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Supplemental Information

Modulation of Curli Assembly and Pellicle Biofilm Formation by Chemical and Protein Chaperones

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Inventory of Supplemental Information

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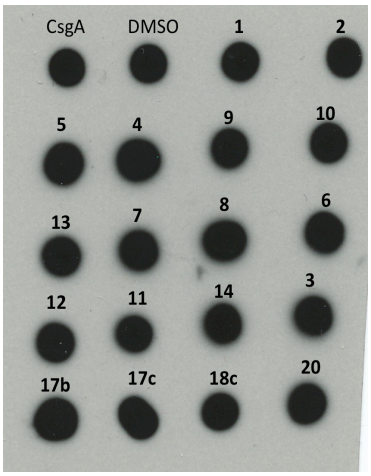
Supplemental Data

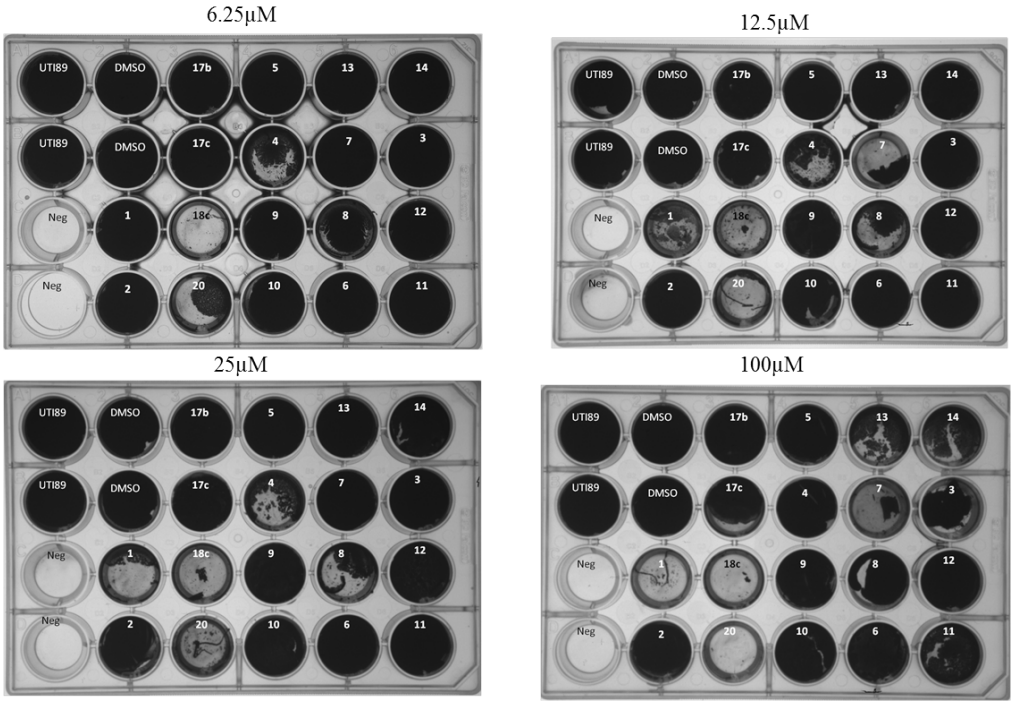
Figure S1, related to Figure 3 and Figure 4.



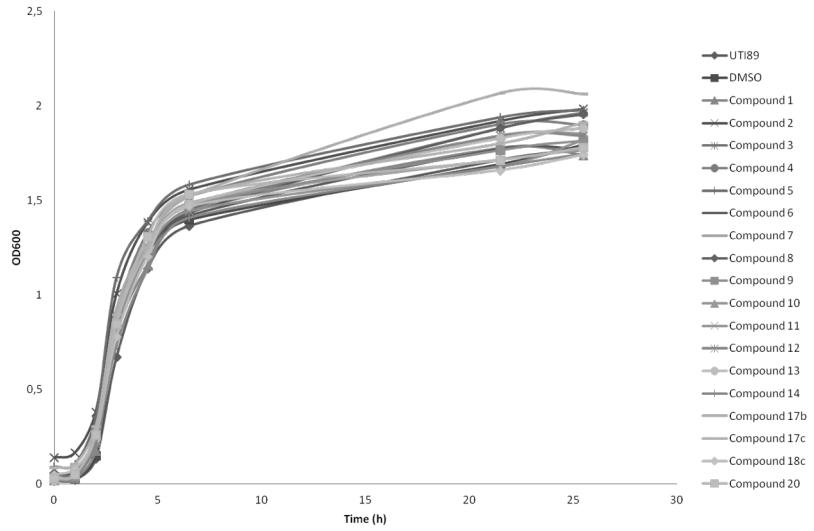
CsgA loading control for gammabody experiments probed with anti-CsgA antibody, detecting all CsgA.

Figure S2, related to Figure 5.

A



B



A. Effect of the compounds on the formation of curli-dependent pellicle biofilm, concentrations not shown in Fig. 5b.

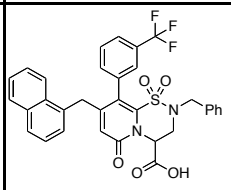
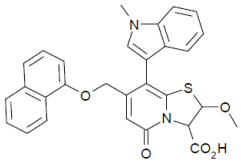
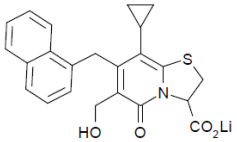
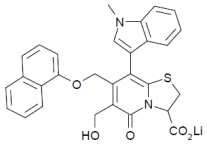
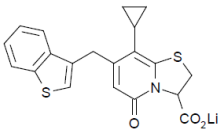
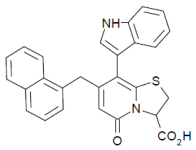
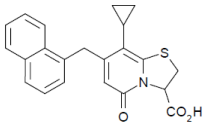
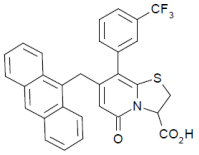
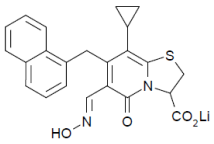
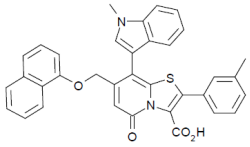
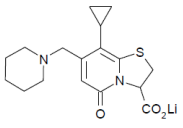
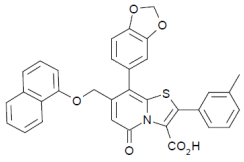
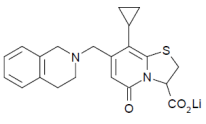
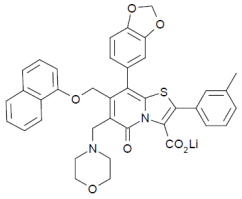
B. Effect of pellicle inhibiting compounds on bacterial growth monitored as optical density at 600nm during incubation with 50µM compound. No compound tested inhibited bacterial growth.

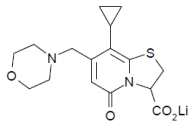
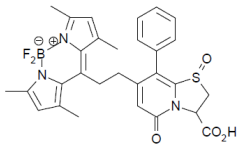
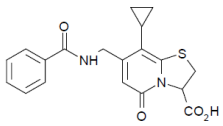
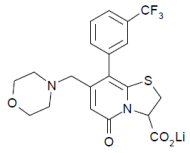
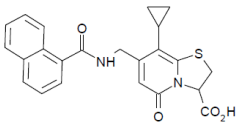
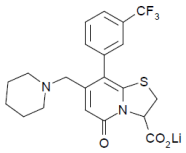
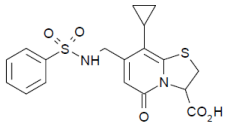
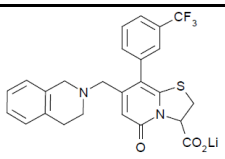
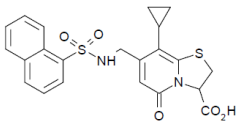
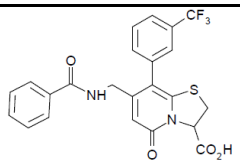
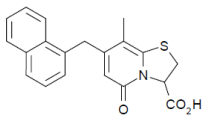
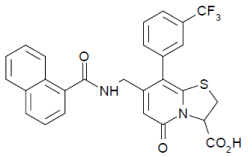
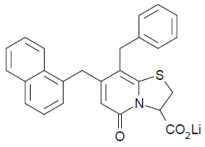
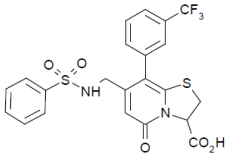
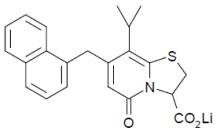
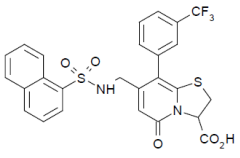
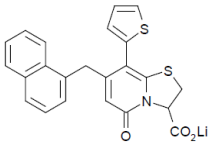
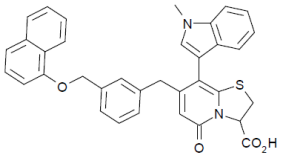
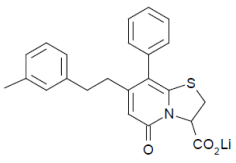
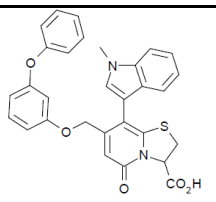
Table S1, related to Figure 1 and Table 1.

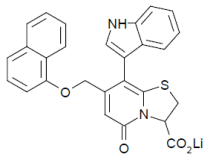
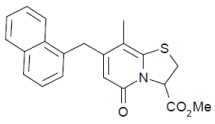
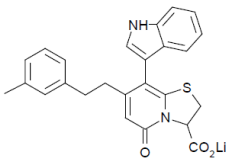
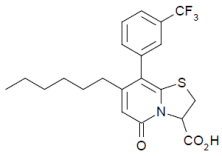
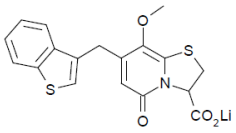
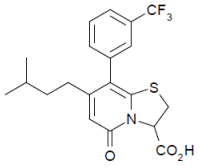
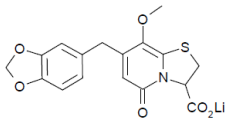
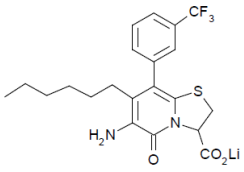
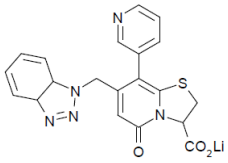
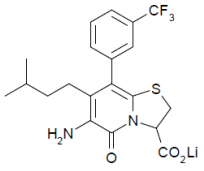
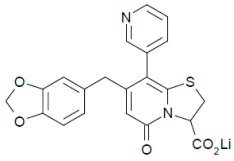
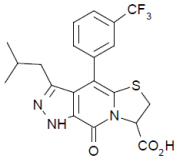
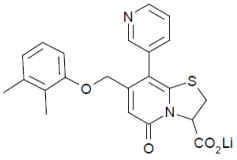
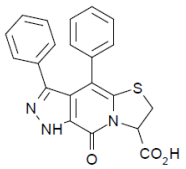
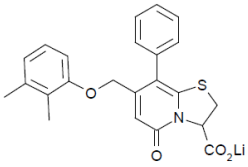
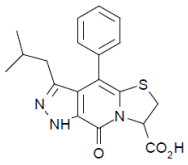
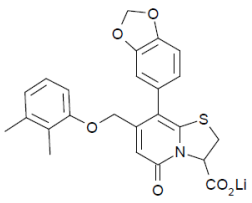
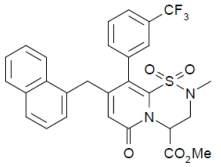
Library of compounds.

ThT fluorescence inhibition after overnight incubation.

- 0-20%, +/- 20-50%, + 50-75%, ++ 75-90%, +++ 90-100%.

ID	Structure	Inhibition	ID	Structure	Inhibition
4 (Sellstedt and Almqvist, 2008)		+	**51** (Chorell, et al., 2012)		++
21 (Pemberton, et al., 2007)		-	**52** (Chorell, et al., 2012)		++
22 (Chorell, et al., 2012)		-	**53** (Chorell, et al., 2012)		++
23 (Pinkner, et al., 2006)		-	**54** (Chorell, et al., 2012)		++
24 (Pemberton, et al., 2007)		-	**55** (Chorell, et al., 2012)		+
25 (Chorell, et al., 2011)		-	**56** (Chorell, et al., 2012)		+/-
26 (Chorell, et al., 2011)		-	**57** (Chorell, et al., 2012)		++

27 (Chorell, et al., 2011)		-	**58** (Chorell, et al., 2012)		-
28 (Chorell, et al., 2011)		-	**59** (Chorell, et al., 2011)		+/-
29 (Chorell, et al., 2011)		-	**60** (Chorell, et al., 2011)		+/-
30 (Chorell, et al., 2011)		-	**61** (Chorell, et al., 2011)		-
31 (Chorell, et al., 2011)		-	**62** (Chorell, et al., 2011)		-
32 (Åberg, et al., 2005)		-	**63** (Chorell, et al., 2011)		-
33 (Åberg, et al., 2005)		+/-	**64** (Chorell, et al., 2011)		-
34 (Chorell, et al., 2012)		-	**65** (Chorell, et al., 2011)		++
35 (Chorell, et al., 2012)		-	**66** (Chorell, et al., 2012)		+
36 (Chorell, et al., 2012)		+/-	**67** (Chorell, et al., 2012)		++

37 (Chorell, et al., 2012)		++	**68** (Åberg, et al., 2005)		-
38 (Chorell, et al., 2012)		++	**69** (Almqvist, et al., 2008)		+/-
39 (Chorell, et al., 2012)		-	**70** (Almqvist, et al., 2008)		+/-
40 (Chorell, et al., 2012)		-	**71** (Almqvist, et al., 2008)		+
41 (Chorell, et al., 2012)		-	**72** (Almqvist, et al., 2008)		+
42 (Chorell, et al., 2012)		-	**73** (Almqvist, et al., 2008)		+/-
43 (Chorell, et al., 2012)		-	**74** (Sellstedt and Almqvist, 2008)		-
44 (Chorell, et al., 2012)		+	**75** (Sellstedt and Almqvist, 2008)		-
45 (Chorell, et al., 2012)		+	**76** (Sellstedt and Almqvist, 2009)		-

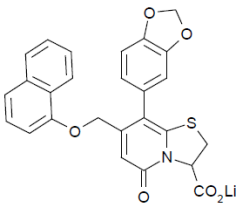
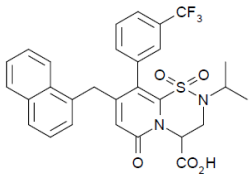
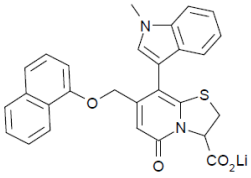
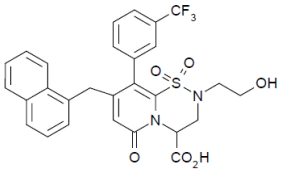
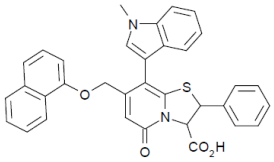
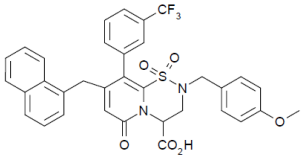
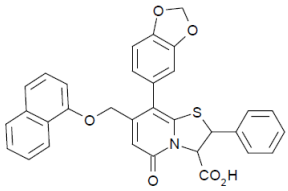
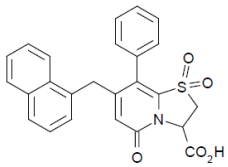
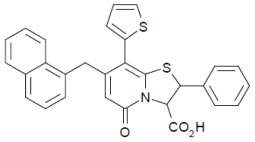
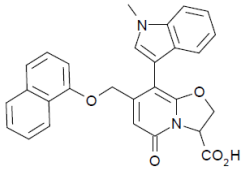
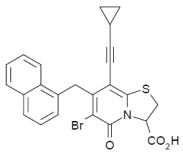
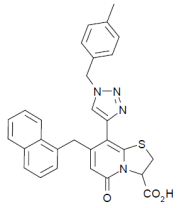
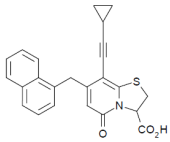
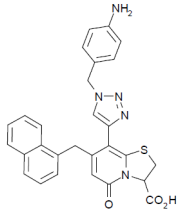
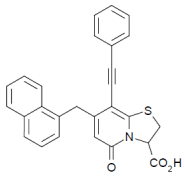
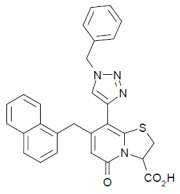
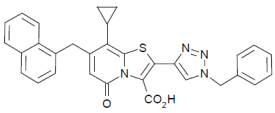
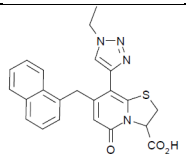
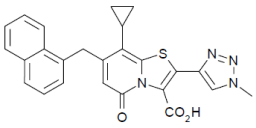
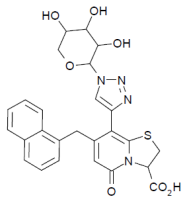
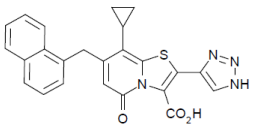
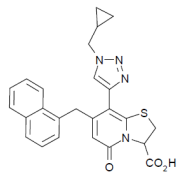
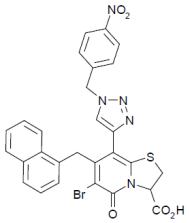
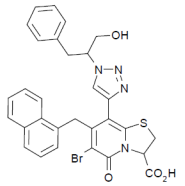
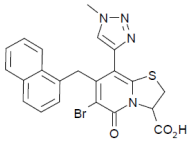
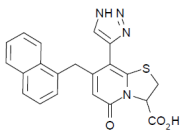
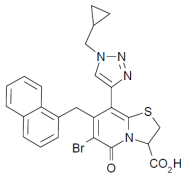
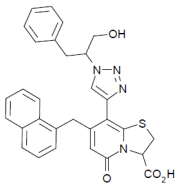
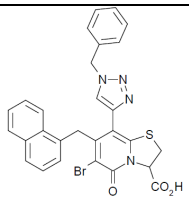
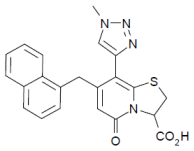
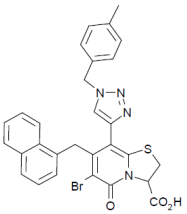
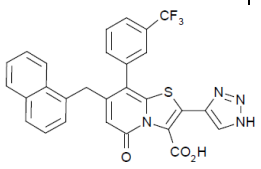
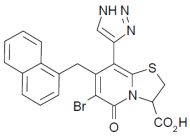
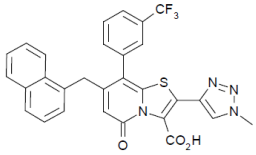
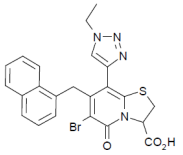
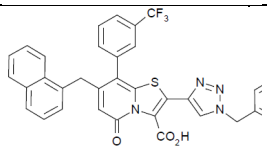
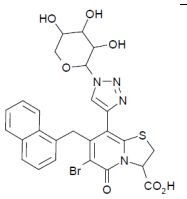
46 (Chorell, et al., 2012)		+	**77** (Sellstedt and Almqvist, 2009)		++
47 (Chorell, et al., 2012)		+	**78** (Sellstedt and Almqvist, 2009)		++
48 (Chorell, et al., 2012)		+/-	**79** (Sellstedt and Almqvist, 2009)		-
49 (Chorell, et al., 2012)		-	**80** (Sellstedt and Almqvist, 2009)		-
50 (Chorell, et al., 2012)		+/-	**81** (Chorell, et al., 2012)		++

Table S2, related to Figure 2.

Compounds synthesized by using acetylenes as the linker moiety to expand the library with triazoles.

Compound	Structure	Inhibition	Compound	Structure	Inhibition
17a		-	**93** (Bengtsson, et al., 2012)		-
18a		-	**94** (Bengtsson, et al., 2012)		-
18b		++	**95** (Bengtsson, et al., 2012)		-
82 (Bengtsson, et al., 2012)		-	**96** (Bengtsson, et al., 2012)		-
83 (Bengtsson, et al., 2012)		-	**97** (Bengtsson, et al., 2012)		-
84 (Bengtsson, et al., 2012)		-	**98** (Bengtsson, et al., 2012)		-
85 (Bengtsson, et al., 2012)		-	**99** (Bengtsson, et al., 2012)		-
86 (Bengtsson, et al., 2012)		-	**100** (Bengtsson, et al., 2012)		+

87 (Bengtsson, et al., 2012)		-	**101** (Bengtsson, et al., 2012)		+
88 (Bengtsson, et al., 2012)		+	**102** (Bengtsson, et al., 2012)		-
89 (Bengtsson, et al., 2012)		+/-	**103** (Bengtsson, et al., 2012)		+
90 (Bengtsson, et al., 2012)		+/-	**104** (Bengtsson, et al., 2012)		+
91 (Bengtsson, et al., 2012)		+/-	**105** (Bengtsson, et al., 2012)		-
92 (Bengtsson, et al., 2012)		+/-			

Supplemental Experimental procedures

Compound synthesis and characterization.

General

Unless stated otherwise, all reagents and solvents were used as received from commercial suppliers. DMF was dried in a solvent drying system equipped with activated molecular sieves and an isocyanate scrubber and was collected freshly prior to every reaction. CuI was purified by refluxing with DCM for 20 h in a Soxhlet apparatus and stored under dark and dry conditions. Zinc was activated by stirring with 10% HCl (aq) for 2 min, then filtering and washing with H_2O, MeOH and Et_2O. The Zinc metal was then dried under vacuum for 16h and stored in a desiccator under inert atmosphere (N_2). Preparatory HPLC was performed on a C18 reversed-phase column (25cm x 21.2mm, 5 μm) with H_2O:MeCN mixtures as eluent. Microwave heated reactions were performed in a microwave reactor; temperatures were monitored with an IR-probe. TLC was performed on silica gel detected with UV-light. Column chromatography was employed on normal phase silica gel (eluents given in brackets). Optical rotation was measured with a polarimeter at 20 °C and 589nm. IR was recorded on a spectrometer equipped with an ATR device. ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz or a 500 MHz spectrometer at 298 K and calibrated using the residual peak of solvent as internal standard [$CDCl_3$ ($CHCl_3$ δ_H 7.26 ppm, $CDCl_3$ δ_C 77.16 ppm), d6-DMSO (d5-DMSO δ_H 2.5 ppm, d6-DMSO δ_C 39.5 ppm)]. HRMS was performed using a mass spectrometer with electrospray ionization (ES^+), sodiumformate was used as calibration chemical.

(3*R*)-6-Bromo-8-cyclopropylethynyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3R-carboxylic acid methyl ester (16a). 15 (100 mg, 0.18 mmol), CuI (6.8 mg, 0.036 mmol), $Pd(PPh_3)_2Cl_2$ (12.6 mg, 0.018 mmol) and Et_3N (50 μl, 0.36 mmol) was dissolved in DMF (2.5 ml) and cyclopropylacetylene (46 μl, 0.54 mmol) in DMF (1.5 ml) was added dropwise over 5 minutes and the reaction was stirred at rt for 22 h. The reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography on silica gel (Heptane:EtOAc 80:20→ 55:45) gave **16a** (73.3 mg, 82%). $[\alpha]_D$ -144 (*c* 0.51, $CDCl_3$); IR 2952, 1748, 1644, 1577, 1474, 1212, 1001, 790, 773 cm^{-1}; ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.14 (d, ***J*** = 8.8 Hz, 1H), 7.89 (d, ***J*** = 8.8 Hz, 1H), 7.74 (d, ***J*** = 8.8 Hz, 1H), 7.62-7.56 (m, 1H), 7.55-7-50 (m, 1H), 7.36-7.31 (m, 1H), 6.94 (dd, ***J*** = 7.2, 0.92 Hz, 1H), 5.71 (dd, ***J*** = 8.8, 2.4 Hz, 1H), 4.65 (d, ***J*** = 3.3 Hz, 2H), 3.85 (s, 3H), 3.79 (dd, ***J*** = 11.6, 0.68 Hz, 1H), 3.57 (d, ***J*** = 12.0, 2.0 Hz, 1H), 1.11-1.03 (m, 1H), 0.55-0.49 (m, 2H), 0.16-0.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 157.2, 150.1, 133.9, 132.3, 132.0, 129.0, 127.2, 126.2, 125.8, 125.7, 125.7, 124.2, 123.2, 113.5, 102.0, 100.2, 69.1, 65.3, 53.7, 39.0, 31.9, 8.8 (split, 2C), 0.1 LRMS (M+H) 495

(3*R*)-6-Bromo-7-naphthalen-1-ylmethyl-5-oxo-8-phenylethynyl-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid methyl ester (16b). 15 (50 mg, 0.09 mmol), CuI (3.4 mg, 0.018 mmol), $Pd(PPh_3)_2Cl_2$ (6.3 mg, 0.009 mmol) and Et_3N (25 μl, 0.18 mmol) was dissolved in DMF (1.25 ml) and Phenylacetylene (30 μl, 0.27 mmol) in DMF (0.75 ml) was added drop wise over 5 minutes. The vial was capped and stirred at rt under nitrogen atmosphere for 22 h. The reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. Purification by flash column chromatography on silica gel (Heptane:EtOAc 7:3) gave **16b** (39 mg, 82%). $[\alpha]_D$ -132 (*c* 0.53, $CDCl_3$); IR 2922, 2337, 1733, 1652, 1578, 1474, 1353, 1228, 1166, 993, 753 cm^{-1}; ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.17 (d, ***J*** = 8 Hz, 1H), 7.90 (d, ***J*** = 8 Hz, 1H), 7.75 (d, ***J*** = 8 Hz, 1H), 7.55 (q, ***J*** = 6.8 Hz, 2H), 7.36 (d, ***J*** = 8 Hz, 1H), 7.197-7.138 (m, 1H), 7.123-7.014 (m, 3H), 6.83

(d, ***J*** = 7.6 Hz, 2H), 5.75 (dd, ***J*** = 8.8, 2 Hz, 1H), 4.86 (d, ***J*** = 2 Hz, 2H), 3.910-3.803 (m, 4H), 3.62 (dd, ***J*** = 12, 2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 157.2, 153.4, 151.5, 133.9, 132.2, 132.0, 131.1 (2C), 129.0, 128.4, 128.2 (2C), 127.3, 126.4, 125.8 (2C), 124.3, 123.2, 122.2, 113.8, 99.6, 97.3, 82.9, 65.4, 53.8, 38.1, 32.0 LRMS (M+H) 531

(3*R*)-methyl 6-bromo-7-(naphthalen-1-ylmethyl)-5-oxo-8-((3-(trifluoromethyl)phenyl)ethynyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylate (16c). **15** (0.18mmol, 100mg), $Pd(PPh_3)_2Cl_2$ (0.018mmol, 12mg), CuI (0.036mmol, 7mg) and TEA (0.36mmol, 50μl) were dissolved in dry DMF (1.5ml) and stirred at rt for 10 min. 3-Ethynyl-α,α,α-trifluorotoluene (0.54mmol, 78μl) dissolved in dry DMF (1ml) was added and the reaction was heated with an oilbath at 50 ^{o}C for 4 h. The reaction mixture was diluted with saturated $NaHCO_3$ (aq) and extracted with EtOAc, the organic phase was dried (Na_2SO_4), filtered and concentrated. The crude material was purified with column chromatography on silica gel (heptane:EtOAc 80:20→50:50) to give **16c** as a yellow foam (98mg, 91%) $[\alpha]_D$ -65 (c0.5, $CHCl_3$) IR λ 1752, 1737, 1648, 1578, 1478, 1436, 1327 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$)δ 8.16 (d, ***J***= 8.4Hz, 1H), 7.92-7.88 (m, 1H), 7.76 (d, ***J***= 8.0Hz, 1H), 7.59-7.50 (m, 2H), 7.41-7.34 (m, 2H), 7.21-7.14 (m, 1H), 7.04 (d, ***J***= 6.8Hz, 1H), 6.99 (s, 1H), 6.89 (d, ***J***= 8.0Hz, 1H), 5.76 (dd, ***J***= 8.6, 2.4Hz, 1H), 4.81-4.70 (m, 2H), 3.88 (s, 3H), 3.86 (dd, ***J***= 12, 8.6Hz, 1H), 3.64 (dd, ***J***= 12, 2.4 ^{13}C-NMR (100 MHz, $CDCl_3$)δ 167.9, 157.1, 153.3, 152.1, 134.1, 134.0, 132.1, 131.9, 130.8 (q, ***J***= 31Hz, 1C), 129.1, 128.7, 127.7 (split, 1C), 127.5, 126.5, 126.0, 125.8, 124.9 (split, 1C), 124.4, 123.6 (q, ***J***= 271Hz. 1C), 123.2, 123.0, 113.9, 98.8, 95.7, 84.5, 65.4, 53.8, 38.1, 32.1 LRMS (M+H) 599

(3*R*)-6-Bromo-8-cyclopropylethynyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3R-carboxylic acid (17a). 16a (93.0 mg, 0.188 mmol) was dissolved in THF (5 ml) and 1 M LiOH (0.376 ml, 0.376 mmol) was added. The mixture was stirred at room temperature for 41 h. The suspension was diluted with EtOAc and brine was added. 1 M HCl was added until pH = 1. The organic phase was dried (Na_2SO_4), filtered and concentrated. Purification by HPLC gave **17a** (56.0 mg, 63%). $[\alpha]_D$ -10 (*c* 0.10, DMSO); IR 1732, 1611, 1557, 1480, 1396, 1232, 1121, 766 cm^{-1}; ^{1}H NMR, (400 MHz, d_6-DMSO) δ 8.24 (d, ***J*** = 8.3 Hz, 1H), 7.97 (d, ***J*** = 7.9 Hz, 1H), 7.82 (d, ***J*** = 8.1 Hz, 1H), 7.68-7.55 (m, 2H), 7.40 (t, ***J*** = 7.6 Hz, 1H), 6.82 (d, ***J*** = 7.0 Hz, 1H), 5.63 (d, ***J*** = 8.9 Hz, 1H), 4.62-4.49 (m, 2H), 3.99 (dd, ***J*** = 11.8, 9.5 Hz, 1H), 3.66 (d, ***J*** = 11.8 Hz, 1H), 1.22-1.13 (m, 1H), 0.60-0.46 (m, 2H), 0.07-0.05 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.1, 155.9, 152.8, 152.4, 133.3, 132.2, 131.4, 128.6, 126.9, 126.4, 125.9, 125.5, 123.2 (split, 2C), 111.9, 100.8, 97.6, 69.2, 65.1, 37.5, 31.7, 8.4 (split, 2C), -0.5; HRMS (ES) calcd [M + Na] for $C_{24}H_{18}BrNNaO_3S$ 502.0088, obsd 502.0086

(3*R*)-6-Bromo-7-naphthalen-1-ylmethyl-5-oxo-8-phenylethynyl-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (17b). 16b (91.0 mg, 0.17 mmol) was dissolved in THF (5 ml) and 1 M LiOH (0.34 ml, 0.34 mmol) was added. The mixture was stirred at room temperature for 40 h. The suspension was diluted with EtOAc and brine was added. 1 M HCl was added until pH = 1. The organic phase was dried (Na_2SO_4), filtered and concentrated. Purification by HPLC gave **17b** (55 mg, 67%). $[\alpha]_D$ -9 (*c* 0.10, DMSO); IR 1746, 1611, 1594, 1557, 1479, 1208, 1169, 790 cm^{-1}; ^{1}H NMR, (400 MHz, d_6-DMSO) δ 8.31 (d, ***J*** = 8.1 Hz, 1H), 7.98 (d, ***J*** = 7.7 Hz, 1H), 7.82 (d, ***J*** = 8.2 Hz, 1H), 7.66-7.55 (m, 2H), 7.46-7.38 (m, 1H), 7.27-7.20 (m, 1H), 7.19-7.11 (m, 2H), 6.93 (d, ***J*** = 7.0 Hz, 1H), 6.82 (d, ***J*** = 7.5 Hz, 2H), 5.69 (d, ***J*** = 8.9 Hz, 1H), 4.77-4.62 (m, 2H), 4.05 (dd, ***J*** = 12.0, 9.0 Hz, 1H), 3.72 (d, ***J*** = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.0, 156.0, 153.8, 152.0, 133.4, 132.3, 131.4, 130.4, (2C) 128.7, 128.6, 128.5 (2C), 127.0, 126.5, 125.9, 125.6, 123.4, 123.3, 121.5, 112.2,

96.8, 95.8, 83.5, 65.3, 37.6, 32.0; HRMS (ES) calcd [M + Na] for $C_{27}H_{18}BrNNaO_3S$ 538.0088, obsd 538.0082

(3*R*)-6-bromo-7-(naphthalen-1-ylmethyl)-5-oxo-8-((3-(trifluoromethyl)phenyl)ethynyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (17c). **16c** (0.16mmol, 98mg) and LiI (1.6mmol, 214mg) was dissolved in dry pyridine (3ml) and the reaction was heated in the microwave oven at 130 ^oC for 15 min. The reaction mixture was diluted with water and pH was set to approx 1 with 1M HCl (aq) and then extracted with EtOAc, the organic phase was dried (Na_2SO_4), filtered and concentrated. The crude material was purified by HPLC, the product was freezedried, to give **17c** (70 mg, 75 %) $[\alpha]_D$ -13 (c0.5, CH_2Cl_2:MeOH 9:1) IR λ 1744, 1615, 1562, 1478, 1327 cm^{-1}; ^{1}H-NMR (500 MHz, d_6-DMSO)δ 8.32 (d, ***J***= 6.4 Hz, 1H), 7.95 (d, ***J***= 6.4 Hz, 1H), 7.81 (d, ***J***= 6.4 Hz, 1H), 7.63-7.53 (m, 3H), 7.44-7.36 (m, 2H), 7.14 (d, ***J***= 6.0 Hz, 1H), 6.94 (d, ***J***= 5.6 Hz, 1H), 6.91 (s, 1H), 5.72-5.67 (m, 1H), 4.78-4.62 (m, 2H), 4.09-4.01 (m, 1H), 3.77-3.70 (m, 1H) ^{13}C-NMR (125 MHz, d_6-DMSO)δ 169.1, 156.2, 155.1, 150.9, 133.9, 133.4, 132.6, 131.4, 129.7, 129.4 (q, ***J***= 34Hz, 1C), 128.6, 126.9, 126.4 (split, 2C), 125.9, 125.5, 124.7 (split, 1C), 123.5, 123.4 (q, ***J***= 270Hz, 1C), 123.2, 123.0, 112.4, 95.2, 93.8, 86.0, 68.2, 37.4, 33.6 HRMS (ES) calcd [M + Na] for $C_{28}H_{17}BrF_3NNaO_3S$ 605.9962, obsd 605.9971

(3*R*)-8-cyclopropylethynyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3R-carboxylic acid (18a). **17a** (31.5 mg, 0.066 mmol) and zinc dust (62.0 mg, 0.98 mmol) were added to a round bottom flask containing 3.0 ml AcOH. The mixture was heated to 100ºC for 2 h. AcOH was removed, the residue was dissolved in DMSO and the zinc dust was filtered off. Purification by HPLC gave **18a** (20.0 mg, 75%). $[\alpha]_D$ -3 (*c* 0.50, $CHCl_3$); IR 2362, 1896, 1719, 1648, 1487, 1427, 1201, 1021, 962, 780 cm^{-1}; ^{1}H NMR, (400 MHz, d_6-DMSO) δ 7.99-7.94 (m, 1H), 7.93-7.85 (m, 2H), 7.58-7.46 (m, 3H), 7.36 (d, ***J*** = 7.0 Hz, 1H), 5.50 (s, 1H), 5.43 (dd, ***J*** = 9.0, 1.6 Hz, 1H), 4.35-4.23 (m, 2H), 3.91 (dd, ***J*** = 12.0, 9.1 Hz, 1H), 3.57 (dd, ***J*** = 12.0, 1.6 Hz, 1H), 1.52-1.44 (m, 1H), 0.83-0.77 (m, 2H), 0.52-0.47 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.3, 159.2, 154.8, 153.7, 134.0, 133.4, 131.4, 128.7, 127.4, 127.3, 126.4, 125.8, 125.6, 123.7, 113.3, 101.5, 96.7, 69.4, 63.6, 36.0, 31.4, 8.7 (split, 2C), -0.1; HRMS (ES) calcd [M + Na] for $C_{24}H_{19}NNaO_3S$ 424.0983, obsd 424.0989

(3*R*)-7-naphthalen-1-ylmethyl-5-oxo-8-phenylethynyl-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (18b). 17b (20.0 mg, 0.0388 mmol) and zinc dust (37.0 mg, 0.58 mmol) were added to a round bottom flask containing 3.0 ml AcOH. The mixture was heated to 100ºC for 2 h. AcOH was removed, the residue was dissolved in DMSO and the zinc dust was filtered off. Purification by HPLC gave **18b** (14.1 mg, 83%). $[\alpha]_D$ -2 (*c* 0.50, $CHCl_3$); IR 2361, 1869, 1706, 1643, 1489, 1429, 1239, 935 cm^{-1}; ^{1}H NMR, (400 MHz, d_6-DMSO) δ 8.04-7.96 (m, 2H), 7.88 (d, ***J*** = 8.0 Hz, 1H), 7.57-7.48 (m, 3H), 7.45-7.41 (m, 1H), 7.37-7.32 (m, 3H), 7.31-7.27 (m, 2H), 5.60 (s, 1H), 5.50 (dd, ***J*** = 9.1, 1.6 Hz, 1H), 4.50-4.38 (m, 2H), 3.98 (dd, ***J*** = 11.9, 9.1 Hz, 1H), 3.65 (dd, ***J*** = 11.9, 1.5 Hz, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.2, 159.3, 154.6, 154.5, 133.9, 133.5, 131.4, 130.7 (2C), 128.7 (3C), 128.6, 127.4 (split, 2C), 126.4, 125.8, 125.6, 123.8, 122.1, 113.6, 96.7, 95.9, 83.7, 63.7, 36.0, 31.6; HRMS (ES) calcd [M + Na] for $C_{27}H_{19}NNaO_3S$ 460.0983, obsd 460.0981

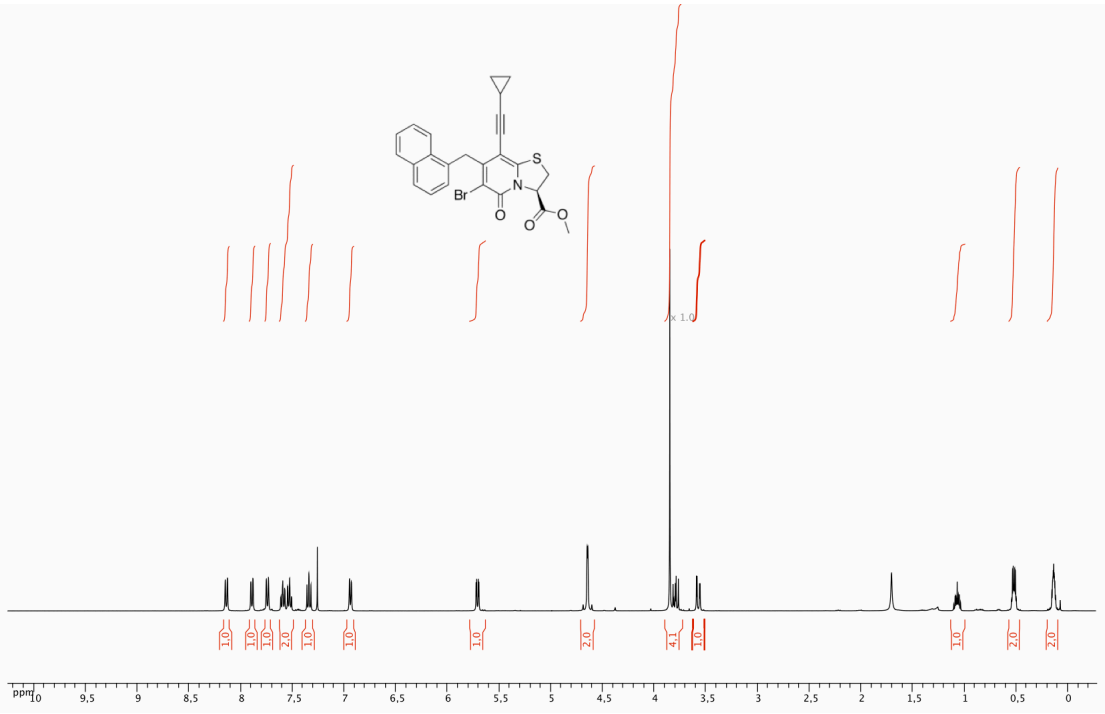
(3*R*)-7-(naphthalen-1-ylmethyl)-5-oxo-8-((3-(trifluoromethyl)phenyl)ethynyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (18c). A suspention of **17c** (0.11mmol, 65mg) and freshly activated Zn dust (1.65mmol, 108mg) in AcOH (4ml) was heated to 100 ^oC with an oilbath for 1 h. The reaction mixture was diluted with water and pH was set to approx 1 with 1M HCl (aq) and then extracted with EtOAc, the organic phase was dried (Na_2SO_4), filtered and concentrated. The crude

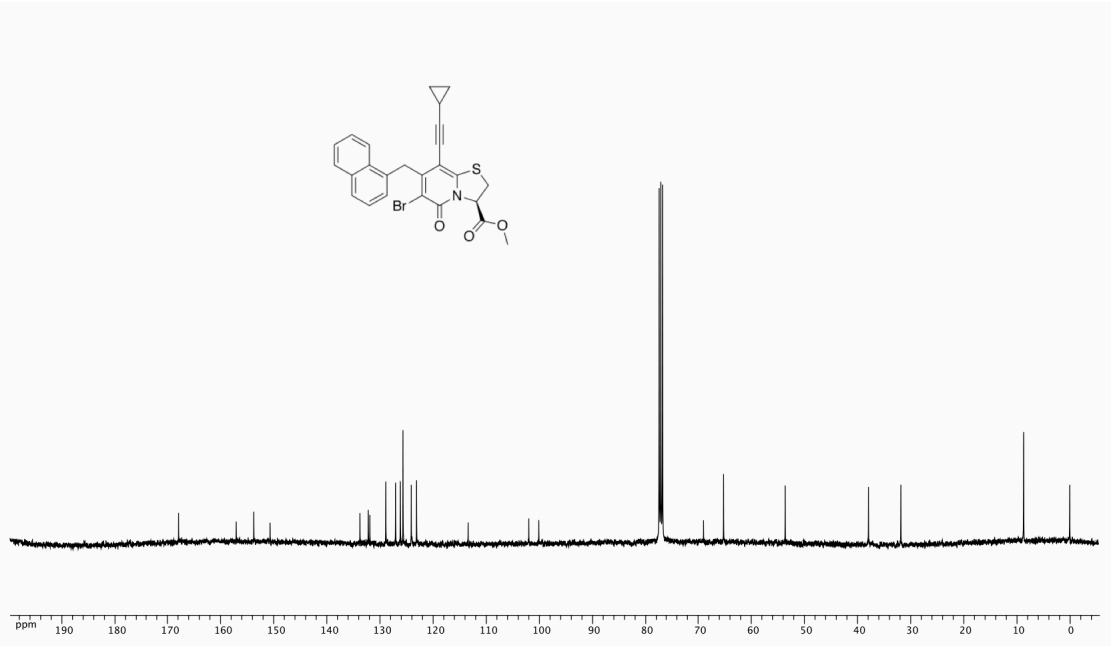
material was purified by HPLC and freezedried, to give **18c** (35mg, 63%) $[\alpha]_D$ 12 (c0.5, CH_2Cl_2:MeOH 9:1) IR λ 1636, 1492, 1421, 1334 cm^{-1}; ^{1}H-NMR (400 MHz, d_6-DMSO)δ 8.07-8.01 (m, 1H), 8.00-7.95 (m, 1H), 7.88 (d, ***J***= 8.4 Hz, 1H), 7.72-7.67 (m, 1H), 7.62-7.45 (m, 6H), 7.41 (d, ***J***= 7.2 Hz, 1H), 5.67 (s, 1H), 5.50 (dd, ***J***= 9.2, 1.4 Hz, 1H), 4.55-4.40 (m, 2H), 3.99 (dd, ***J***= 12, 9.2 Hz, 1H), 3.67 (dd, ***J***= 12, 1.4 Hz, 1H), ^{13}C-NMR (100 MHz, d_6-DMSO)δ 169.2, 159.3, 155.4, 154.4, 134.4, 133.9, 133.4, 131.4, 129.9, 129.6 (q, ***J***= 32 Hz, 1C), 128.6, 127.4, 127.2, 126.8 (split, 1C), 126.4, 125.8, 125.6, 125.0 (split, 1C), 123.8, 123.6 (q, ***J***=271 Hz, 1C), 123.3, 113.9, 95.3, 95.0, 85.6, 64.0, 36.0, 31.8 HRMS (ES) calcd [M + Na] for $C_{28}H_{18}F_3NNaO_3S$ 528.0857, obsd 528.0862

(3*R*)-6-Bromo-7-naphthalen-1-ylmethyl-5-oxo-8-(3-trifluoromethyl-phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid methyl ester (19). **2** (0.2mmol, 100mg) was dissolved in dry MeCN (3ml) and NBS (0.3mmol, 53mg) was added, the reaction was stirred at rt for 30 min and then quenched with saturated $NaHCO_3$ (aq). The mixture was extracted with EtOAc and the organic phase was dried (Na_2SO_4), filtered and concentrated. The crude material was purified by column chromatography on silica gel (heptane:EtOAc 70:30 → 40:60) compound **19** was isolated as a colorless foam (105 mg, 91%) $[\alpha]_D$ -117 (c0.5, $CHCl_3$) IR λ 1618, 1474, 1384, 1326 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.73-7.67 (m, 1H), 7.63-7.57 (m, 1H), 7.54-7.48 (m, 1H), 7.35-7.20 (m, 4H), 7.19-6.91 (m, 4H), 5.70-5.64 (m, 1H), 4.30-4.12 (m, 2H), 3.78 (d, ***J*** = 2.8 Hz, 3H), 3.62 (dd, ***J*** = 12.0, 8.6 Hz, 1H), 3.42-3.35 (m, 1H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.1, 157.6, 152.0 (d, ***J*** = 6 Hz, 1C), 146.7, 136.7 (d, ***J*** = 7 Hz, 1C), 133.7, 133.0 (d, ***J*** = 39 Hz, 1C), 132.2 (d, ***J*** = 8 Hz, 1C), 131.3 (split, 1C), 130.9 (dq, ***J*** = 32, 8 Hz, 1C), 129.2 (split, 1C), 128.7, 127.4, 126.9-126.3 (m, 1C), 126.1, 125.7 (split, 1C), 125.5-125.0 (m, 2C), 124.8, 123.5 (dq, ***J*** = 271, 9 Hz, 1C), 122.5 (d, ***J*** = 5 Hz, 1C), 115.1, 114.7, 65.0, 53.6 (d, ***J*** = 4 Hz, 1C), 37.2, 31.8 LRMS (M+H) 575

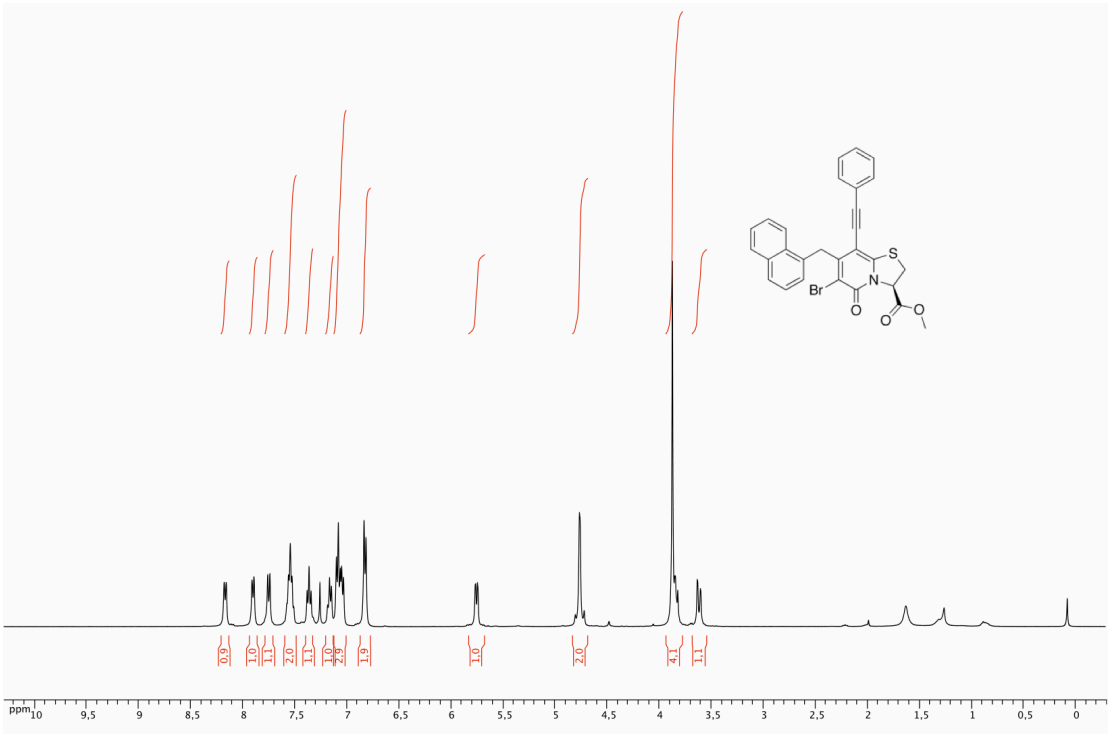
(3*R*)-6-Bromo-7-naphthalen-1-ylmethyl-5-oxo-8-(3-trifluoromethyl-phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (20). **19** (0.17mmol, 99 mg) was dissolved in THF (5 ml) and 1M LiOH (0.34mmol, 0.34ml) was added and the reaction was stirred at rt for 18h. The reaction mixture was diluted with water (pH ~ 1, set with 1M HCl) and extracted with EtOAc, the organic phase was dried (Na_2SO_4), filtered and concentrated. The crude material was purified by column chromatography on silica gel (DCM:MeOH:AcOH 93:6:1) compound **20** was isolated as a colorless foam (85 mg, 89%) $[\alpha]_D$ -22 (c0.5, $CHCl_3$:MeOH 9:1) IR λ 1625, 1480, 1385, 1326 cm^{-1}; ^{1}H-NMR (400 MHz, d_6-DMSO) δ 7.93-7.83 (m, 1H), 7.83-7.70 (m, 2H), 7.56-7.31 (m, 6H), 7.30-7.15 (m, 1H), 7.07-6.92 (m, 1H), 5.72-5.60 (m, 1H), 4.40-4.13 (m, 2H), 3.98-3.84 (m, 1H), 3.63-3.50 (m, 1H) ^{13}C-NMR (100 MHz, d_6-DMSO) δ 169.1, 156.5, 150.8 (split, 1C), 148.2, 137.0 (d, ***J*** = 6 Hz, 1C), 133.8 (d, ***J*** = 19 Hz, 1C), 133.2, 132.4 (d, ***J*** = 6 Hz, 1C), 130.9, 129.7 (split, 1C), 129.1 (dq, ***J*** = 32, 12 Hz, 1C), 128.5, 127.0, 126.3, 126.1, 125.7, 125.3 (d, ***J*** = 7 Hz, 1C), 125.1-124.8 (m, 1C), 124.1 (d, ***J*** = 8 Hz, 1C), 123.5 (dq, ***J*** = 271, 13 Hz, 1C), 122.7, 113.5, 113.2 (split, 1C), 64.9 (split, 1C), 36.8, 31.9 (d, ***J*** = 13 Hz, 1C) HRMS (ES) calcd [M + Na] for $C_{26}H_{17}BrF_3NNaO_3S$ 581.9962, obsd 581.9959

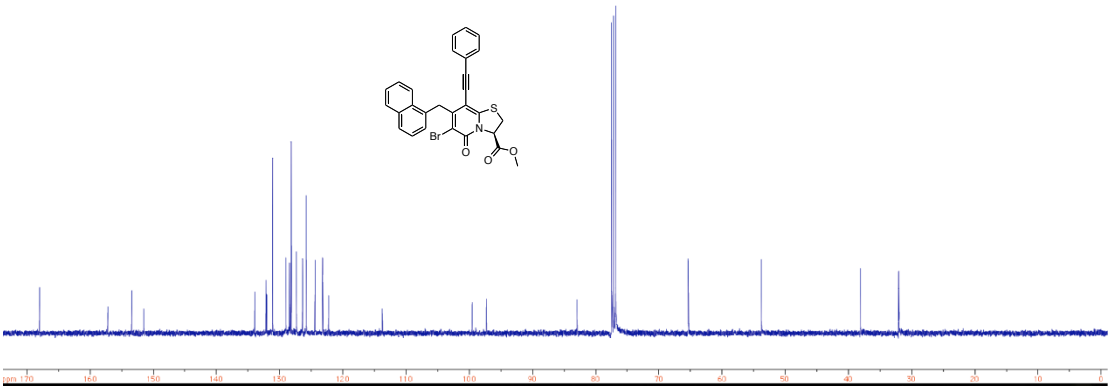
^{1}H-NMR and ^{13}C-NMR of **16a** in $CDCl_3$



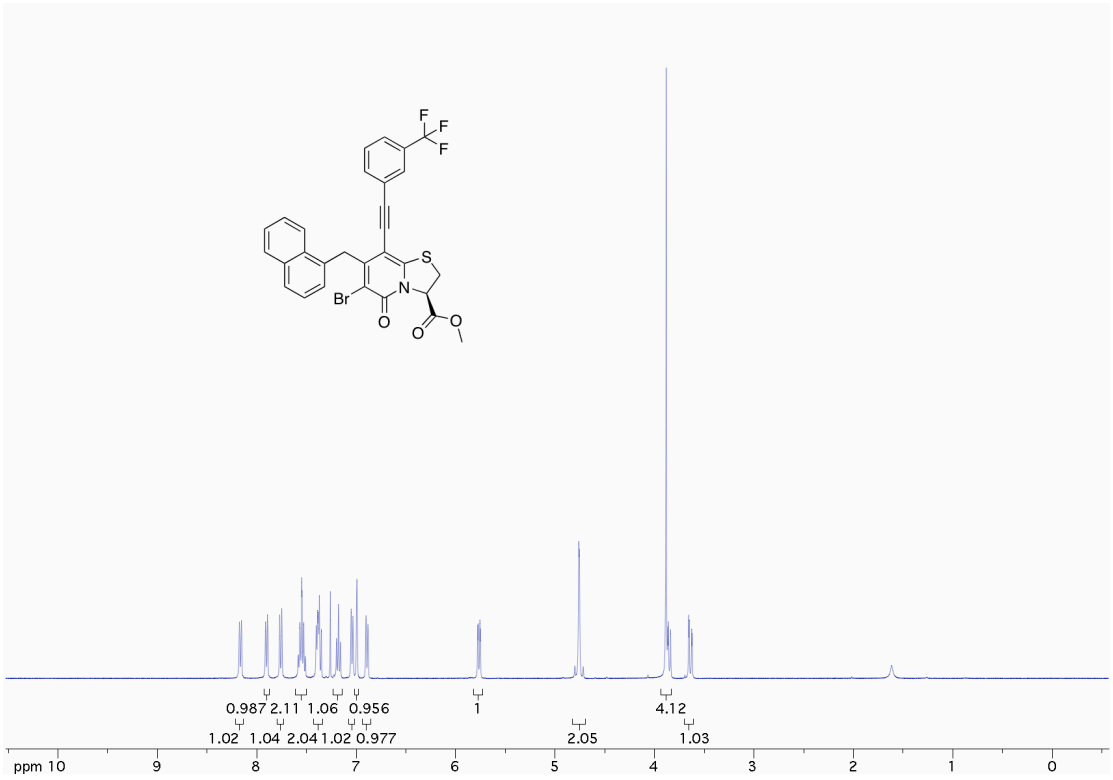


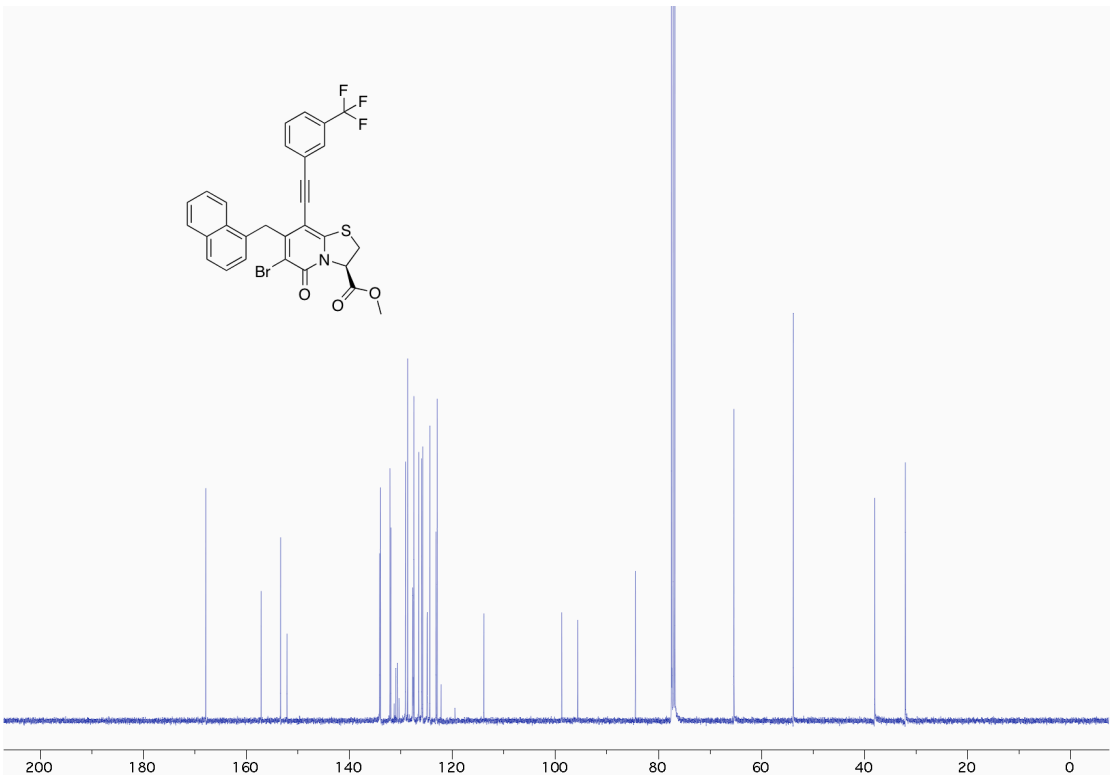
^{1}H-NMR and ^{13}C-NMR of **16b** in $CDCl_3$



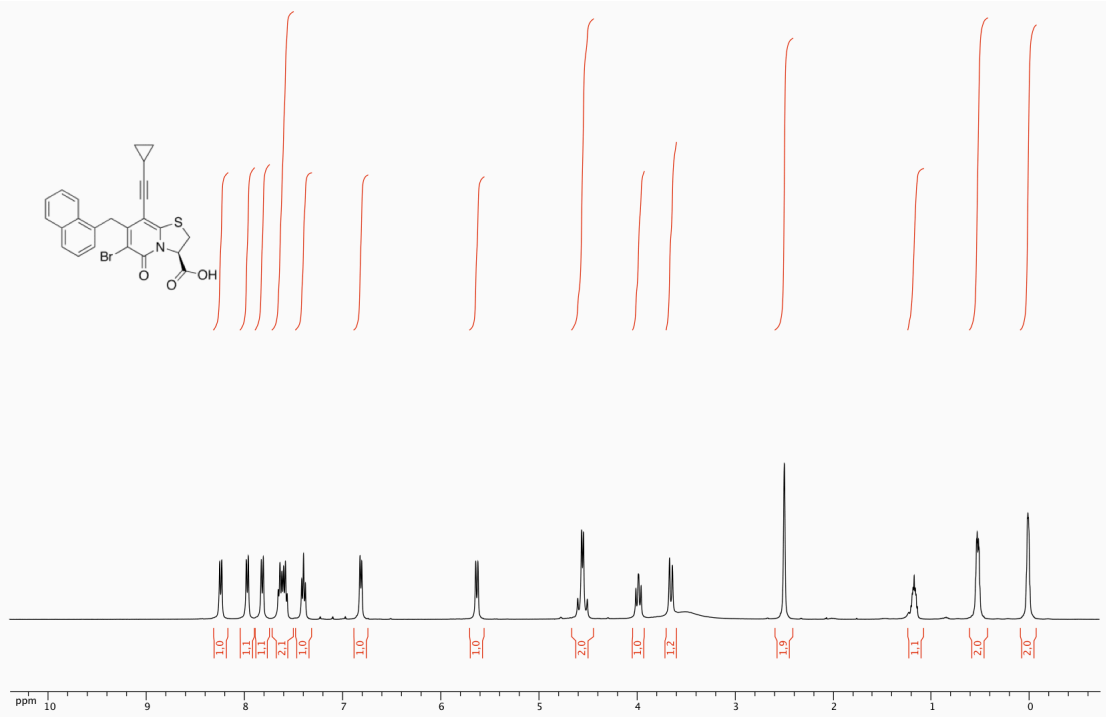


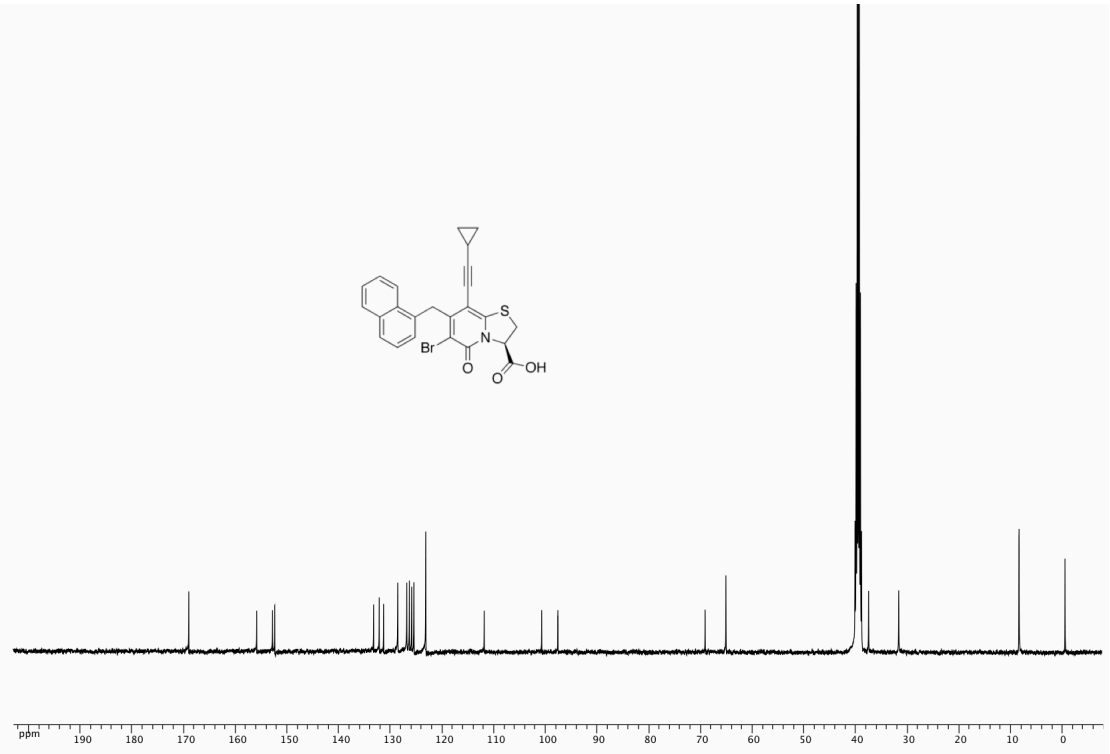
^{1}H-NMR and ^{13}C-NMR of **16c** in $CDCl_3$



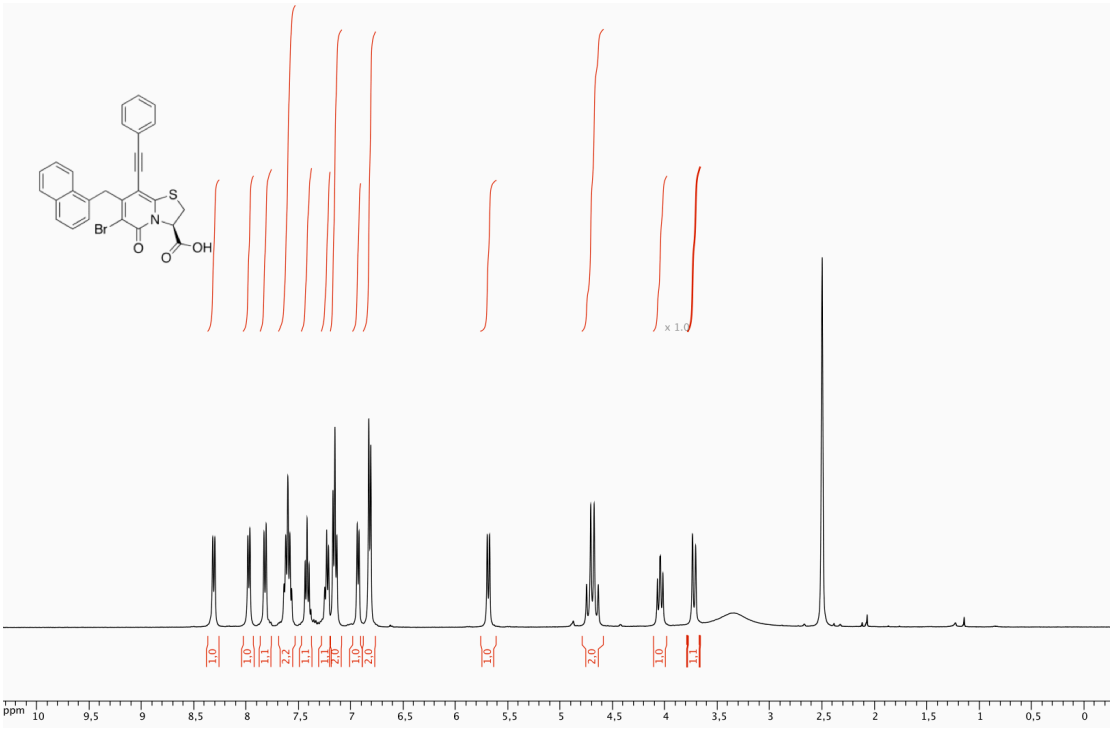


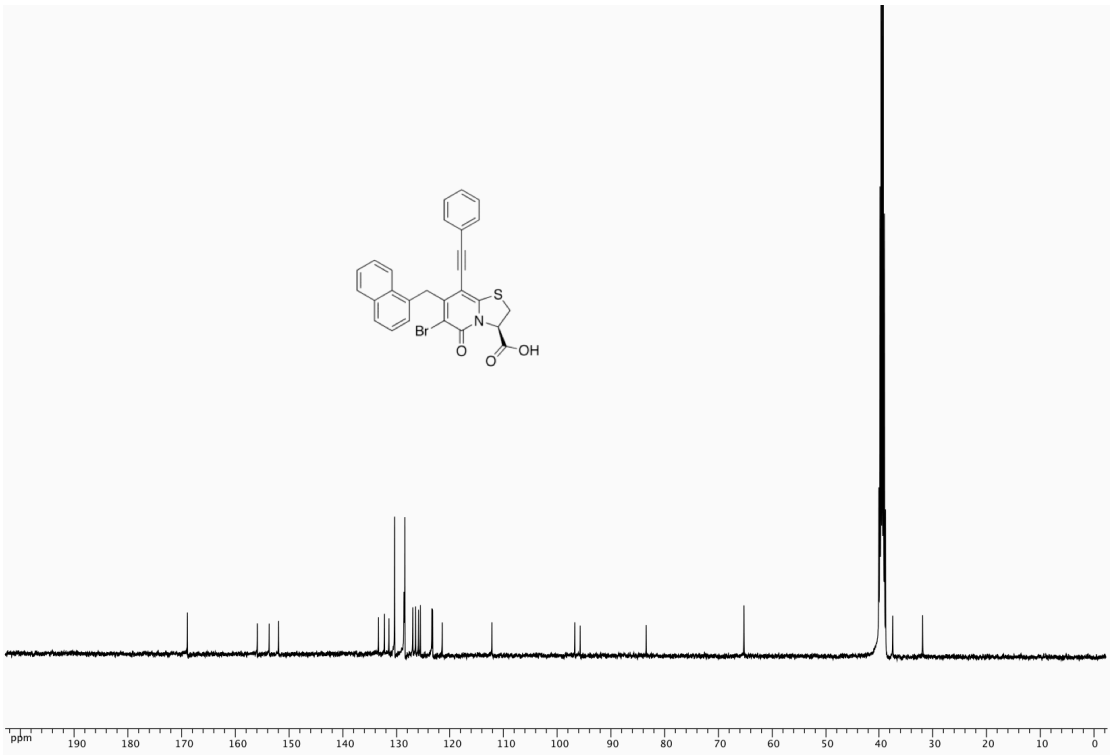
^{1}H-NMR and ^{13}C-NMR of **17a** in d_6-DMSO



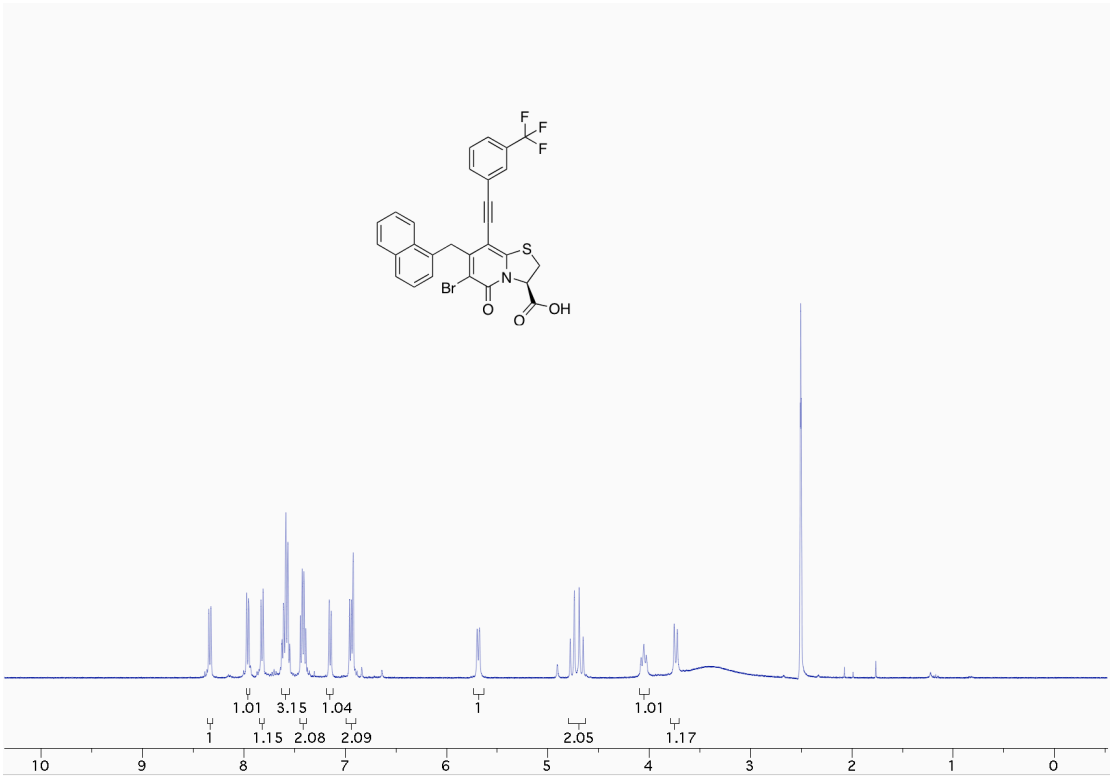


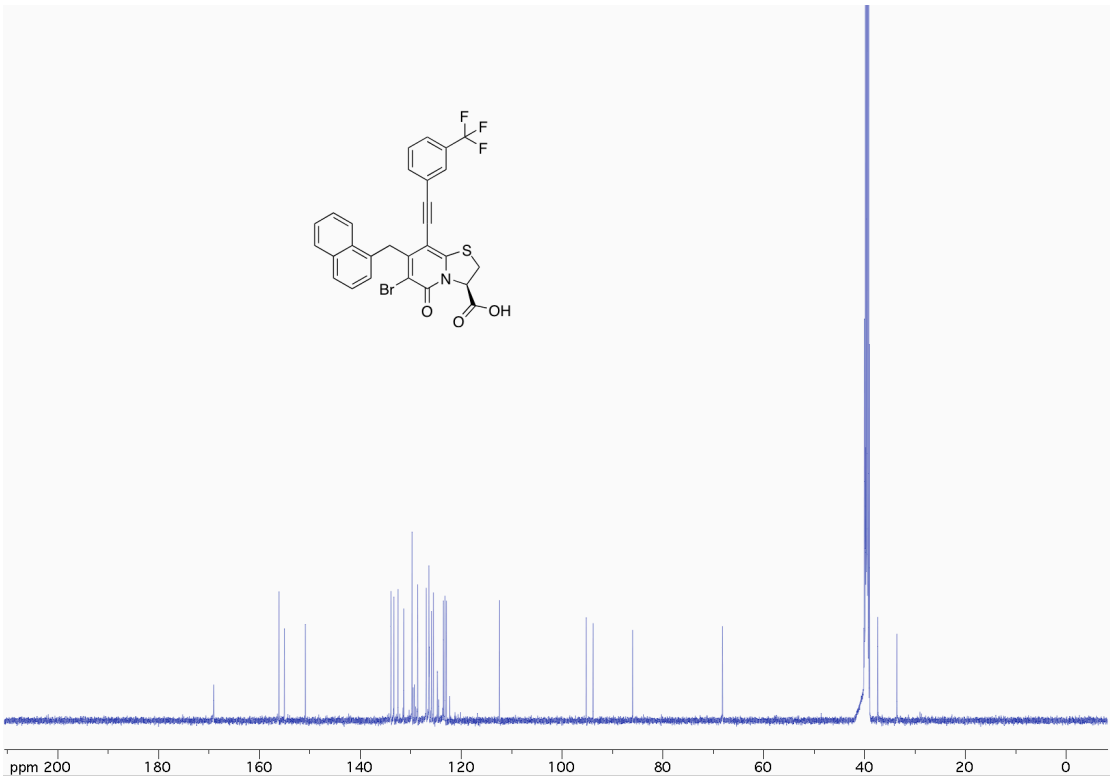
^{1}H-NMR and ^{13}C-NMR of **17b** in d_6-DMSO



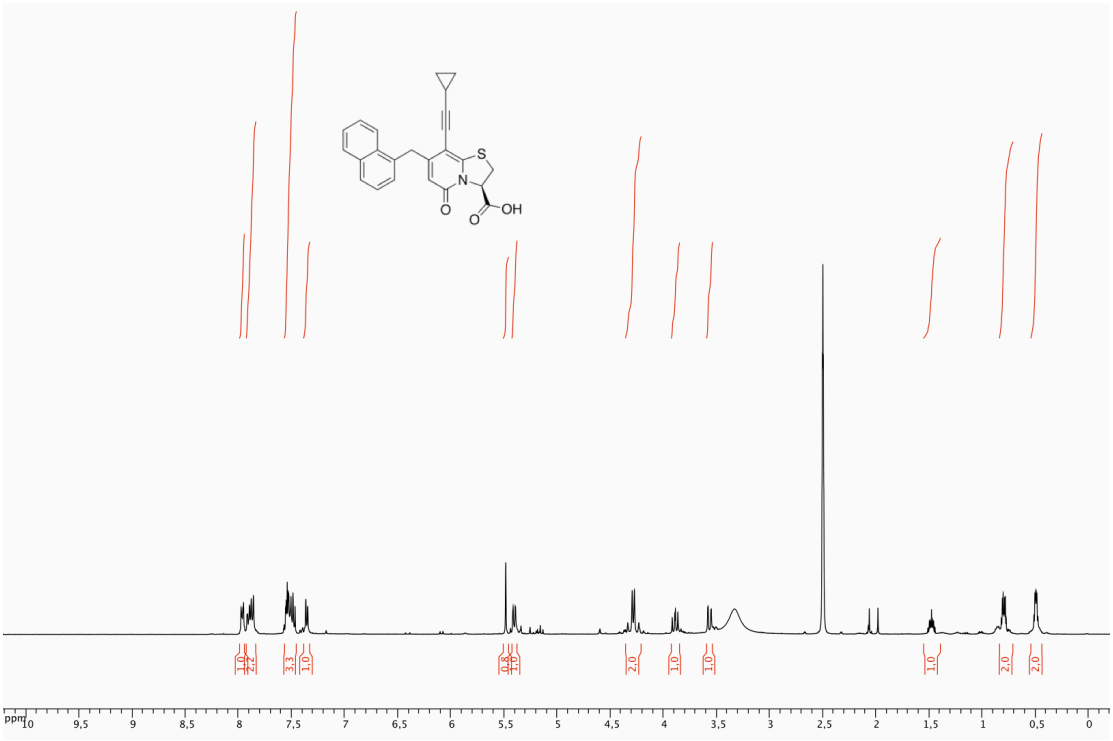


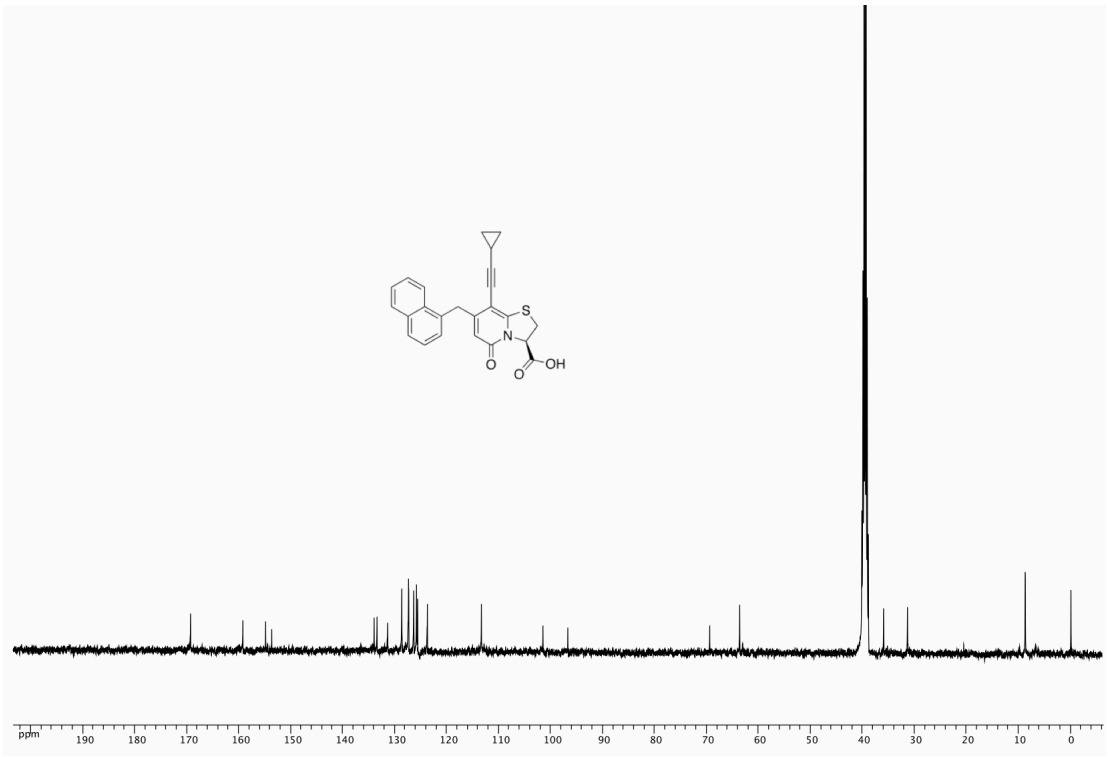
^{1}H-NMR and ^{13}C-NMR of **17c** in d_6-DMSO



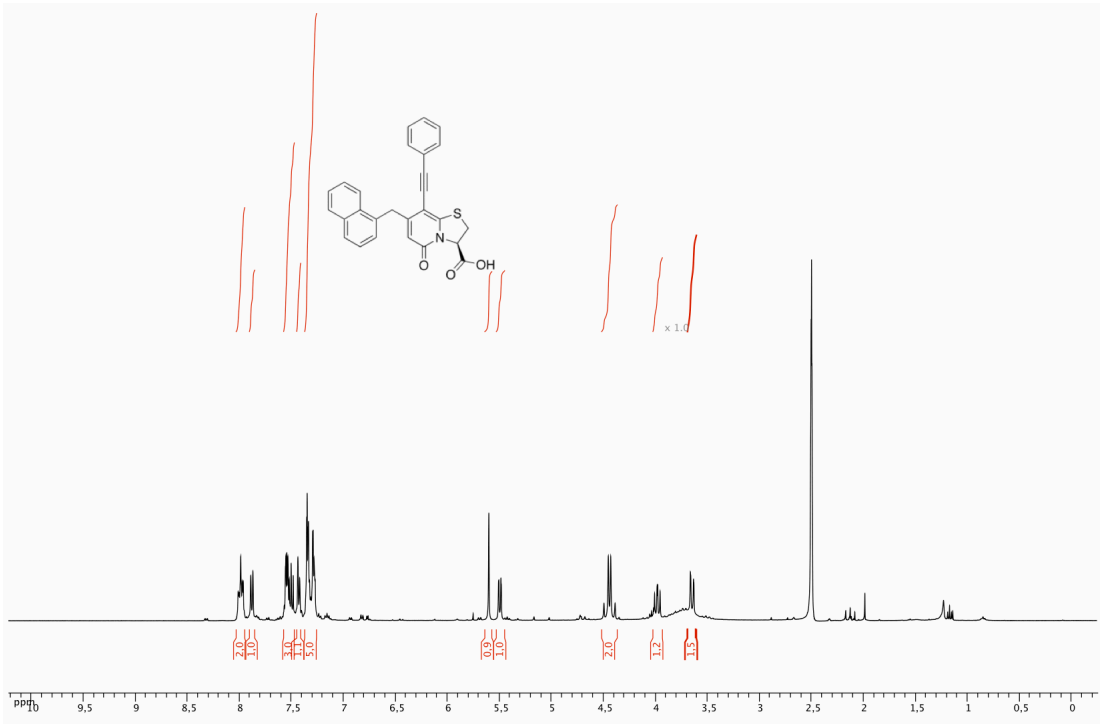


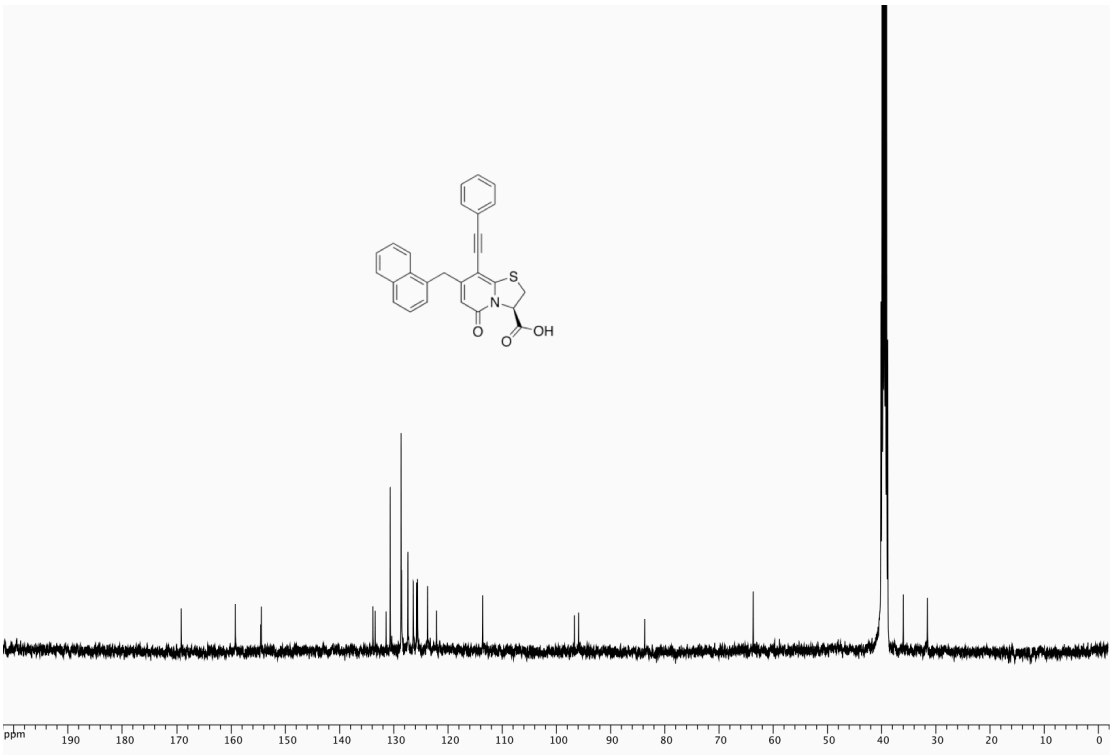
^{1}H-NMR and ^{13}C-NMR of **18a** in d_6-DMSO



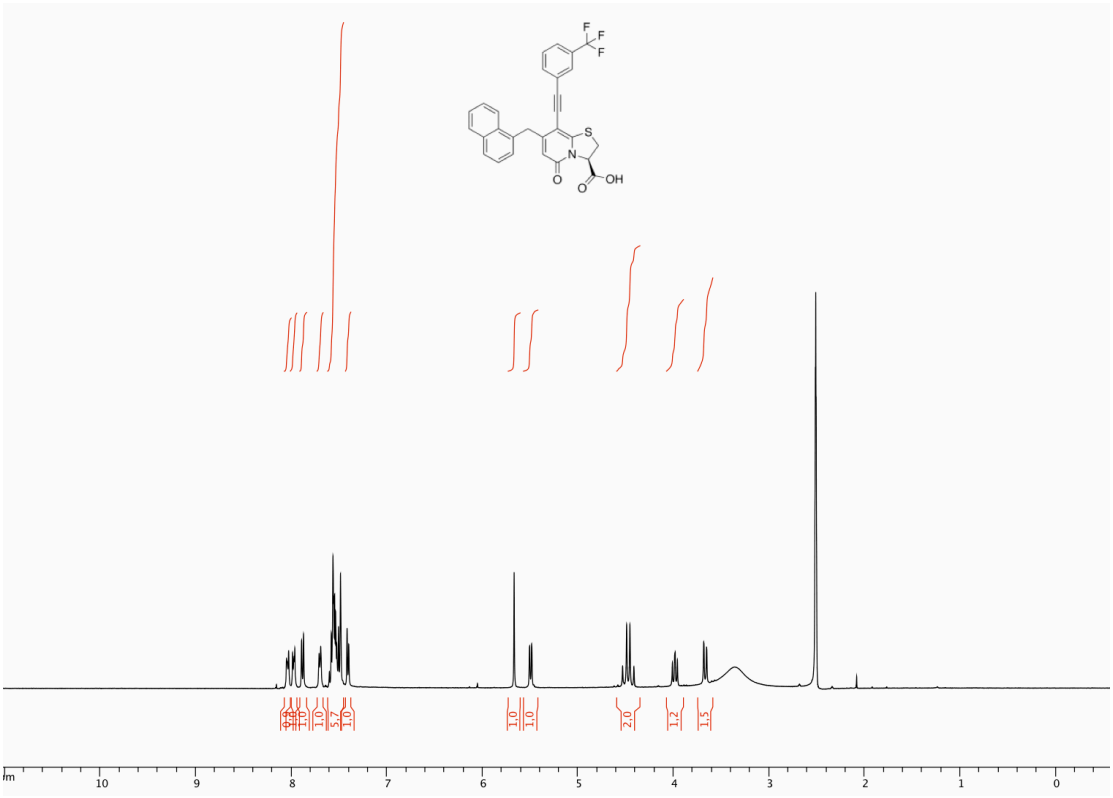


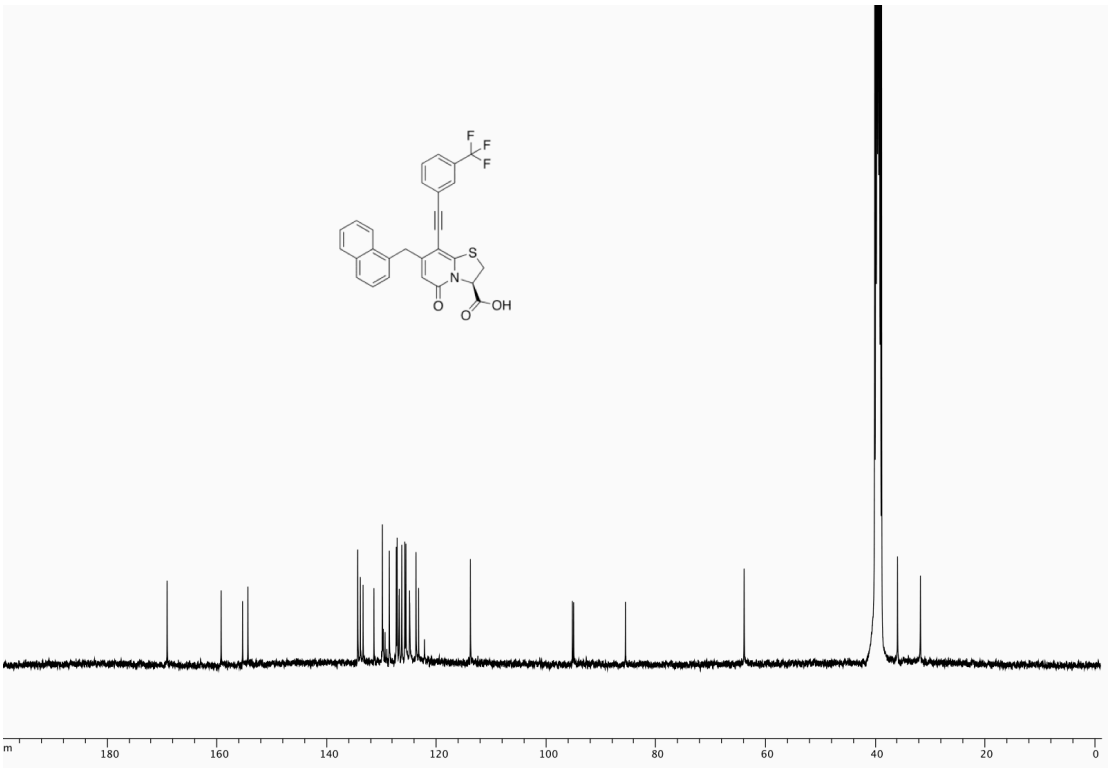
^{1}H-NMR and ^{13}C-NMR of **18b** in d_6-DMSO



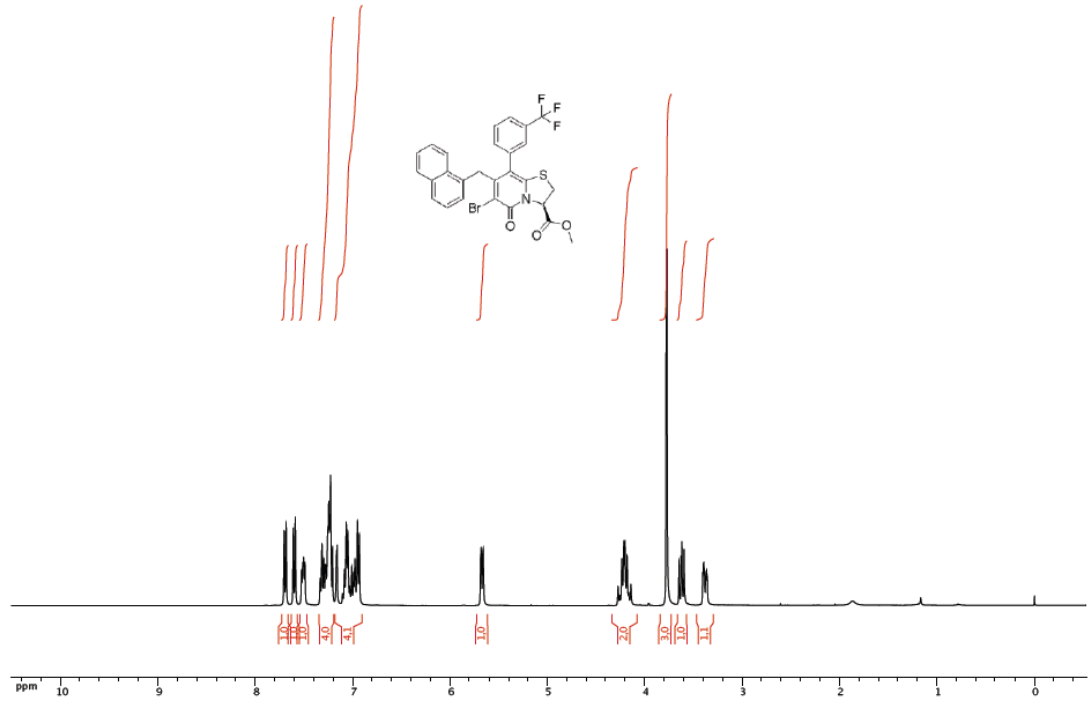


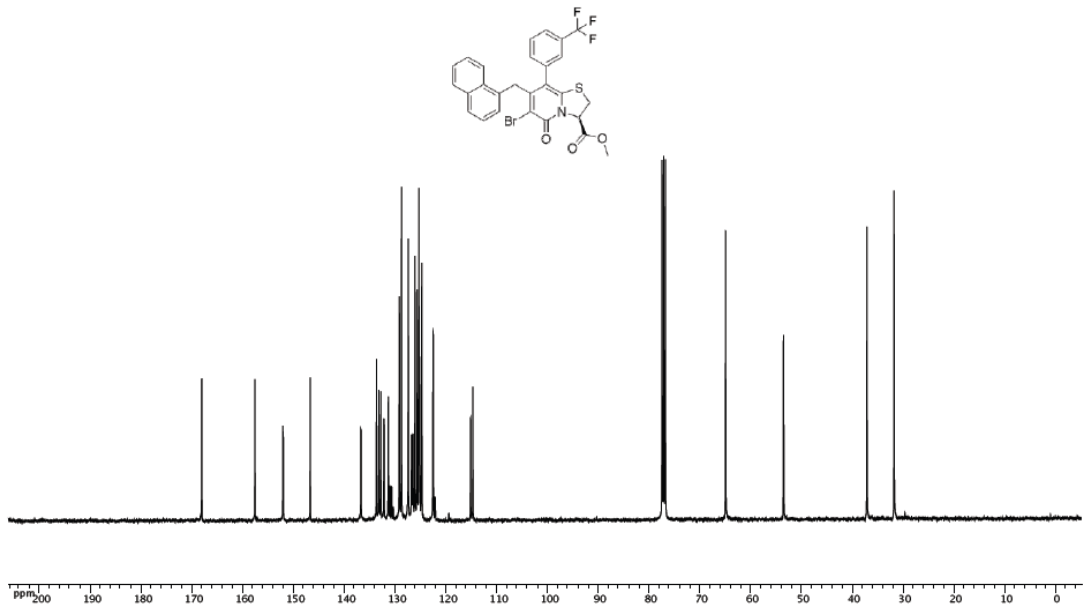
^{1}H-NMR and ^{13}C-NMR of **18c** in d_6-DMSO



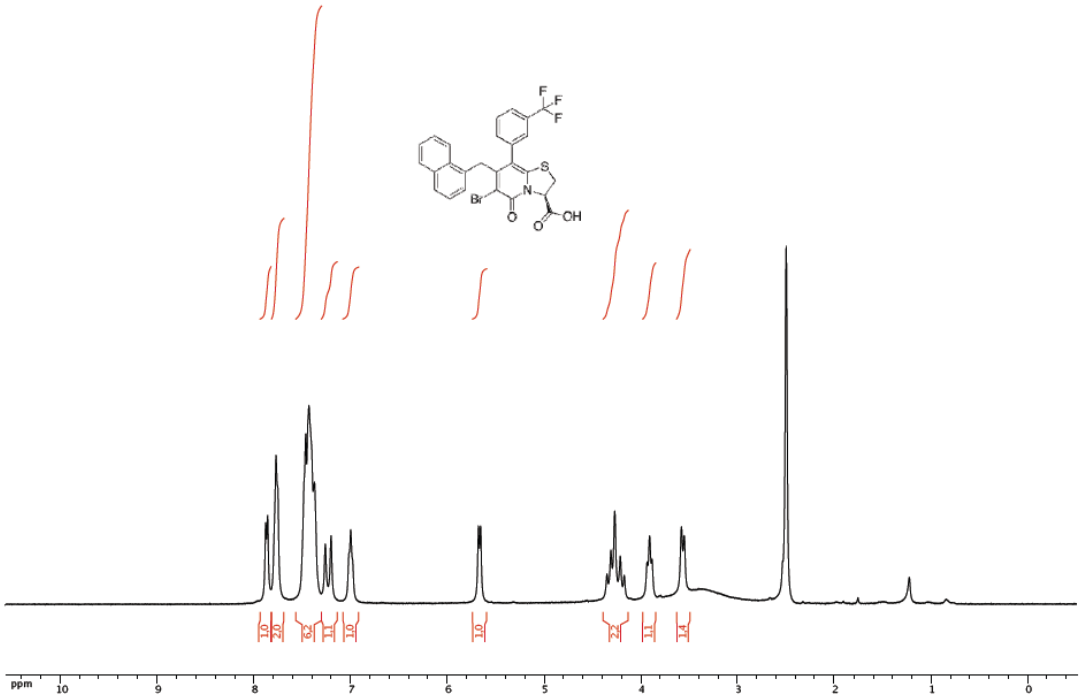


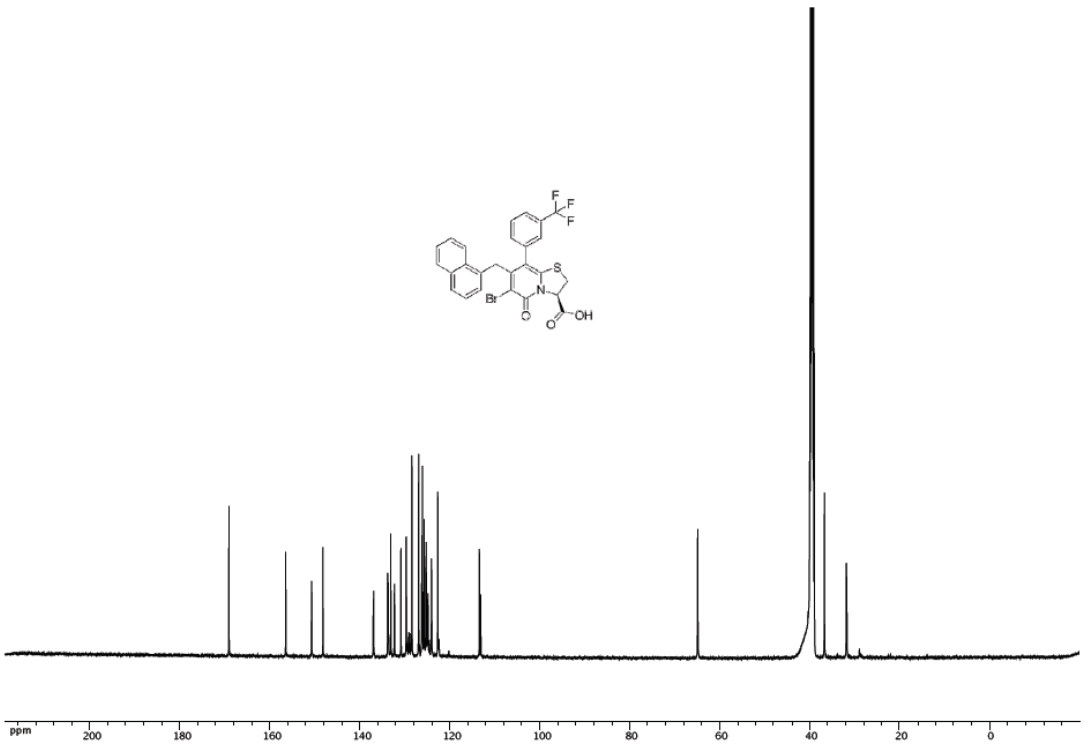
^{1}H-NMR and ^{13}C-NMR of **19** in $CDCl_3$





^{1}H-NMR and ^{13}C-NMR of **20** in d_6-DMSO





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