545) in  $\text{CDCl}_3$ .



**Fig. S39:** HPLC trace for compound **12 (SGT545)**.  $R_t = 7.96$  min.



**Fig. S40:**  $^1\text{H}$  NMR spectrum for compound **13 (SGT542)** in  $\text{CDCl}_3$ .

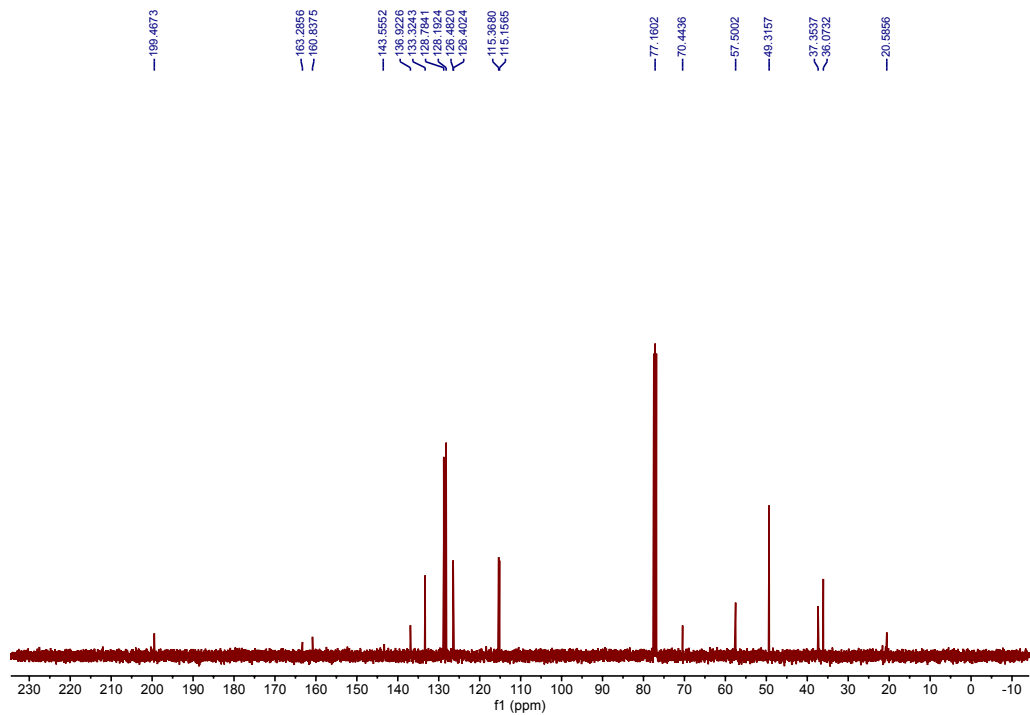


Fig. S41:  $^{13}\text{C}$  NMR spectrum for compound **13** (SGT542) in  $\text{CDCl}_3$ .

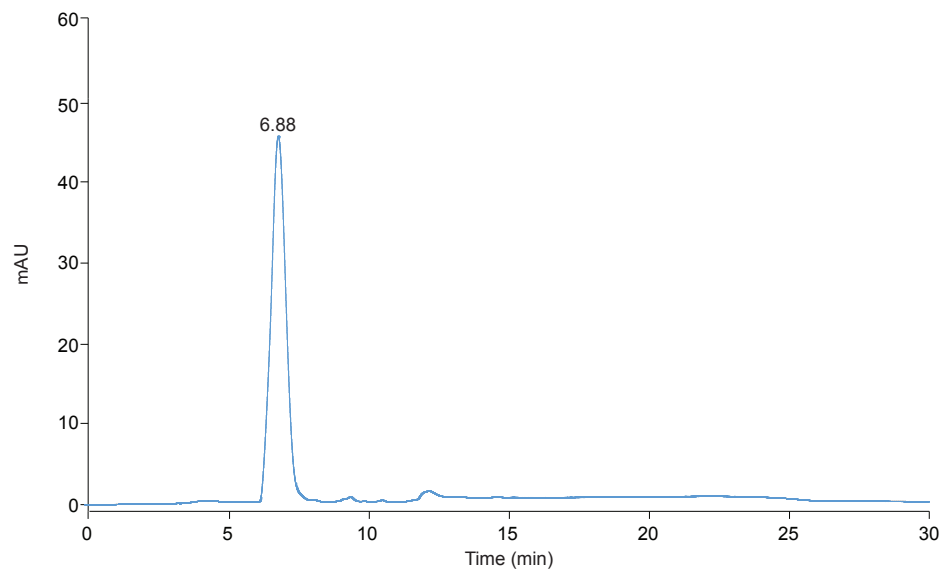


Fig. S42: HPLC trace for compound **13** (SGT542).  $R_t = 6.88$  min.

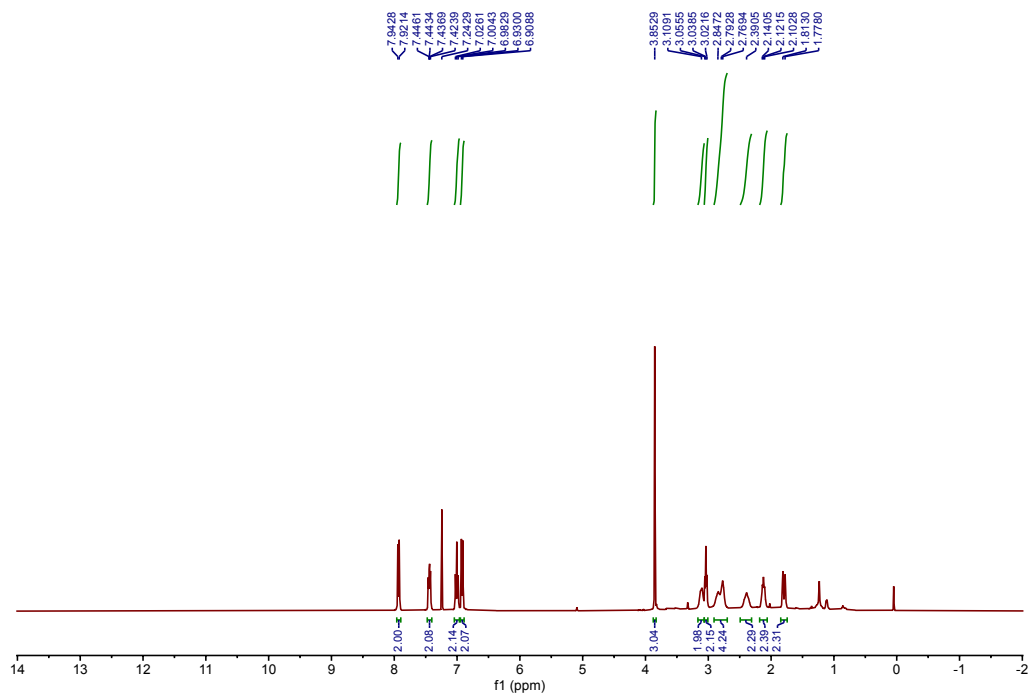


Fig. S43:  $^1\text{H}$  NMR spectrum for compound **14** (SGT541) in  $\text{CDCl}_3$ .

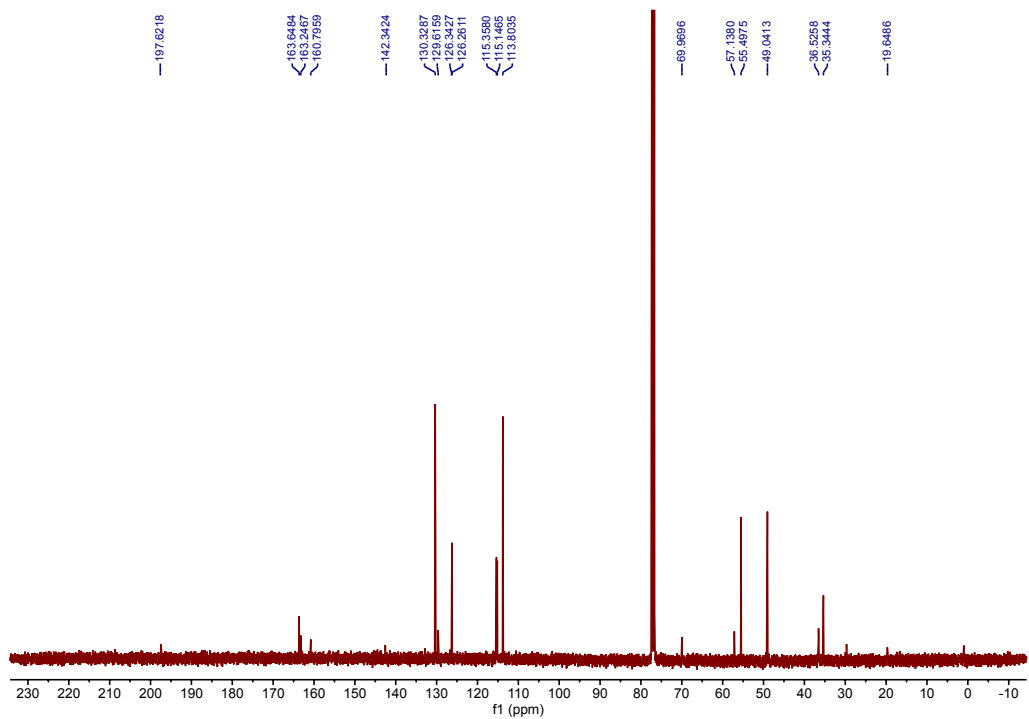


Fig. S44:  $^{13}\text{C}$  NMR spectrum for compound **14** (SGT541) in  $\text{CDCl}_3$ .

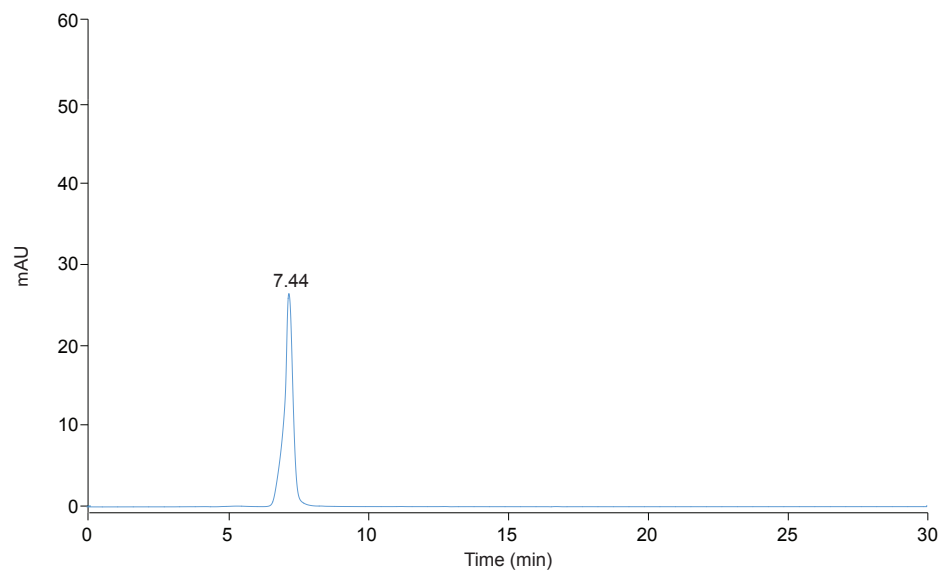


Fig. S45: HPLC trace for compound **14** (SGT541).  $R_t = 7.44$  min.

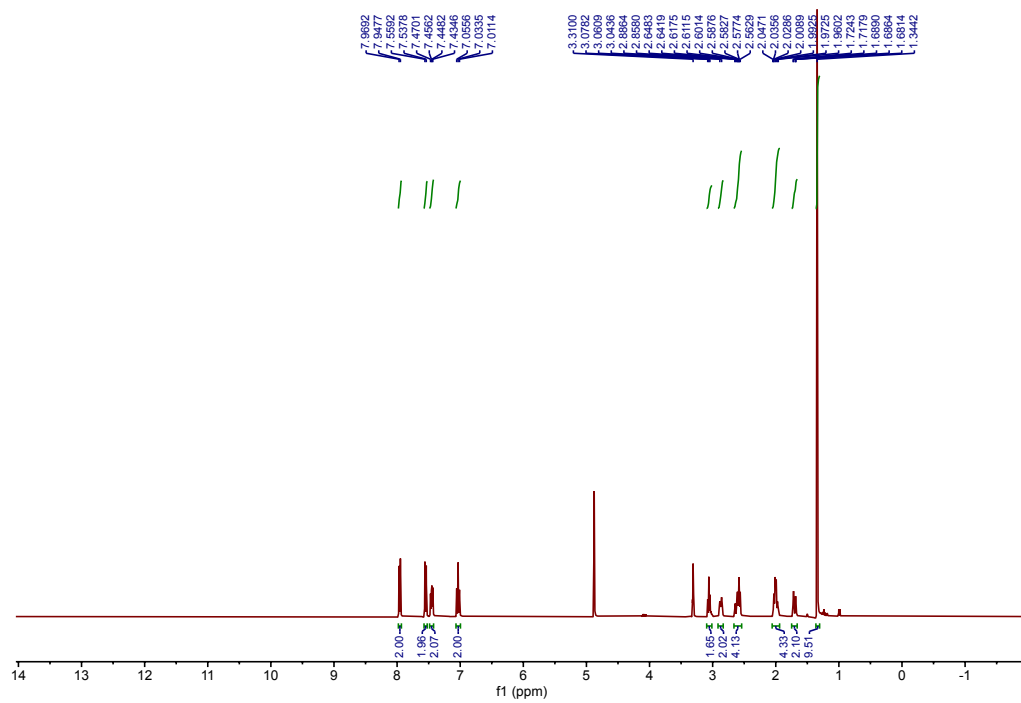


Fig. S46:  $^1\text{H}$  NMR spectrum for compound **15** (SGT540) in  $\text{CD}_3\text{OD}$ .

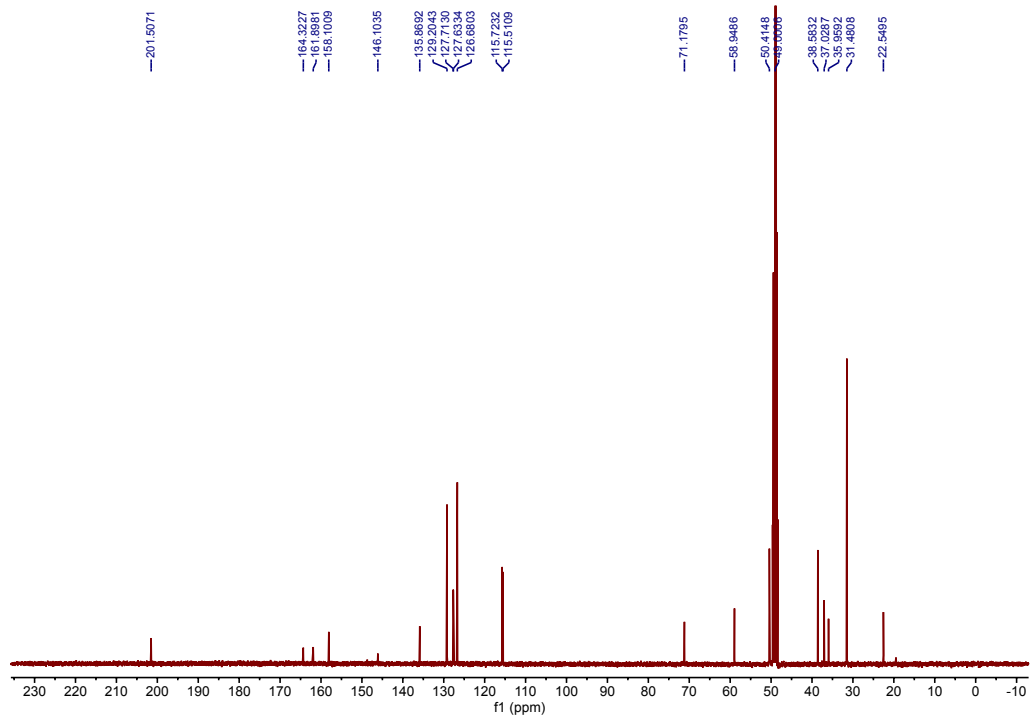


Fig. S47:  $^{13}\text{C}$  NMR spectrum for compound **15** (SGT540) in  $\text{CD}_3\text{OD}$ .

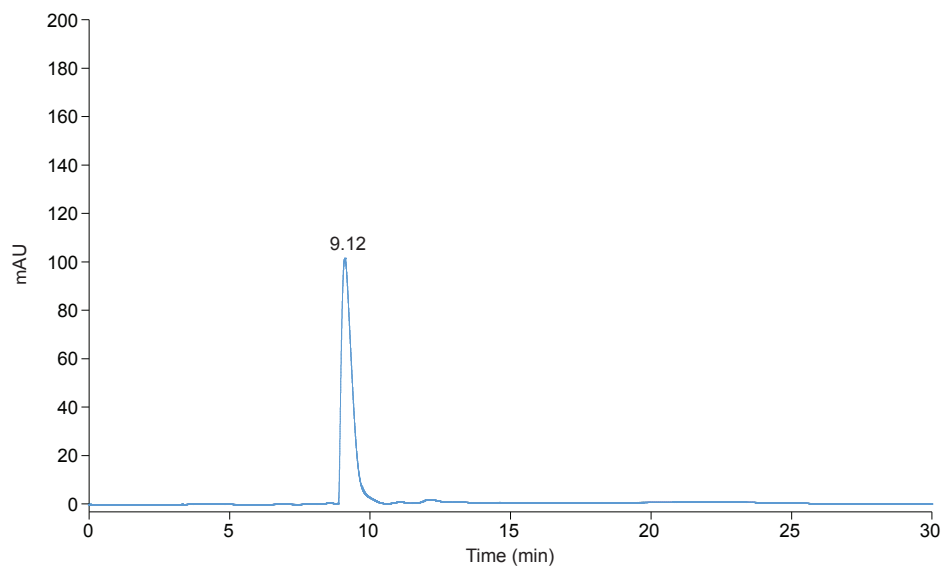


Fig. S48: HPLC trace for compound **15** (SGT540).  $R_t = 9.12$  min.

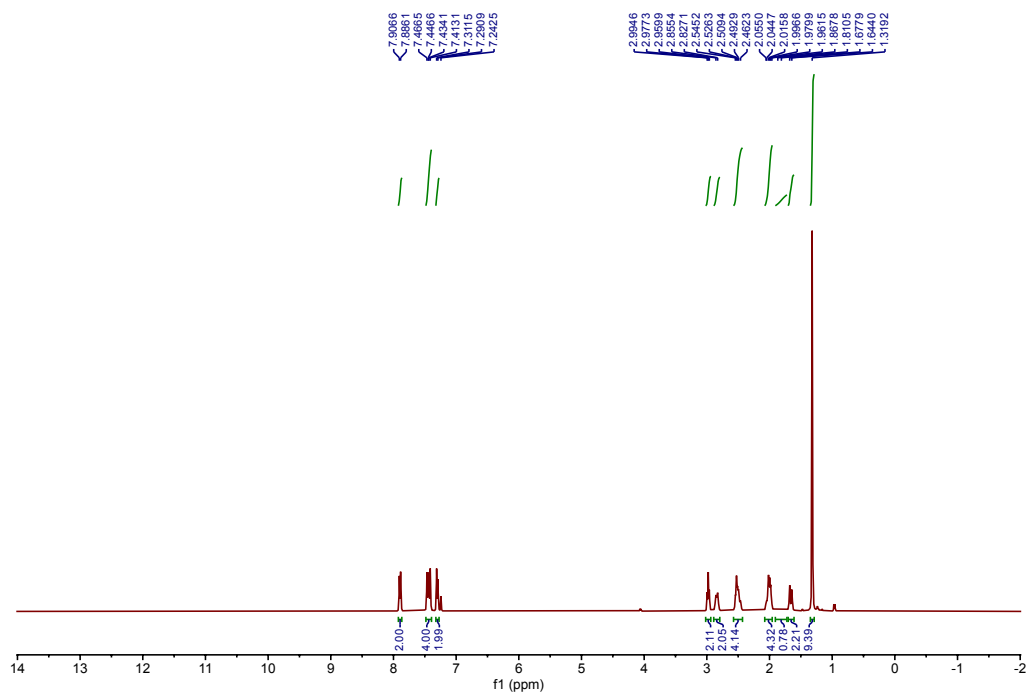


Fig. S49:  $^1\text{H}$  NMR spectrum for compound **21** (SGT546) in  $\text{CDCl}_3$ .

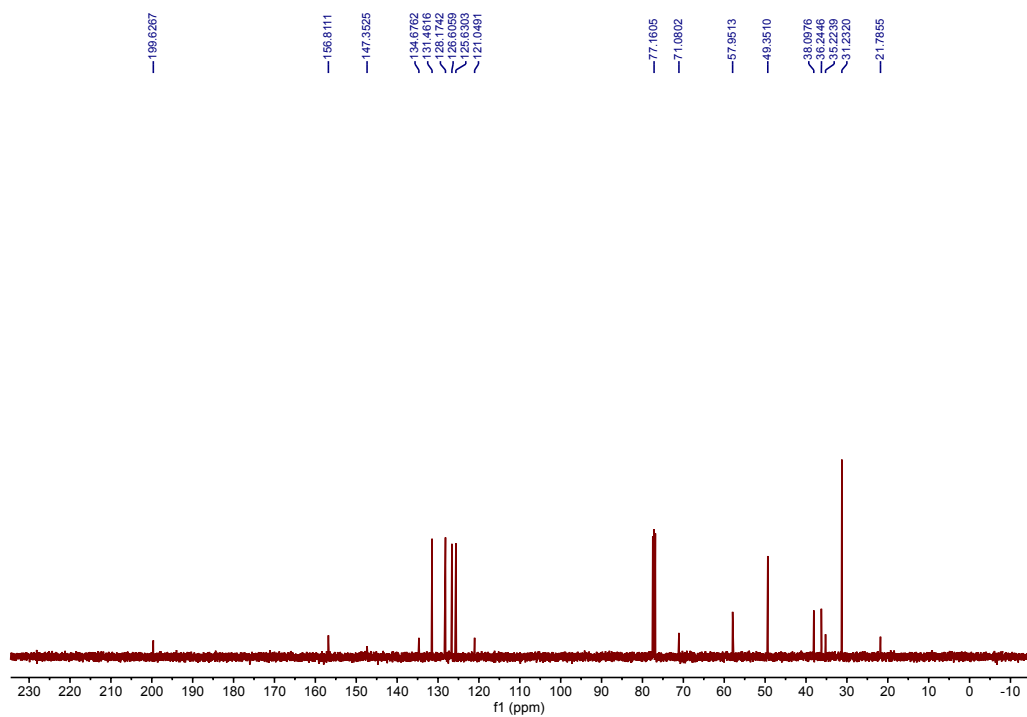
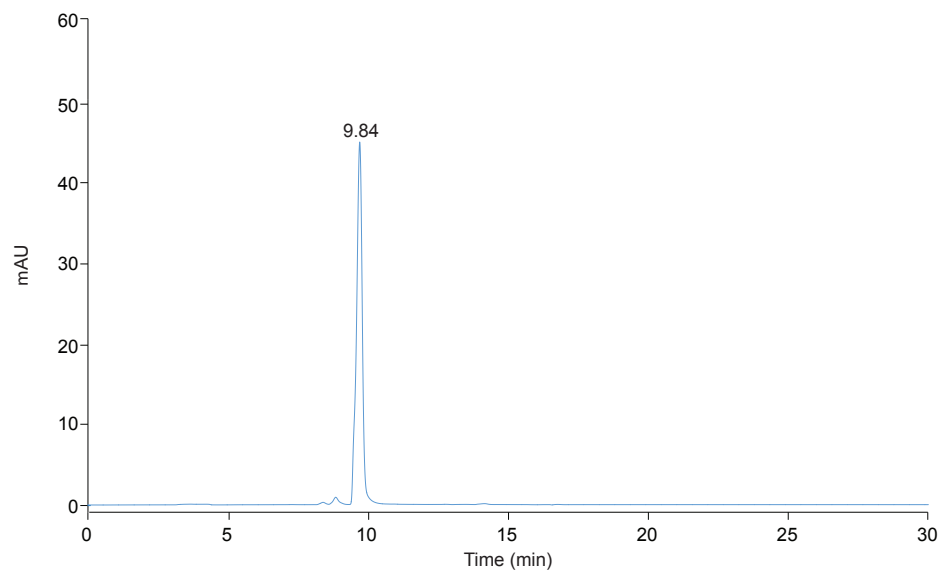
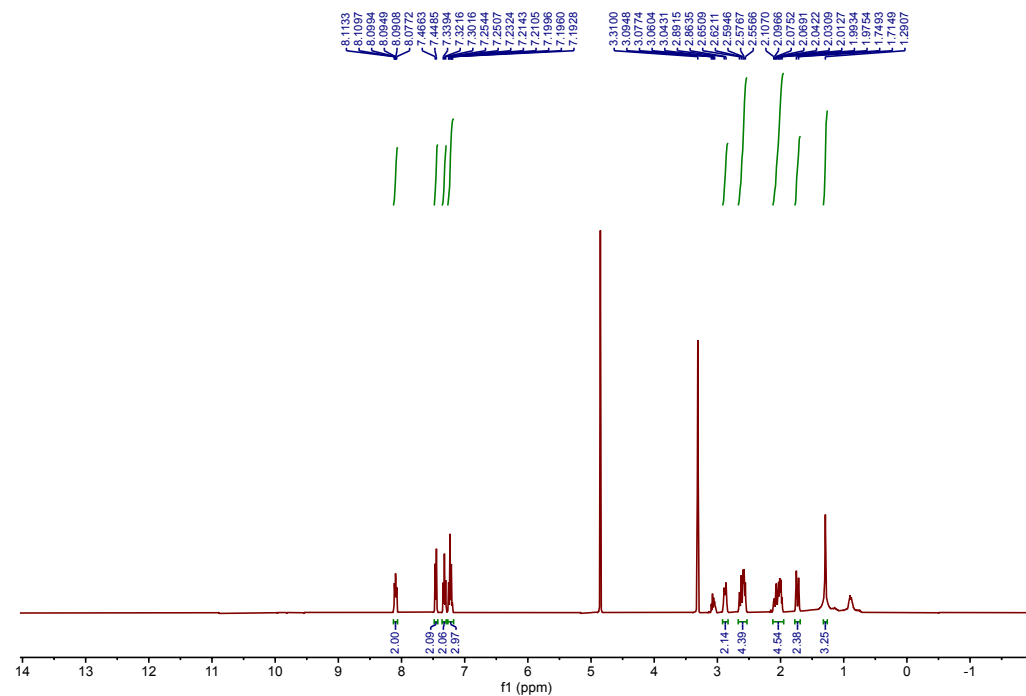


Fig. S50:  $^{13}\text{C}$  NMR spectrum for compound **21** (SGT546) in  $\text{CDCl}_3$ .





**Fig. S51:** HPLC trace for compound **21** (SGT546).  $R_t = 9.84$  min.



**Fig. S52:** <sup>1</sup>H NMR spectrum for compound **22** (SGT564) in CD<sub>3</sub>OD.

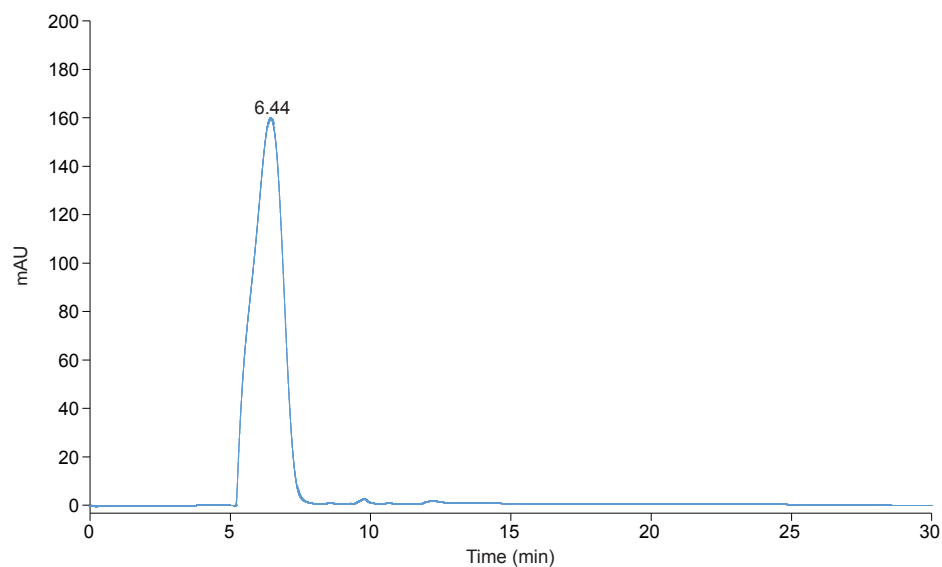


Fig. S53: HPLC trace for compound **22** (SGT564).  $R_t = 6.44$  min.

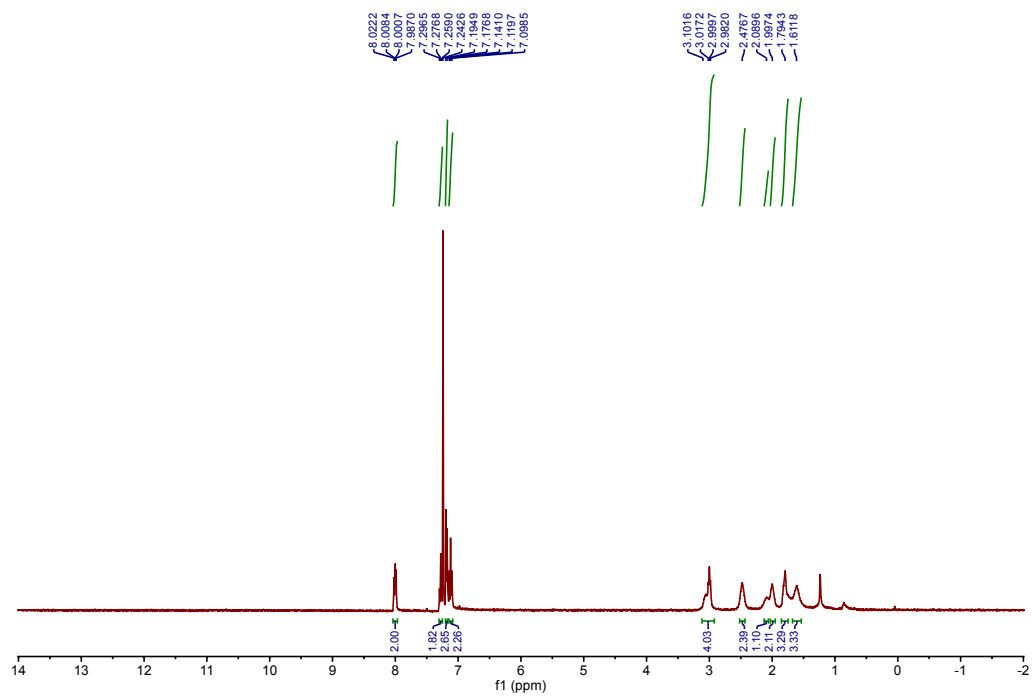


Fig. S54:  $^1\text{H}$  NMR spectrum for compound **23** (SGT565) in  $\text{CDCl}_3$ .

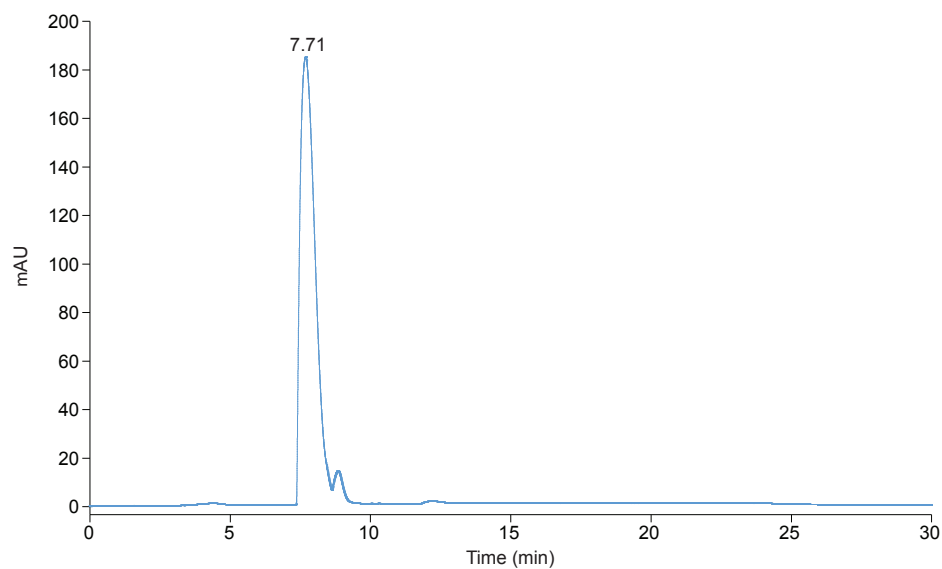


Fig. S55: HPLC trace for compound 23 (SGT565).  $R_t = 7.71$  min.

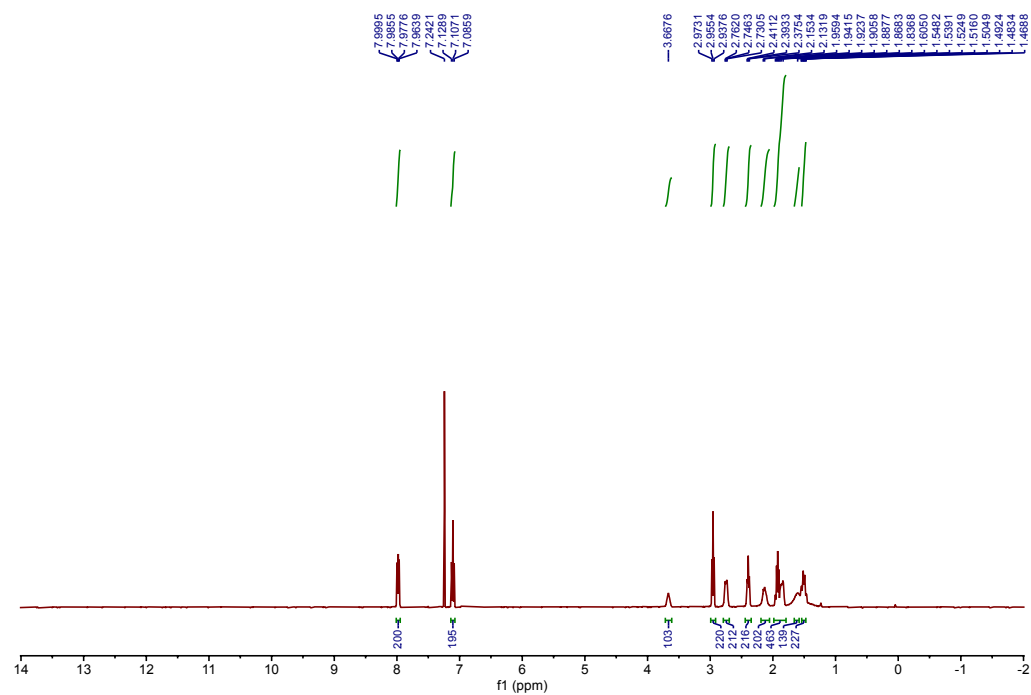


Fig. S56:  $^1\text{H}$  NMR spectrum for compound 24 (SGT566) in  $\text{CDCl}_3$ .

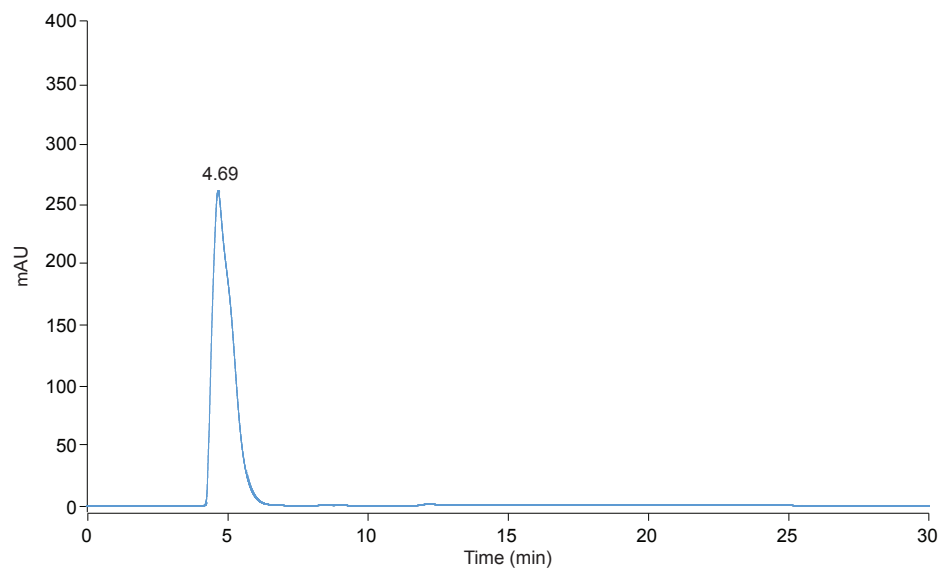


Fig. S57: HPLC trace for compound **24** (SGT566).  $R_t = 4.69$  min.

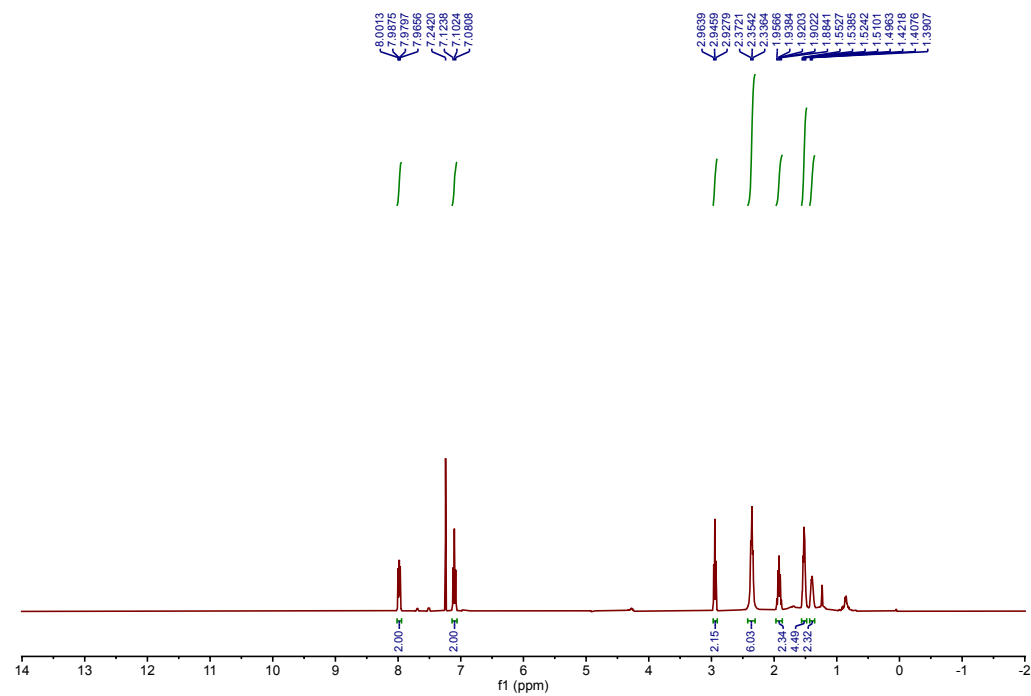
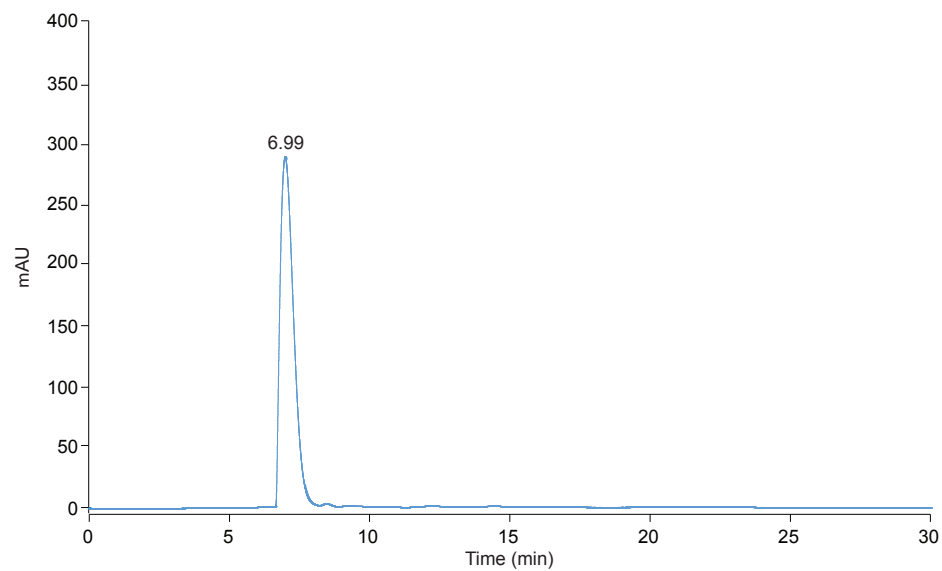
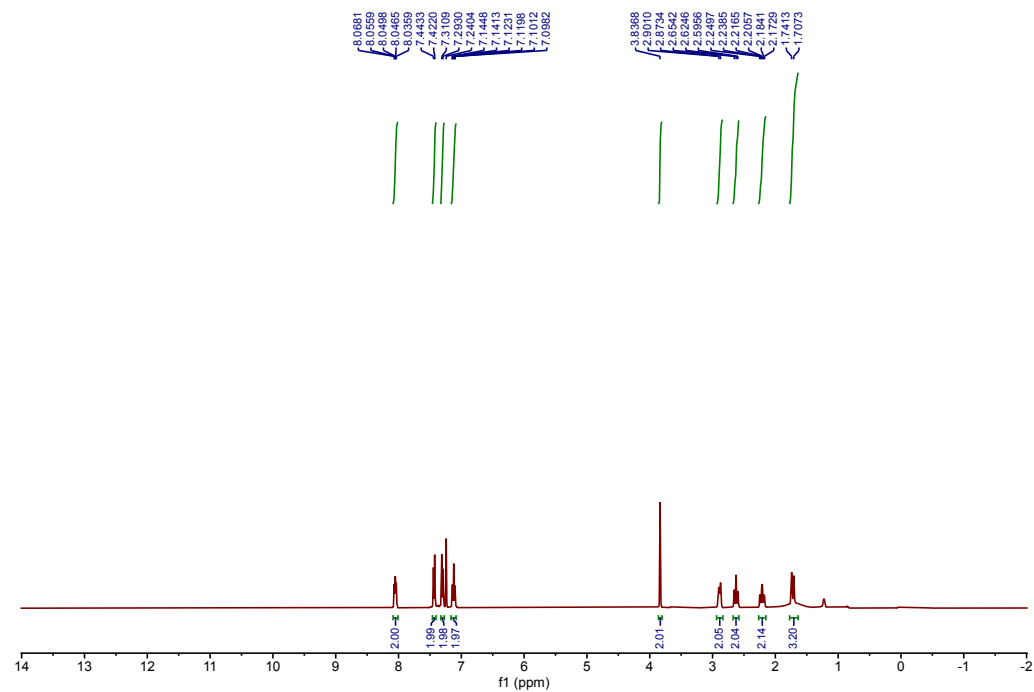


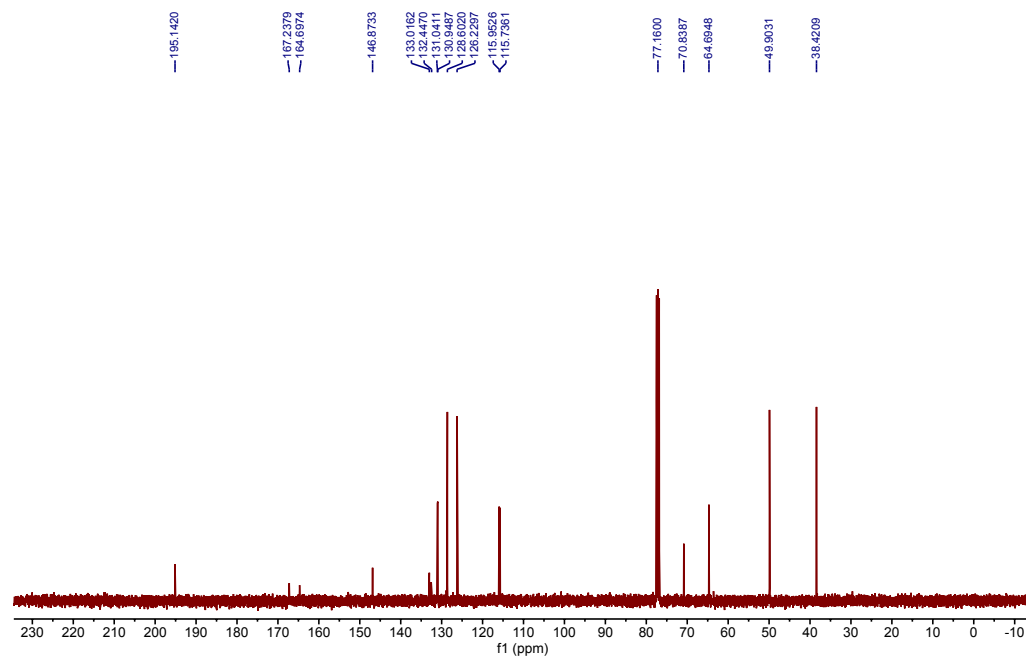
Fig. S58: <sup>1</sup>H NMR spectrum for compound **25** (SGT567) in CDCl<sub>3</sub>.



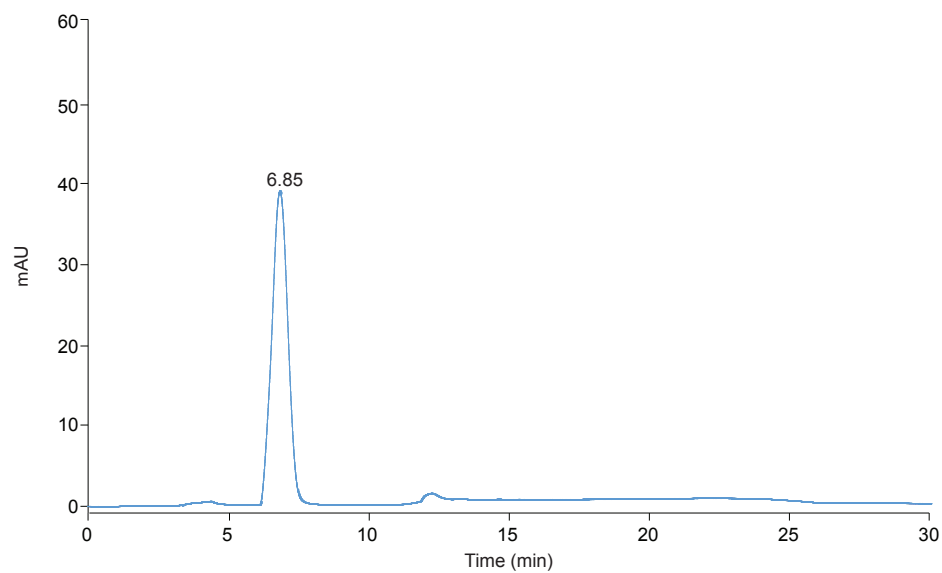
**Fig. S59:** HPLC trace for compound **25** (SGT567).  $R_t = 6.99$  min.



**Fig. S60:**  $^1\text{H}$  NMR spectrum for compound **26** (SGT568) in  $\text{CDCl}_3$ .



**Fig. S61:**  $^{13}\text{C}$  NMR spectrum for compound **26 (SGT568)** in  $\text{CDCl}_3$ .



**Fig. S62:** HPLC trace for compound **26 (SGT568)**.  $R_t = 6.85$  min.

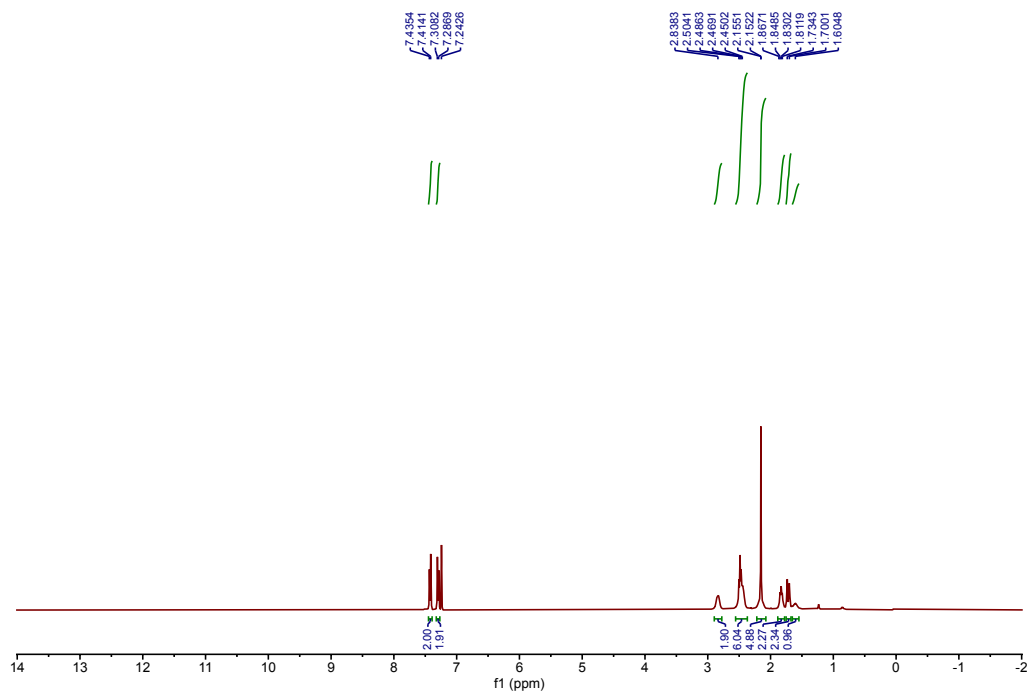


Fig. S63:  $^1\text{H}$  NMR spectrum for compound **27** (SGT569) in  $\text{CDCl}_3$ .

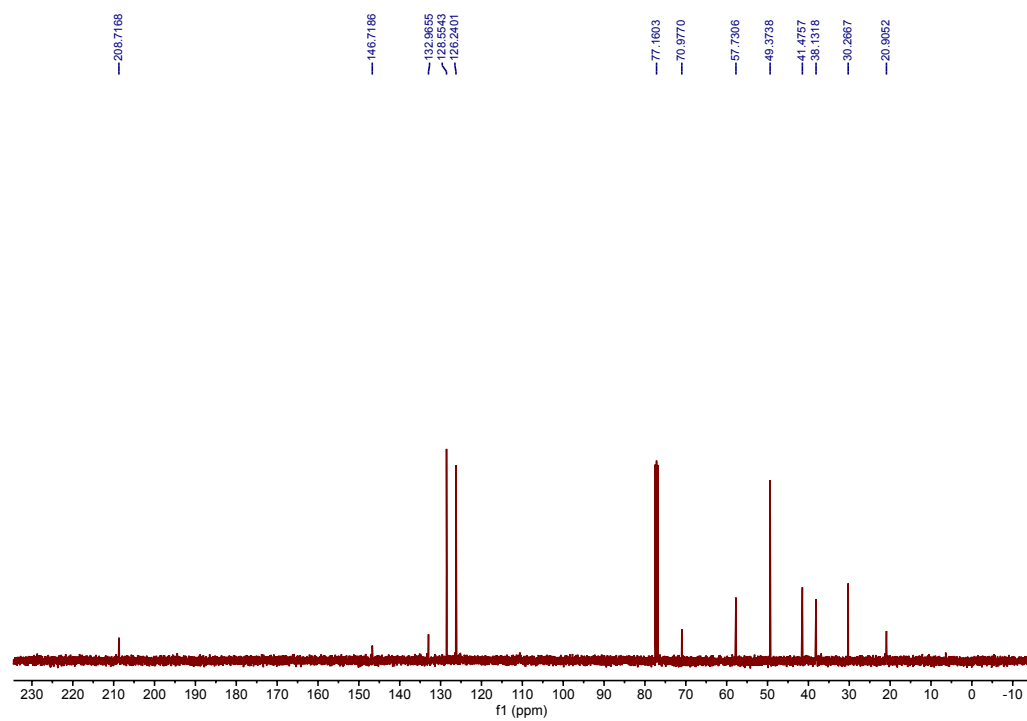
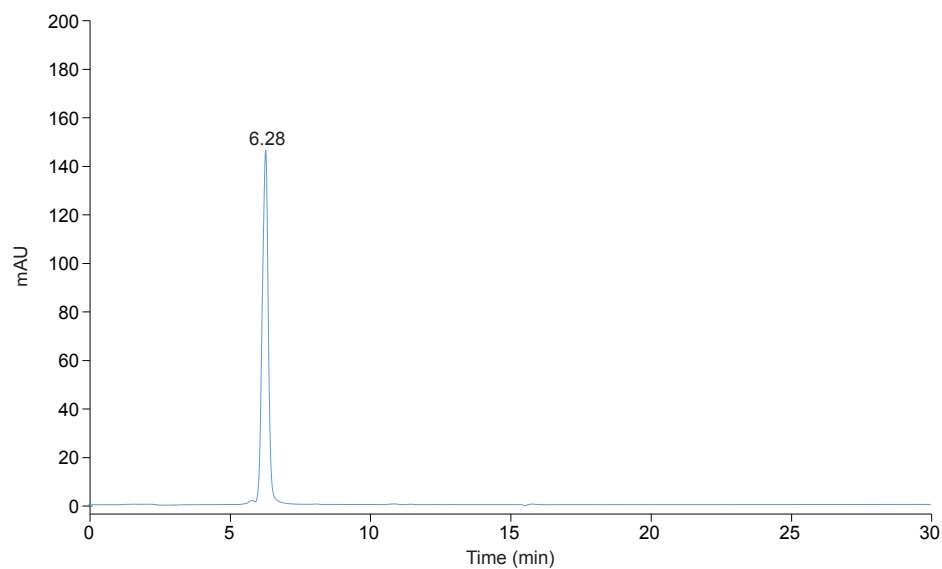
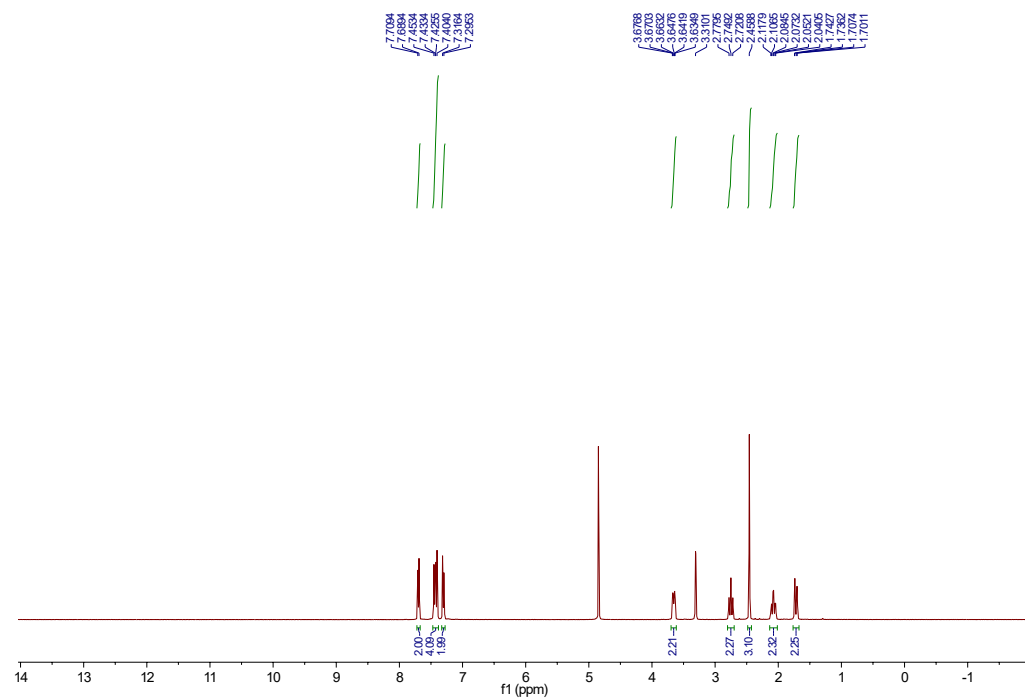


Fig. S64:  $^{13}\text{C}$  NMR spectrum for compound **27** (SGT569) in  $\text{CDCl}_3$ .

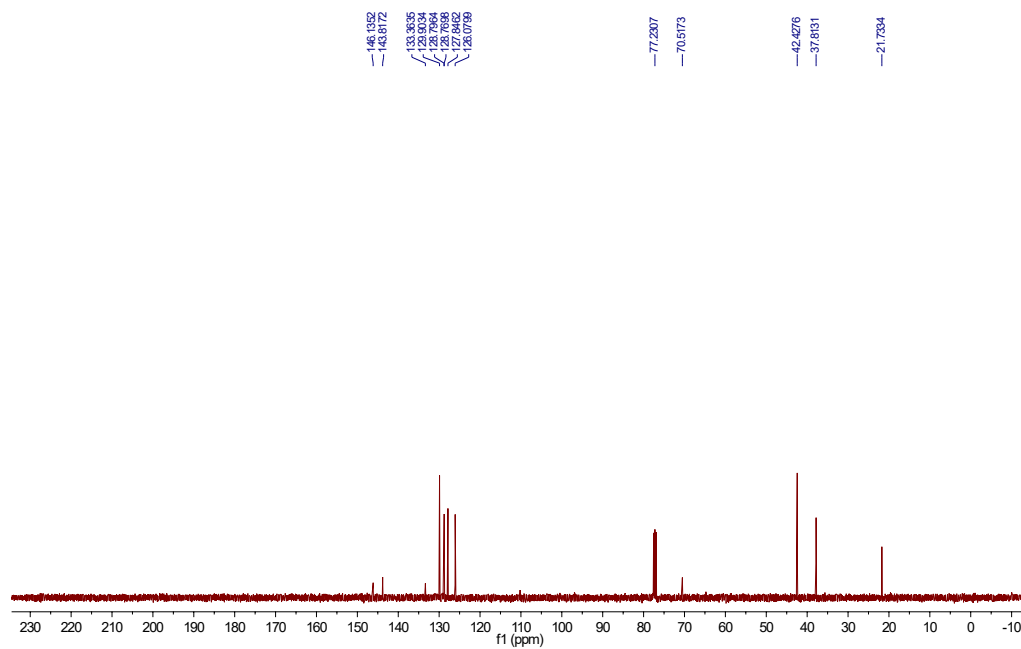


**Fig. S65:** HPLC trace for compound **27 (SGT569)**.  $R_t = 6.28$  min.

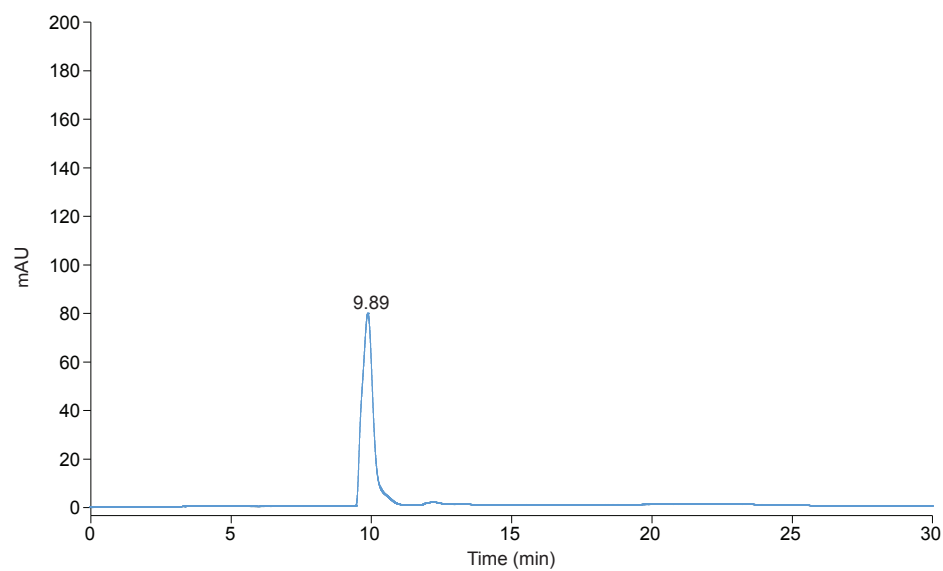


**Fig. S66:** <sup>1</sup>H NMR spectrum for compound **28 (SGT570)** in CD<sub>3</sub>OD.





**Fig. S67:**  $^{13}\text{C}$  NMR spectrum for compound **28** (SGT570) in  $\text{CDCl}_3$ .



**Fig. S68:** HPLC trace for compound **28** (SGT570).  $R_t = 9.89$  min.

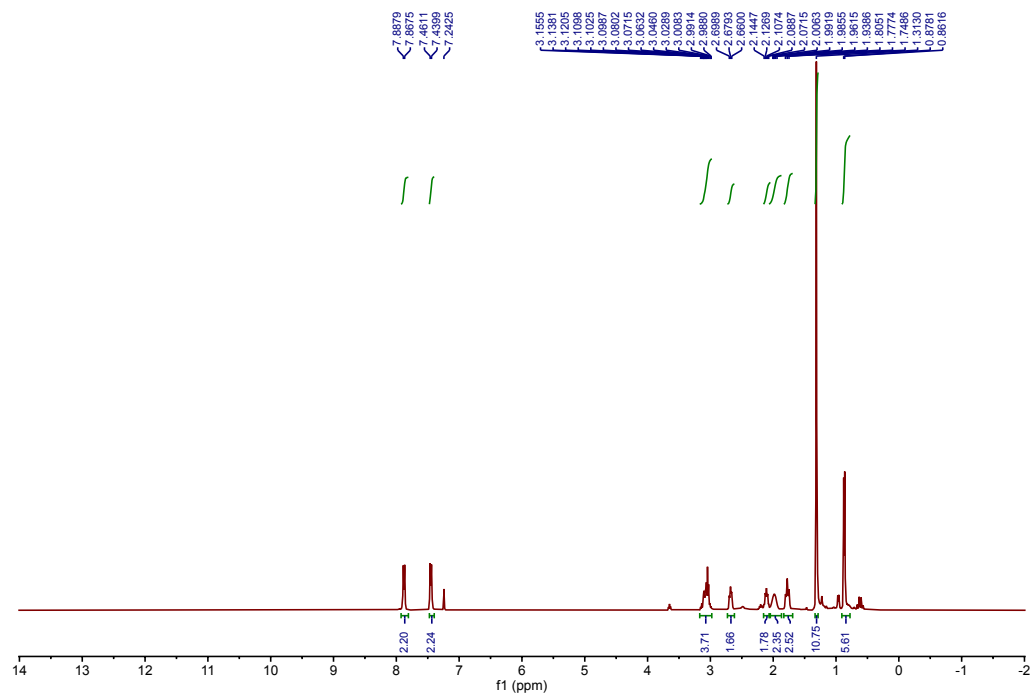


Fig. S69:  $^1\text{H}$  NMR spectrum for compound **29** (SGT571) in  $\text{CDCl}_3$ .

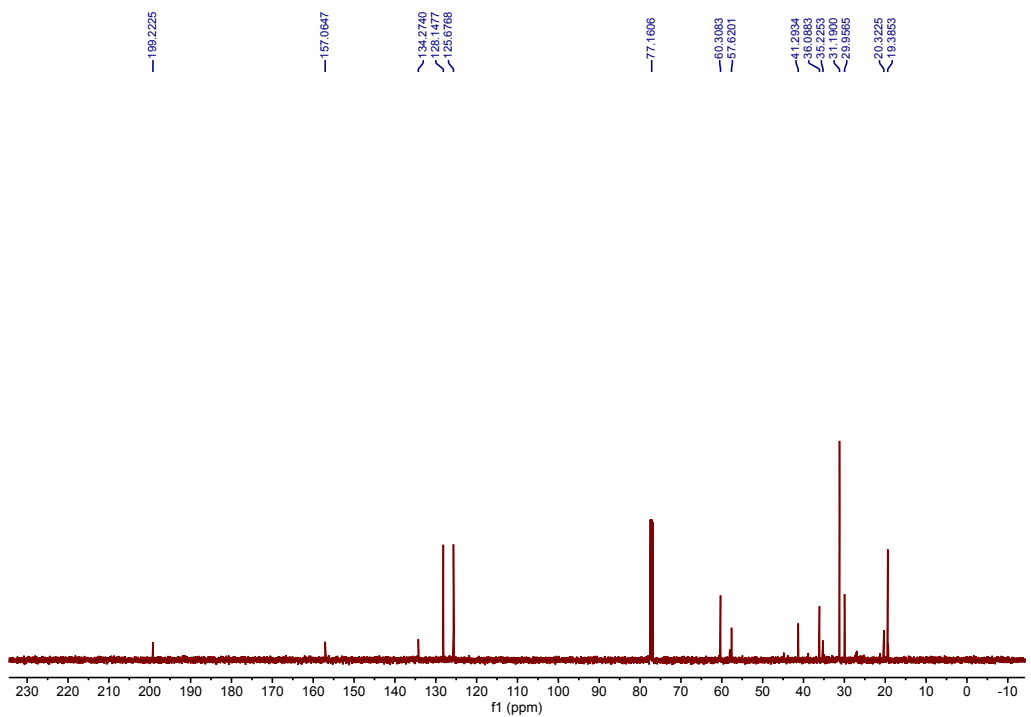


Fig. S70:  $^{13}\text{C}$  NMR spectrum for compound **29** (SGT571) in  $\text{CDCl}_3$ .

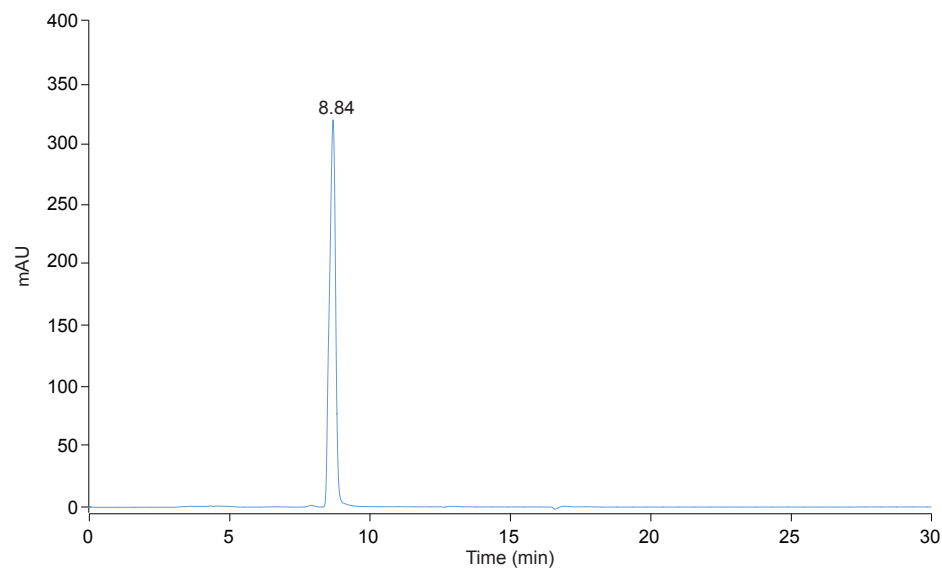


Fig. S71: HPLC trace for compound **29** (SGT571).  $R_t = 8.84$  min.

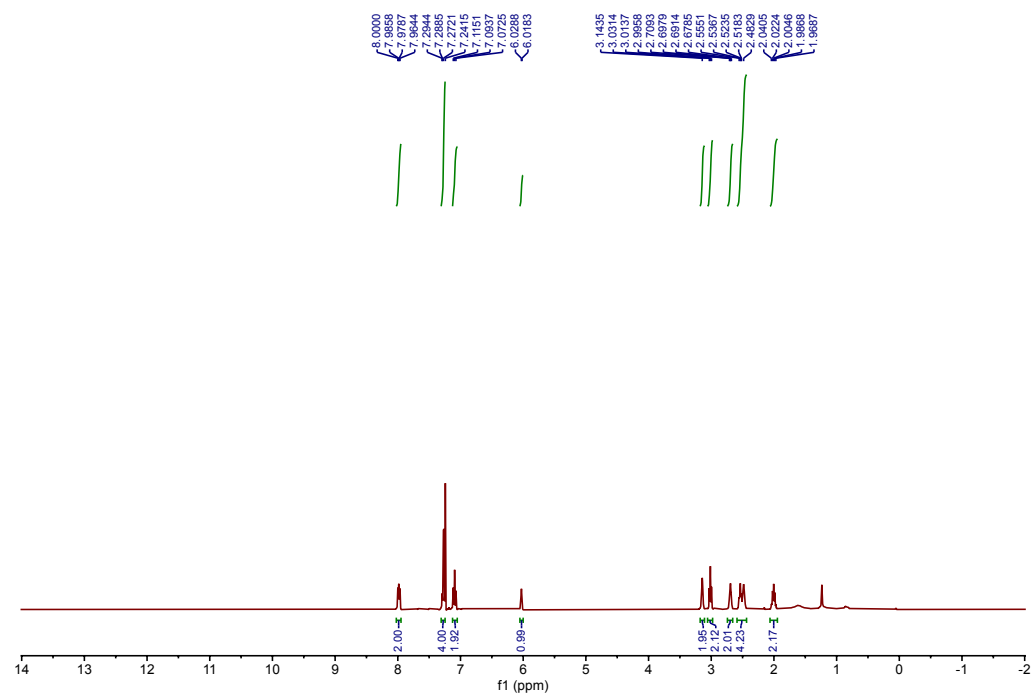


Fig. S72:  $^1\text{H}$  NMR spectrum for compound **30** (SGT572) in  $\text{CDCl}_3$ .

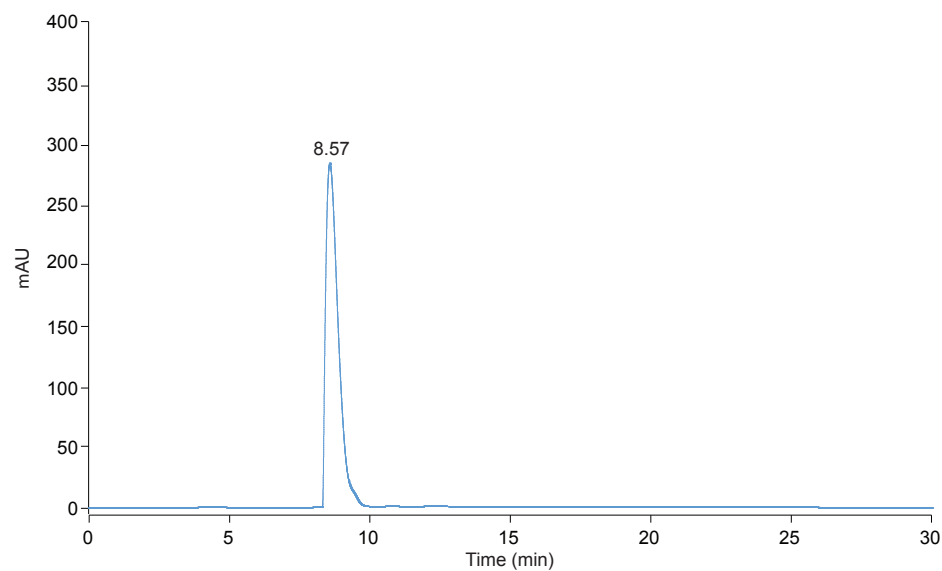


Fig. S73: HPLC trace for compound **30** (SGT572).  $R_t = 8.57$  min.

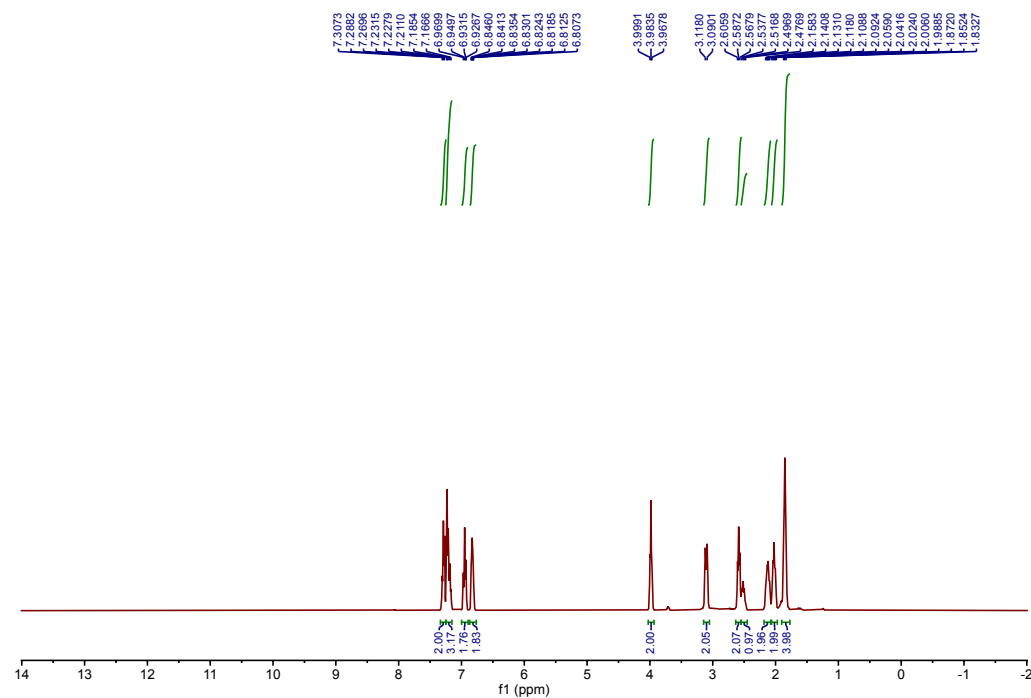
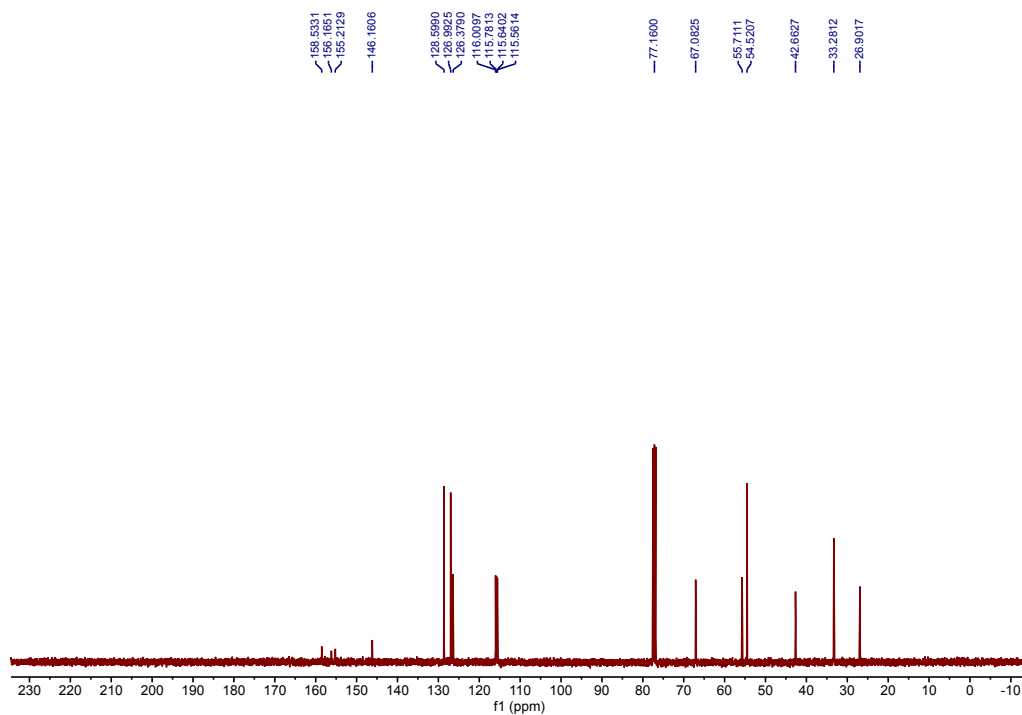
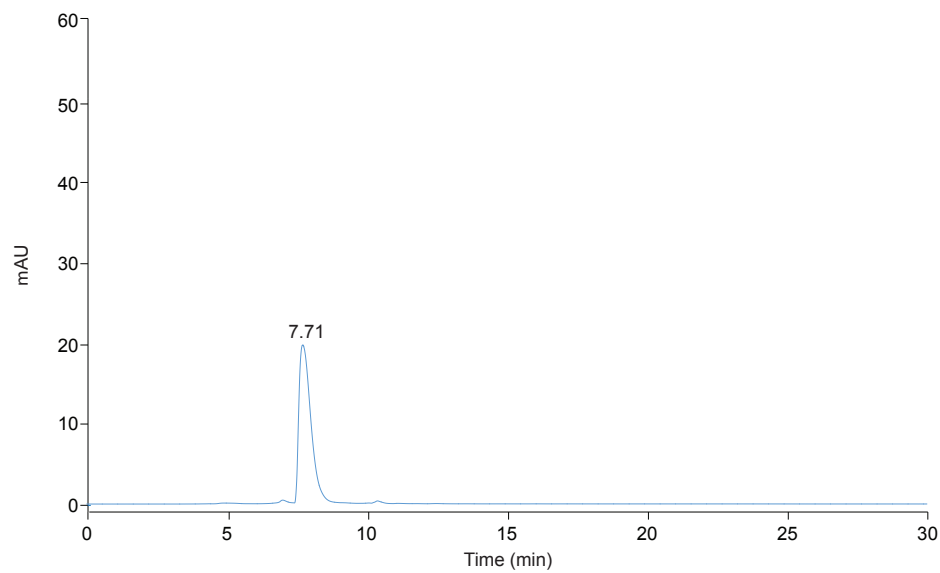


Fig. S74:  $^1\text{H}$  NMR spectrum for compound **31** (SGT573) in  $\text{CDCl}_3$ .



**Fig. S75:**  $^{13}\text{C}$  NMR spectrum for compound **31** (SGT573) in  $\text{CDCl}_3$ .



**Fig. S76:** HPLC trace for compound **31** (SGT573).  $R_t = 7.71$  min.

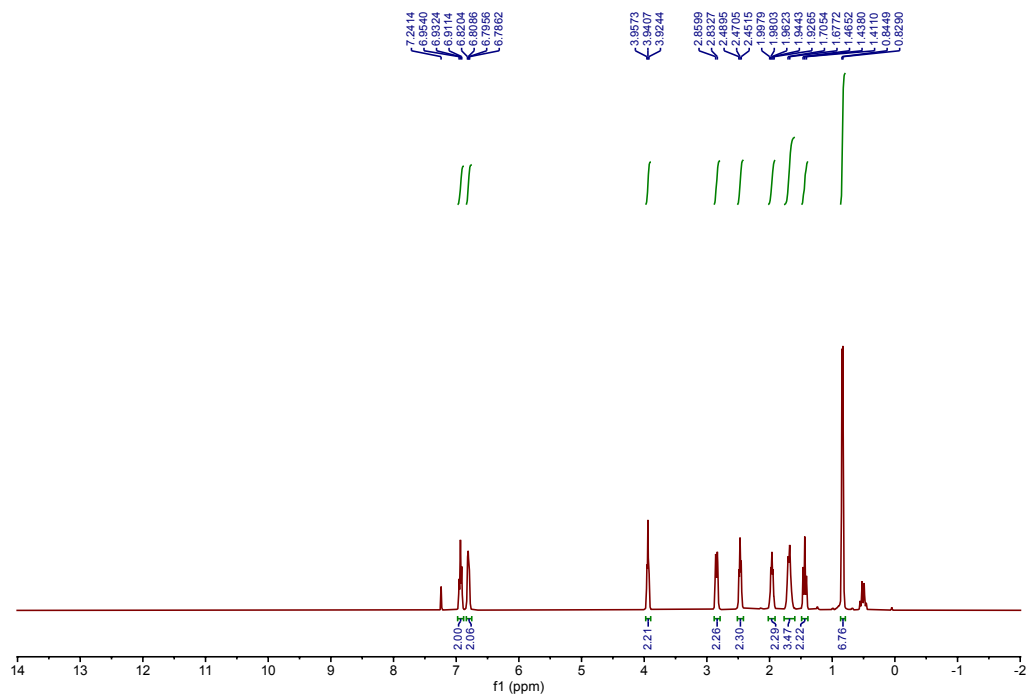


Fig. S77:  $^1\text{H}$  NMR spectrum for compound **32** (SGT574) in  $\text{CDCl}_3$ .

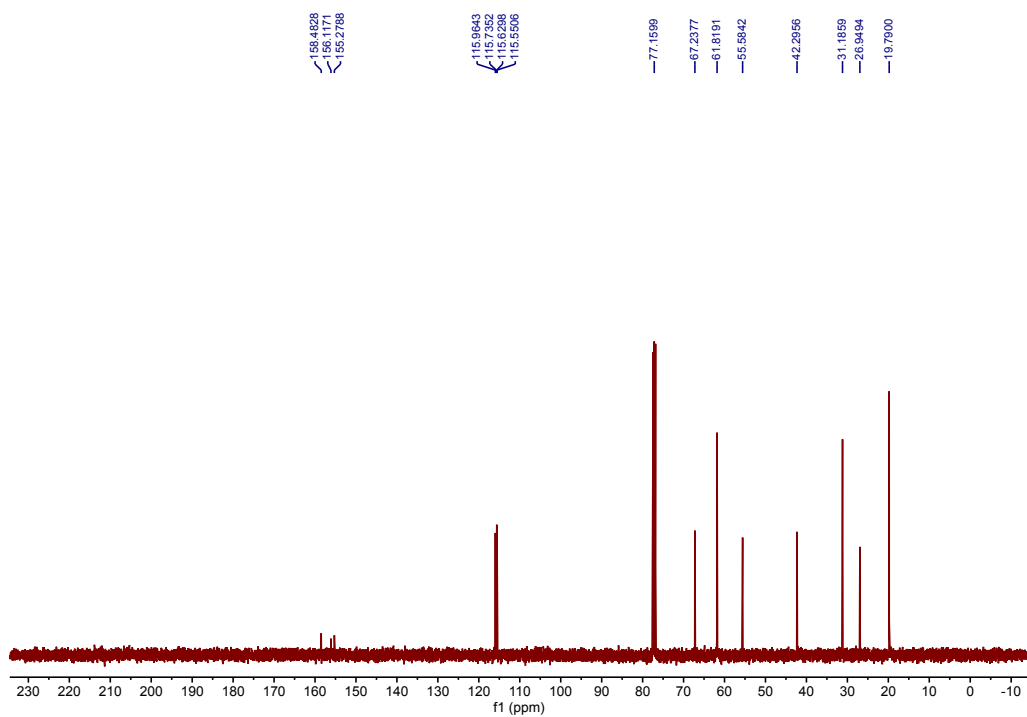


Fig. S78:  $^{13}\text{C}$  NMR spectrum for compound **32** (SGT574) in  $\text{CDCl}_3$ .

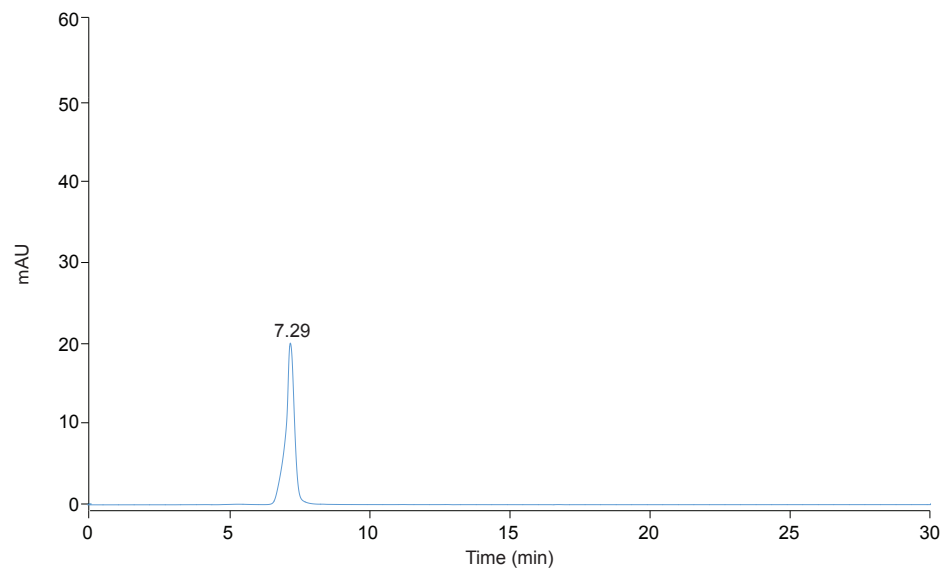


Fig. S79: HPLC trace for compound **32** (SGT574).  $R_t = 7.29$  min.

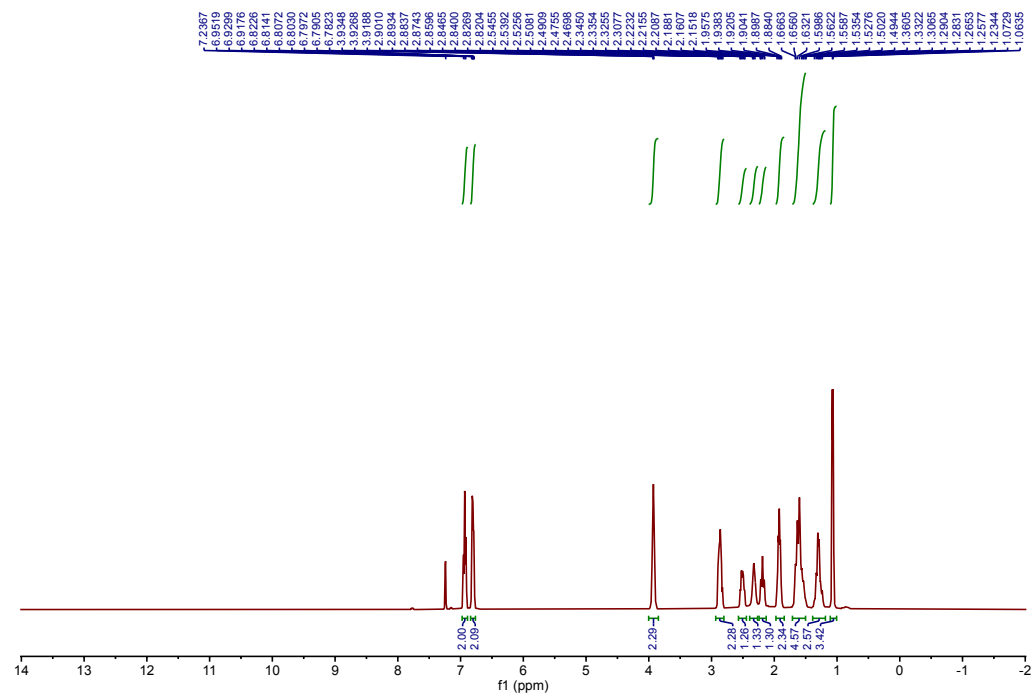
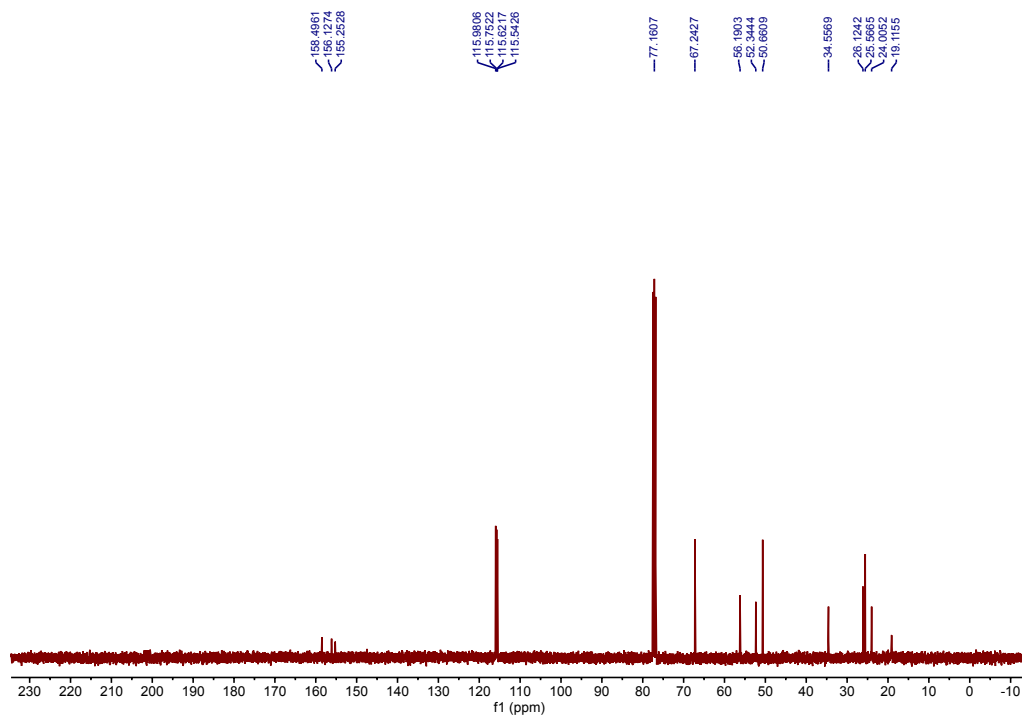
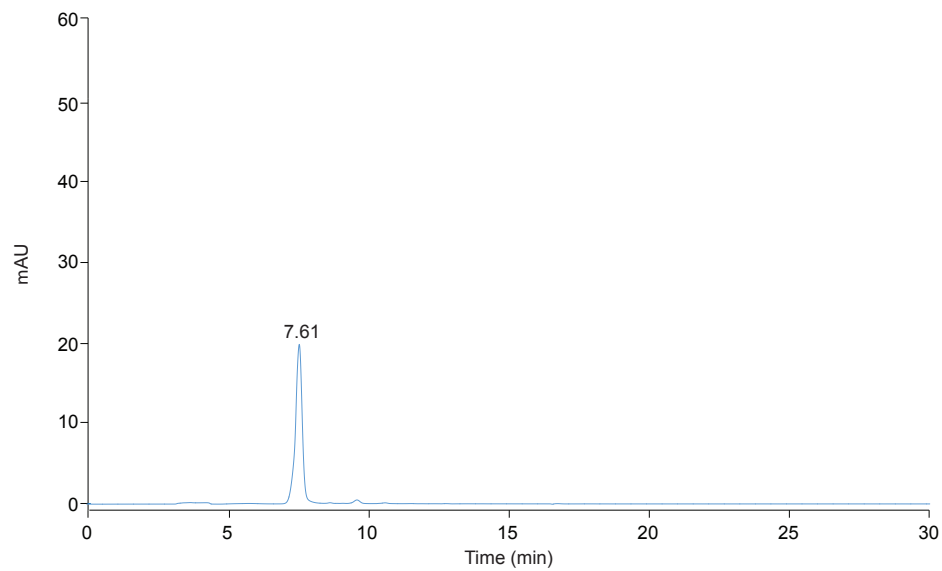


Fig. S80:  $^1\text{H}$  NMR spectrum for compound **33** (SGT575) in  $\text{CDCl}_3$ .



**Fig. S81:**  $^{13}\text{C}$  NMR spectrum for compound **33** (SGT575) in  $\text{CDCl}_3$ .



**Fig. S82:** HPLC trace for compound **33** (SGT575).  $R_t = 7.61$  min.



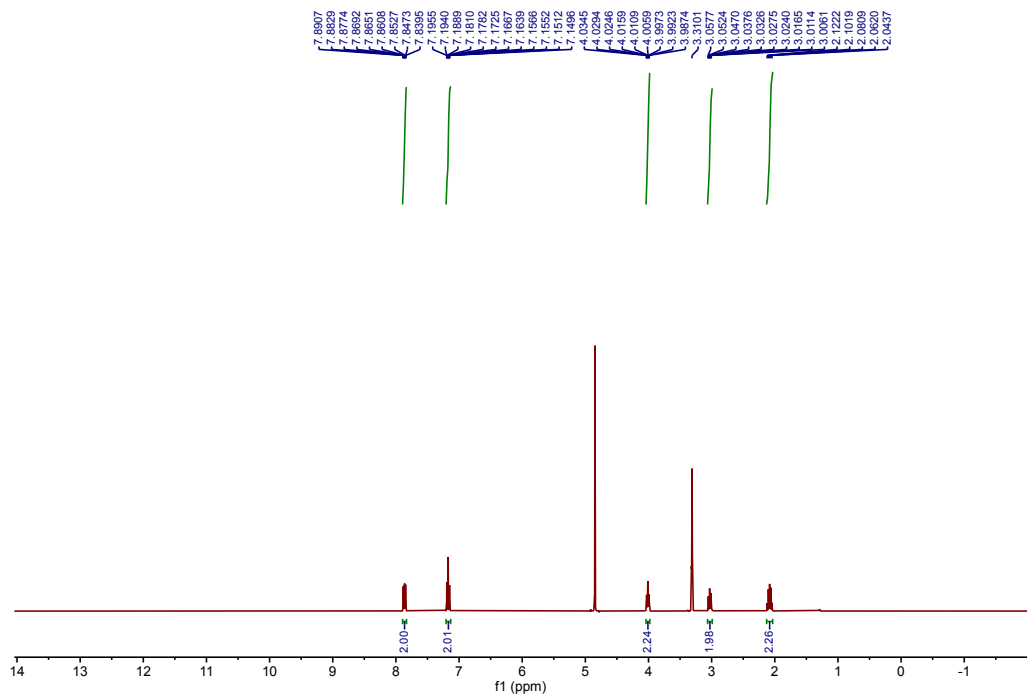


Fig. S83:  $^1\text{H}$  NMR spectrum for compound **34** (SGT1416) in  $\text{CD}_3\text{OD}$ .

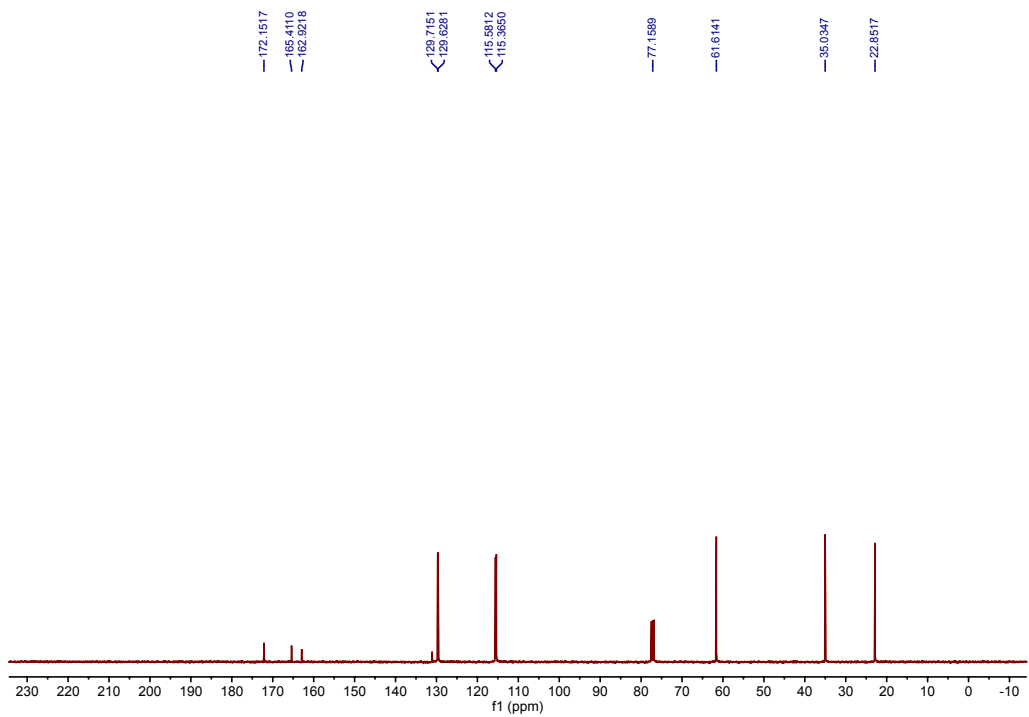


Fig. S84:  $^{13}\text{C}$  NMR spectrum for compound **34** (SGT1416) in  $\text{CDCl}_3$ .

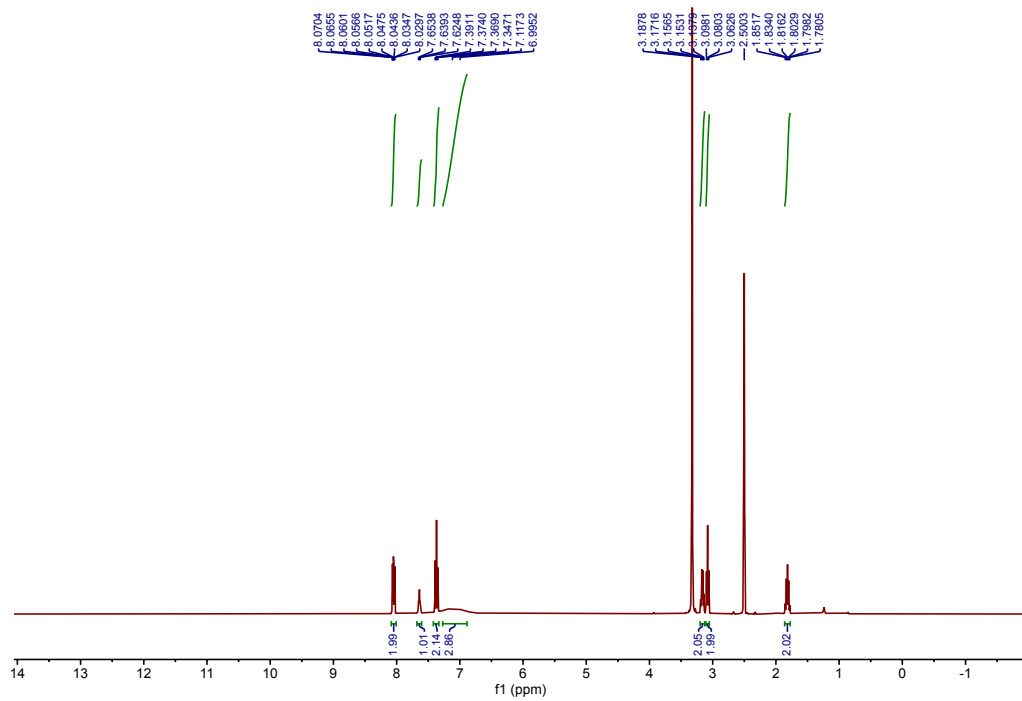


Fig. S85:  $^1\text{H}$  NMR spectrum for compound **35** (SGT1264) in  $(\text{CD}_3)_2\text{SO}$ .

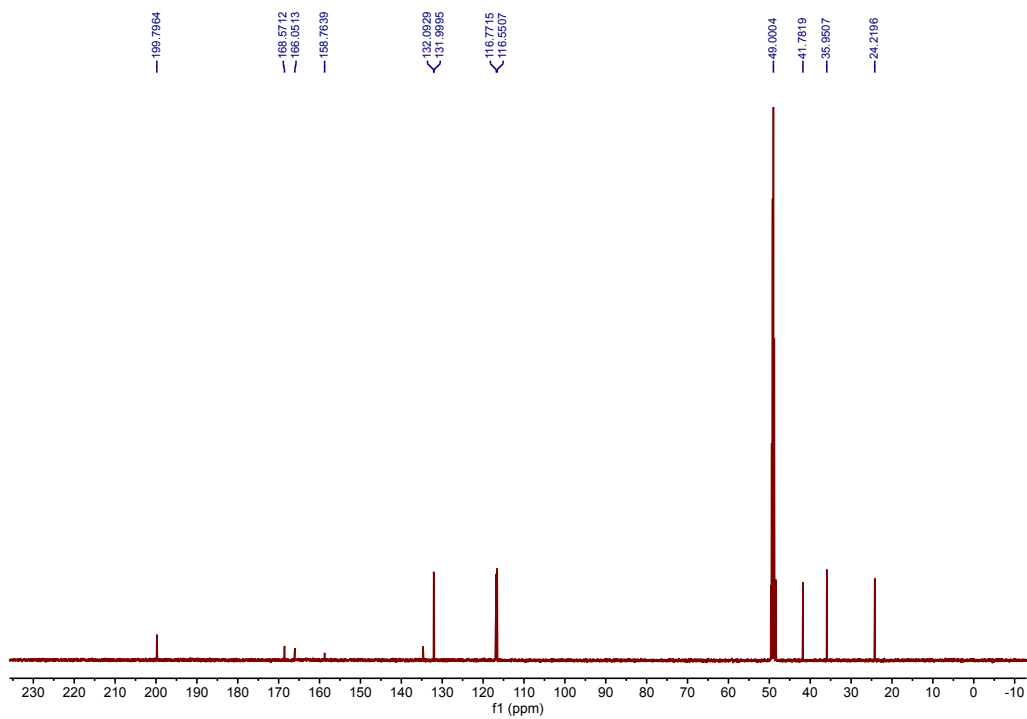
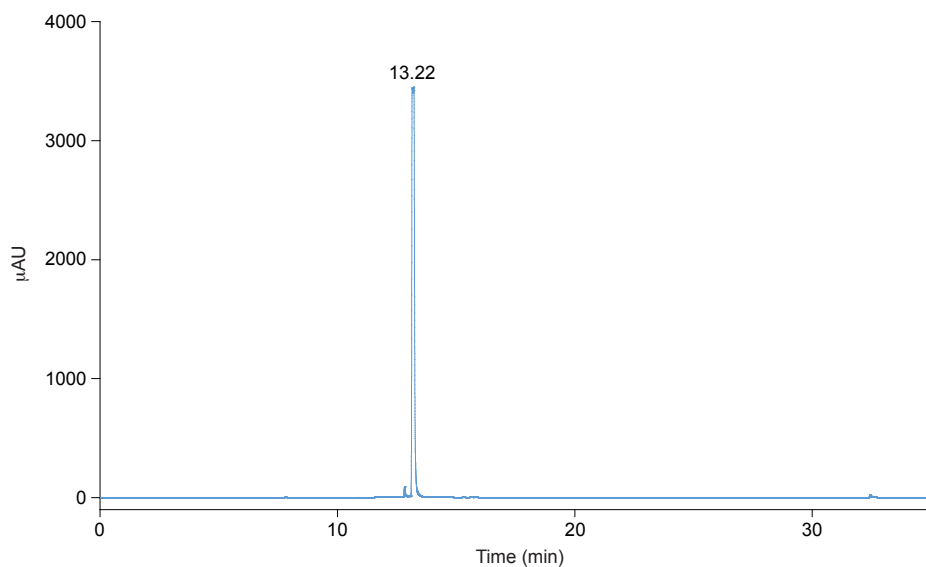
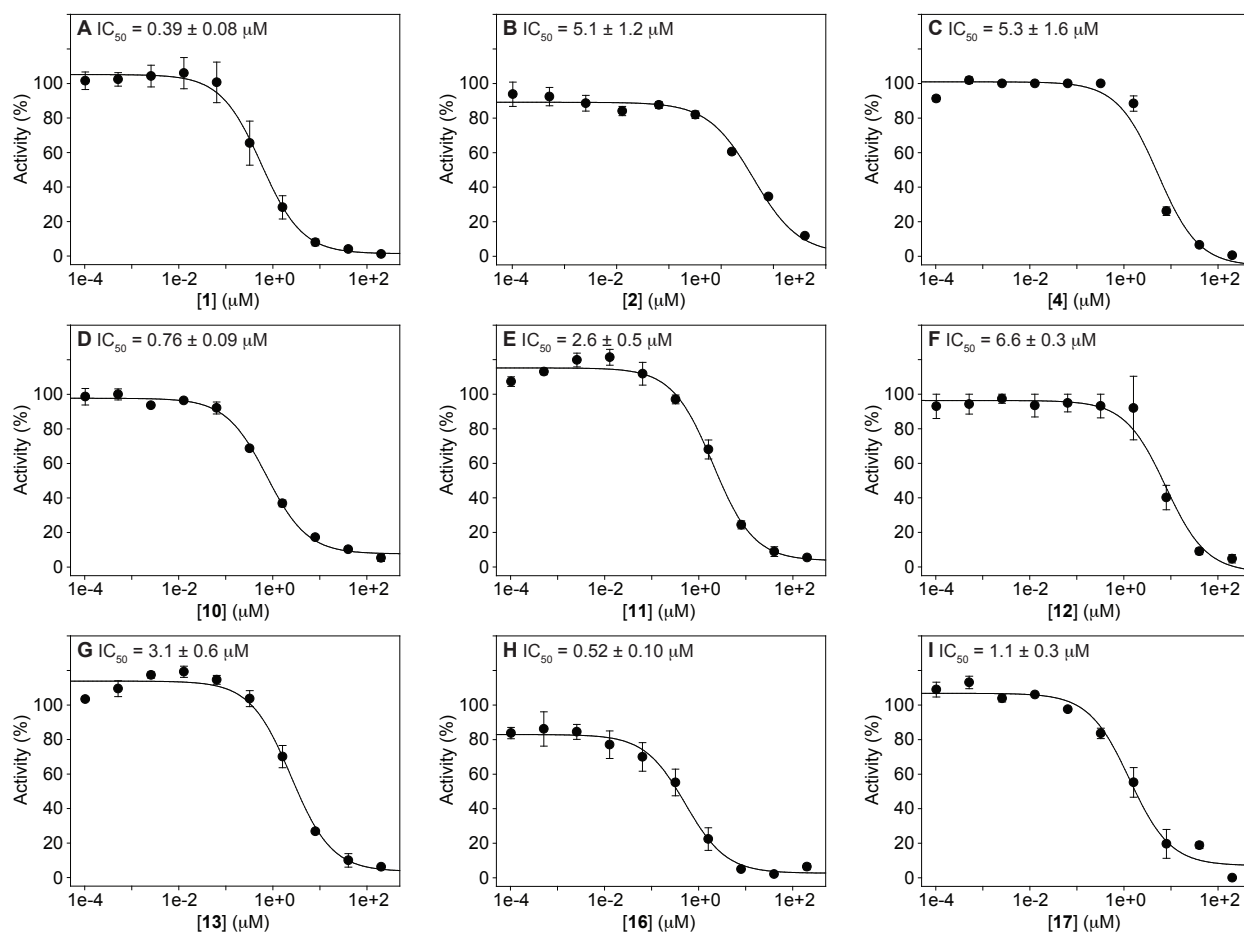


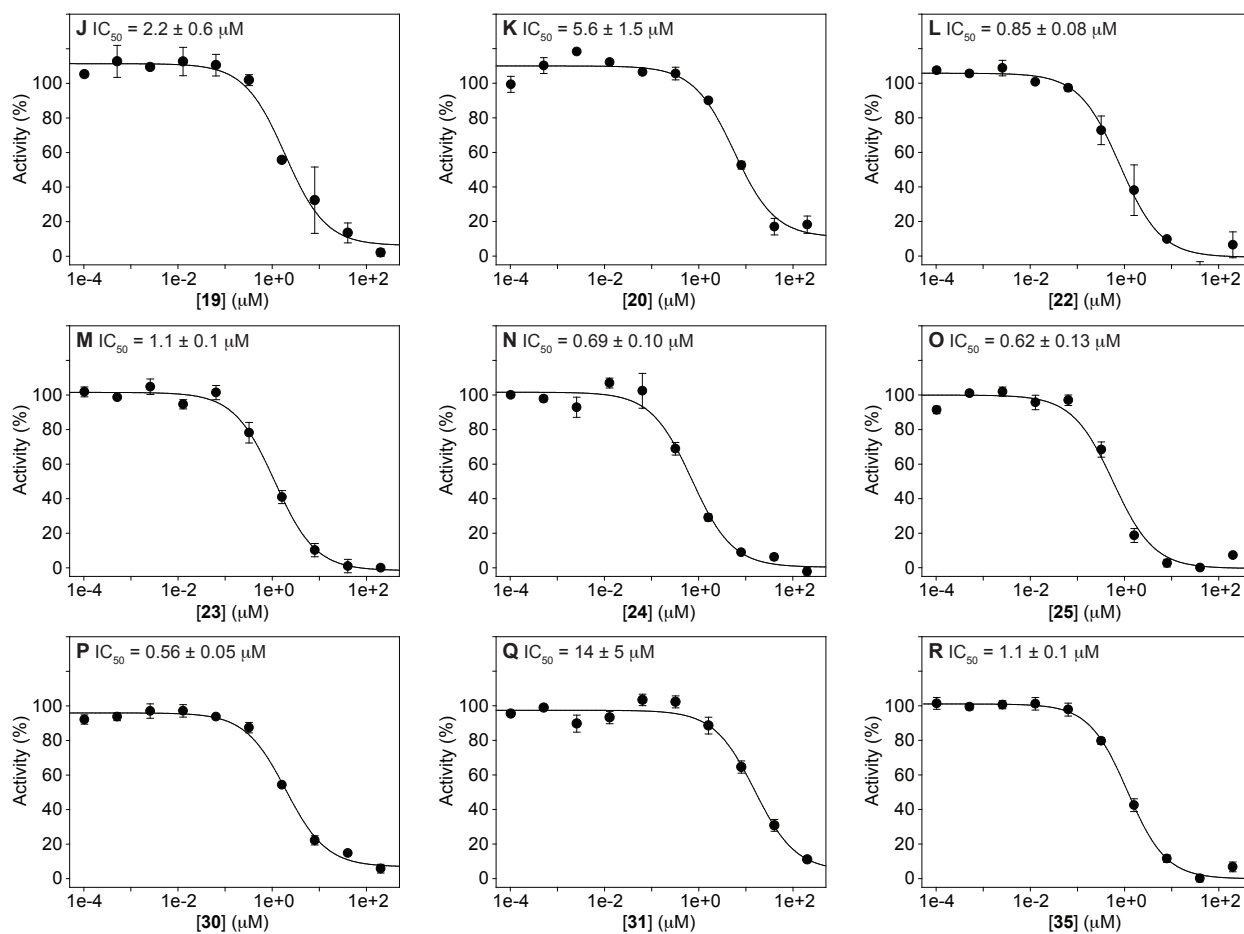
Fig. S86:  $^{13}\text{C}$  NMR spectrum for compound **35** (SGT1264) in  $\text{CD}_3\text{OD}$ .



**Fig. S87:** HPLC trace for compound **35** (SGT1264).  $R_t = 13.22$  min.



**Fig. S88:**  $IC_{50}$  curves for compounds **A. 1**, **B. 2**, **C. 4**, **D. 10**, **E. 11**, **F. 12**, **G. 13**, **H. 16**, and **I. 17** against Eis by using KAN as a substrate.



**Fig. S89:** IC<sub>50</sub> curves for compounds J, K, L, M, N, O, P, Q, and R against Eis by using KAN as a substrate.