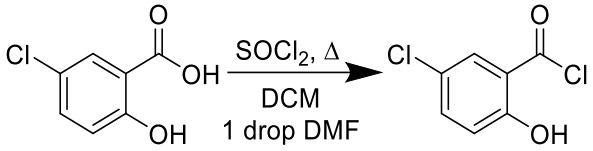
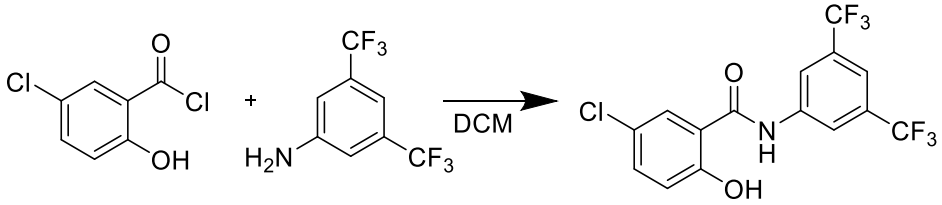
Synthesis of 5-chloro-2-hydroxybenzoyl chloride



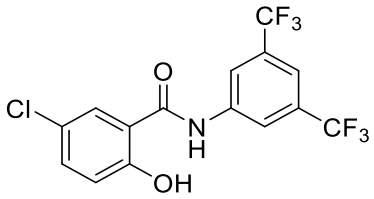
The synthesis of 5-chloro-2-hydroxybenzoyl chloride began by first adding 5-chloro-2-hydroxybenzoic acid (2 mmol) to a round bottom flask with 5mL dichloromethane and a drop of dimethylformamide. To the reaction mixture, thionyl chloride (10 mmol) was added dropwise and the reaction was heated at 50°C for 12 hours. Reaction progress was checked after 12 hours via TLC (70% EtOAc/Hexanes) or for complete dissolution of 5-chloro-2-hydroxybenzoic acid. Upon complete dissolution of the acid, the DCM was removed via rotary evaporation to yield the acid chloride crude. No further purification was carried out and the acid chloride was used within 1 hour of synthesis.

Synthesis of N-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide (IMD-0354)



The synthesis of IMD-0354 began by first dissolving 3,5-bis(trifluoromethyl)aniline (2.2 mmol) in 5mL dichloromethane. The previously generated 5-chloro-2-hydroxybenzoyl chloride was then dissolved in 3 mL of dichloromethane and added dropwise to the reaction mixture. After stirring for 1 hour the reaction formed a precipitate which was filtered, rinsed 2x with 3 mL DCM and dried to afford IMD-0354 in 89% yield.

Spectral characterization of N-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide (IMD-0354)



^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 11.46 (s br., 1H), 10.87 (s, 1H), 8.45 (s, 2H), 7.88 (d, 1H, *J*= 2.6 Hz), 7.83 (s, 1H), 7.49 (dd, 2H, *J*= 2.6, 8.8 Hz), 7.06 (d, 1H, *J*=8.8 Hz)

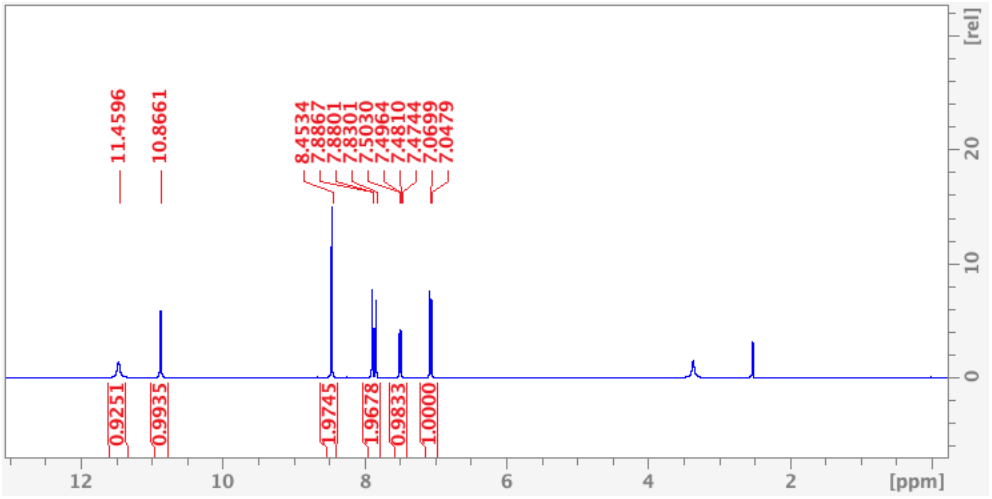
^{13}C NMR (100 MHz ,DMSO-*d*$_6$): δ 165.99, 156.83, 140.71, 133.71, 131.18 (q, *J*= 32 Hz), 129.03, 123.68 (q, *J*= 271 Hz), 123.25, 120.80 (d, *J*=3.6 Hz), 120.51, 119.52, 117.30 (t, *J*=3.6 Hz)

^{19}F NMR (376 MHz, DMSO-*d*$_6$): δ -61.6602

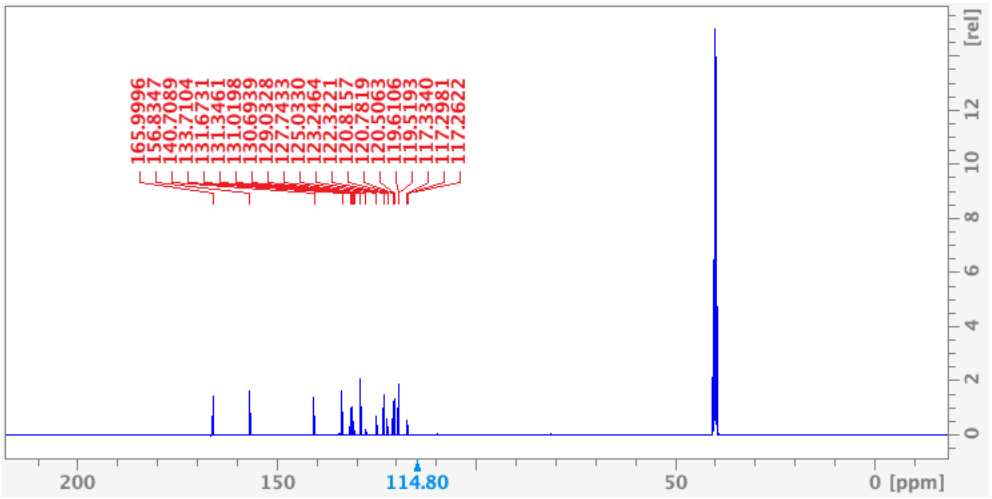
HRMS (ESI) *m/z*: calculated for $C_{15}H_8ClF_6NO_2$ $[M-1]^-$: 382.0064, found 382.0107

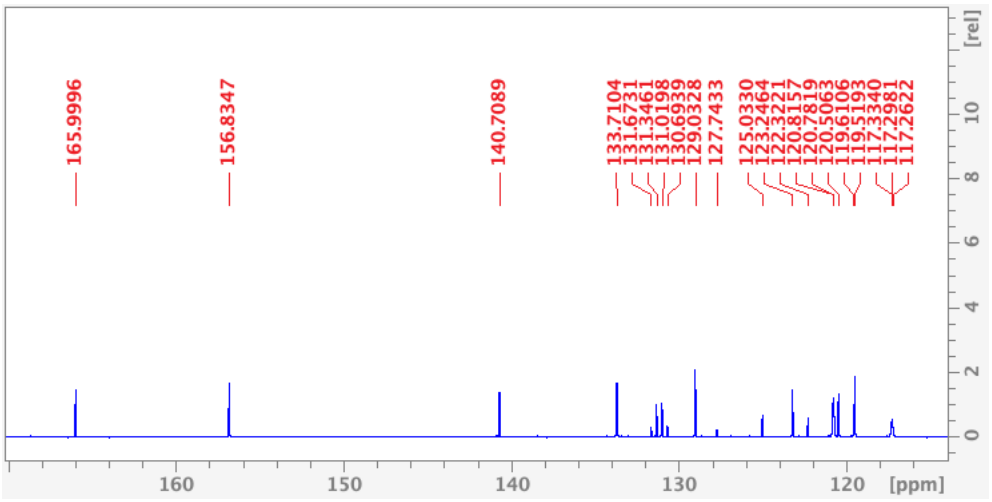
CHN Anal. Calcd for $C_{15}H_8ClF_6NO_2$: C, 46.96; H, 2.10; N, 3.65. Found C, 46.70; H, 2.01; N, 3.51.

^{1}H NMR

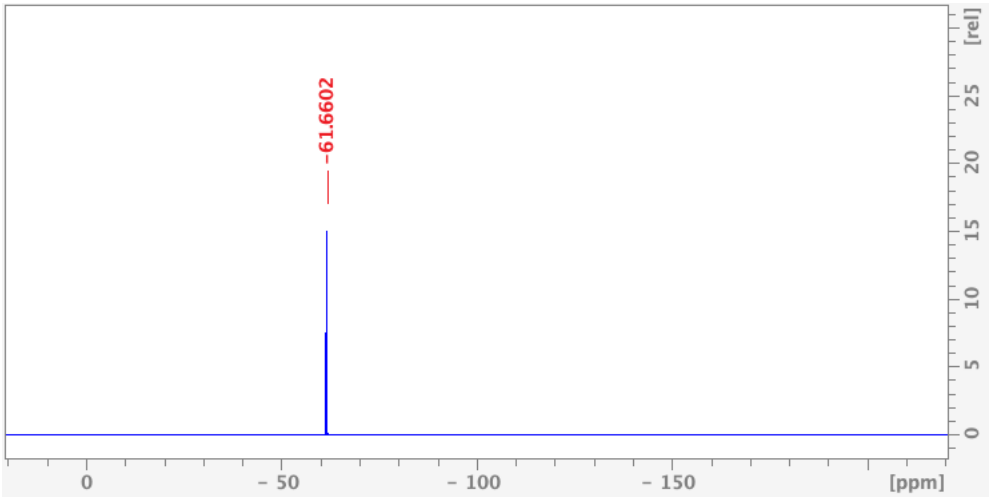


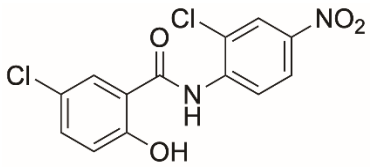
^{13}C NMR





^{19}F NMR





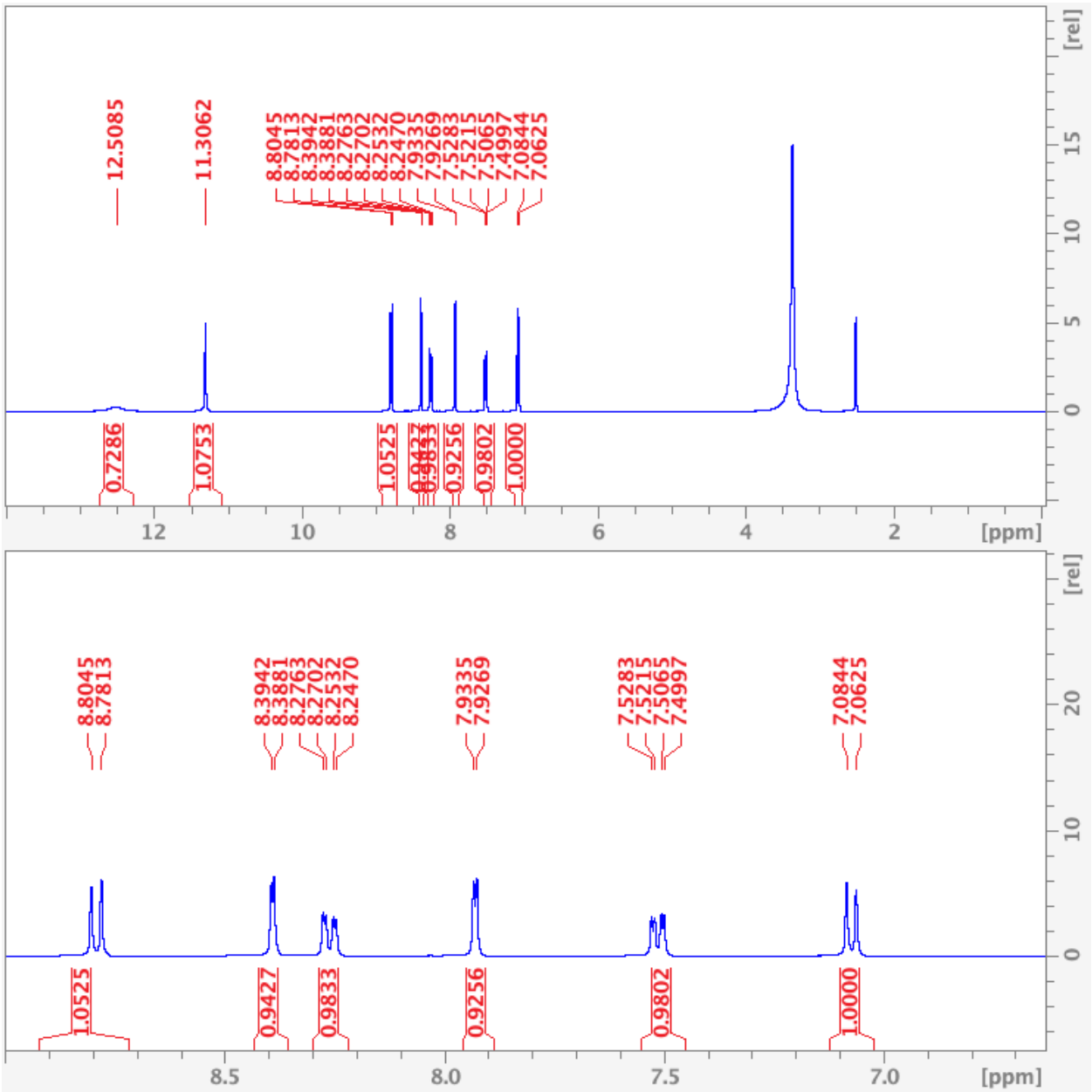
Spectral characterization of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (niclosamide)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 12.52 (s, 1H), 11.31 (s, 1H), 8.79 (d, 1H, *J*= 9.2Hz), 8.39 (d, 1H, *J*= 2.5Hz), 8.26 (dd, 1H, *J*= 2.5Hz, 9.2Hz), 7.93 (d, 1H, *J*= 2.7Hz), 7.51 (dd, 1H, *J*= 2.7Hz, 8.7Hz), 7.07 (d, 1H, *J*= 8.8Hz)

HRMS (ESI) *m/z*: calculated for $C_{13}H_8Cl_2N_2O_4$ [M-1]$^-$: 324.9777, found 324.9817

CHN Anal. Calcd for $C_{13}H_8Cl_2N_2O_4 \cdot H_2O$: C, 45.24; H, 2.92; N, 8.12. Found C, 45.80; H, 2.76; N, 8.19.

^{1}H NMR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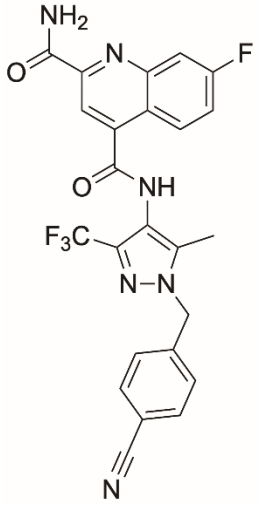


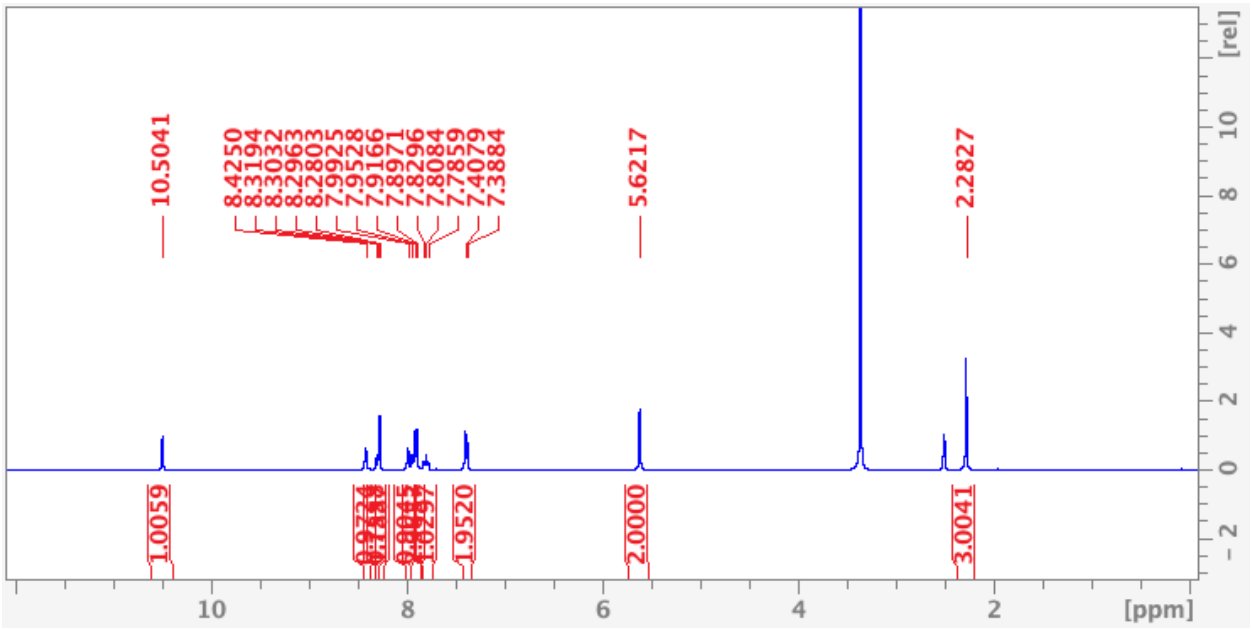
Spectral characterization of N^4-(1-(4-cyanobenzyl)-5-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-7-fluoroquinoline-2,4-dicarboxamide (BAY-876)

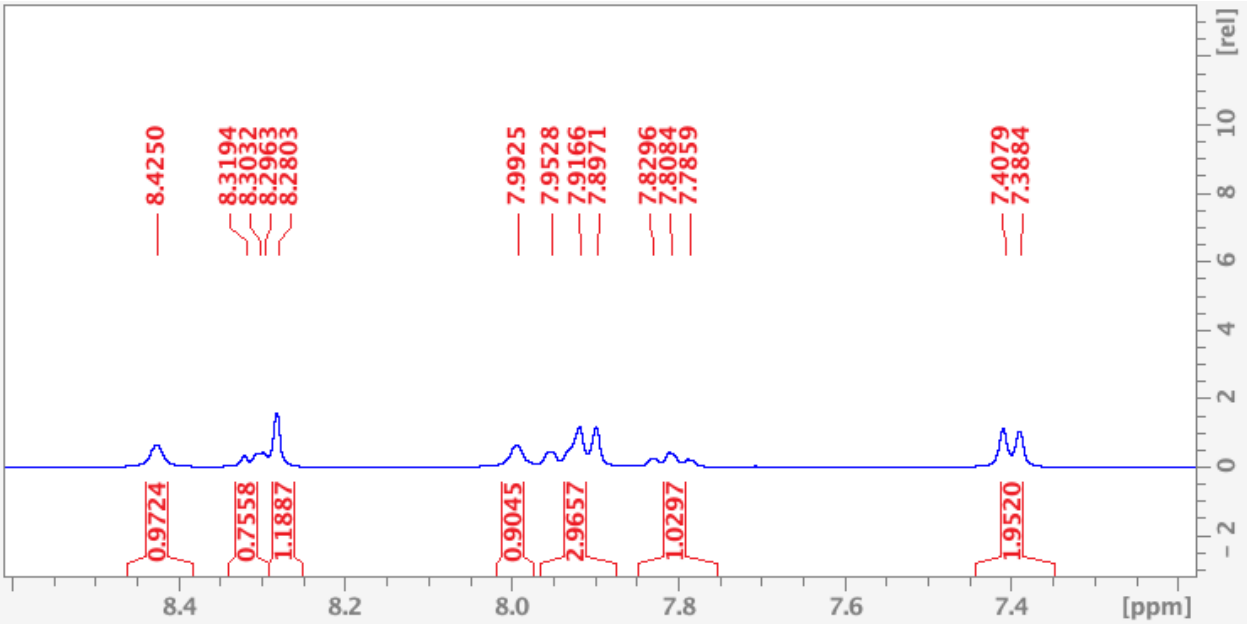
^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H), 8.43 (s, 1H), 8.32-8.28 (m, 2H), 7.99 (s, 1H), 7.95-7.89 (m, 3H), 7.81 (t, 1H, *J*= 9Hz), 7.40 (d, 2H, *J*= 7.8Hz), 5.62 (2H, s), 2.283 (s, 3H)

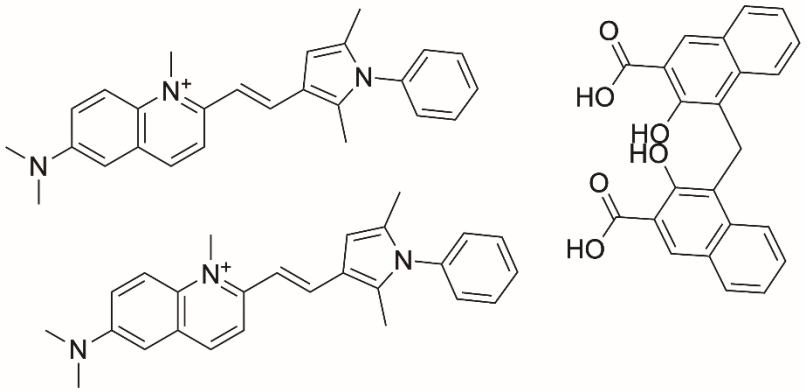
HRMS (ESI) *m/z*: calculated for $C_{24}H_{16}F_4N_6O_2$ $[M+1]^+$: 497.1344, found 497.1343

CHN Anal. Calcd for $C_{24}H_{16}F_4N_6O_2$: C, 58.07; H, 3.25; N, 16.93. Found C, 57.91; H, 3.15; N, 17.06.

^{1}H NMR







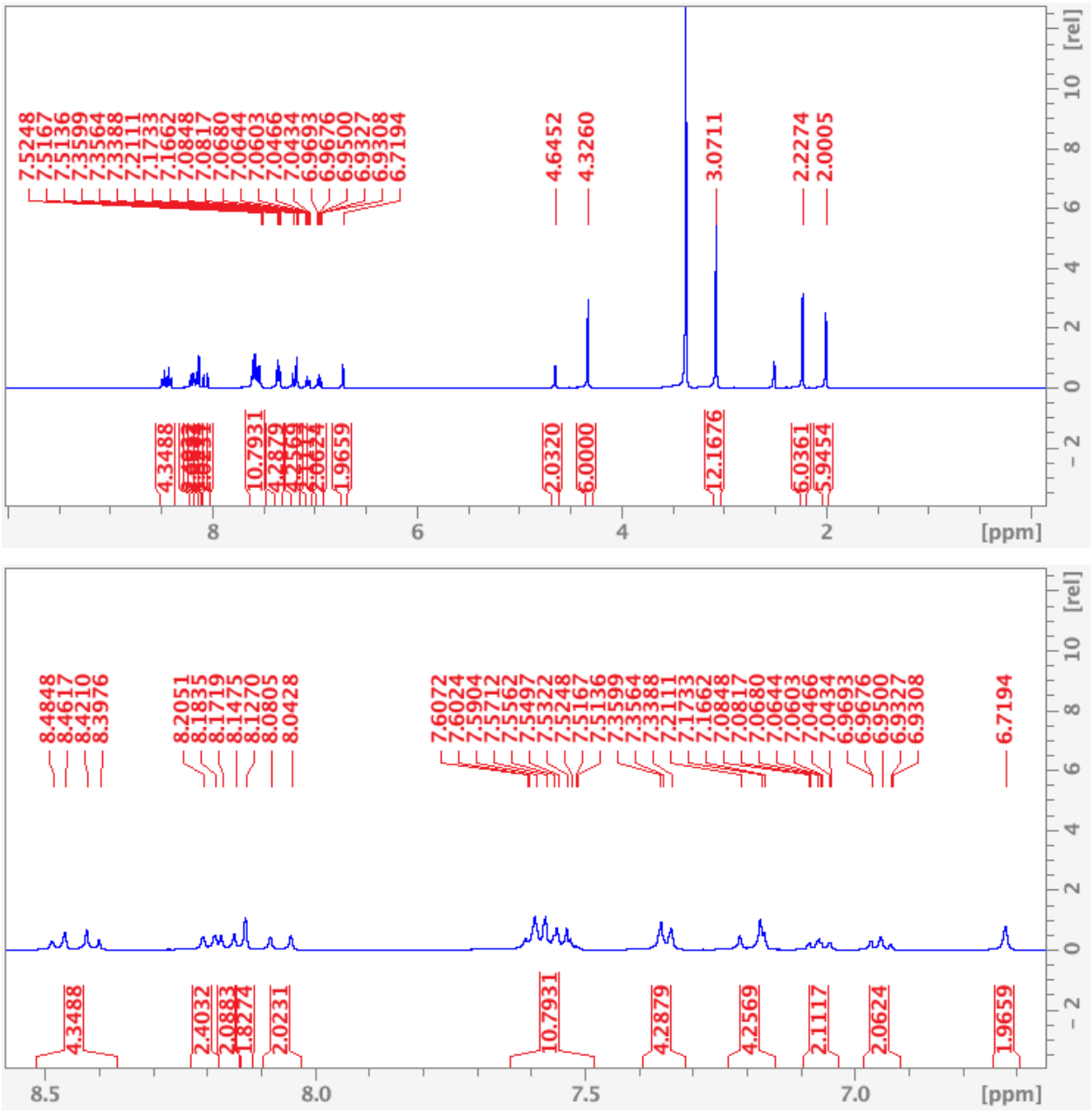
Spectral characterization of* 4,4'-methylenebis(3-hydroxy-2-naphthoic acid), (E)-2-(2-(2,5-dimethyl-1-phenyl-1H-pyrrol-3-yl)vinyl)-6-(dimethylamino)-1-methylquinolin-1-ium salt *(pyrvinium pamoate)

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (q, 4H, *J*= 9.2Hz, 16.3Hz), 8.19 (d, 2H, *J*= 8.6Hz), 8.16 (d, 2H, *J*= 9.8Hz), 8.13 (s, 2H), 8.06 (d, 2H, *J*= 15.1Hz), 7.61-7.52 (m, 10H), 7.36-7.34 (m, 4H), 7.19 (d, 2H, 18Hz), 7.17 (s, 2H), 7.08-7.04 (m, 2H), 6.97-6.93 (m, 2H), 6.72 (s, 2H), 4.65 (s, 2H), 4.33 (s, 6H), 3.07 (s, 12H), 2.23 (s, 6H), 2.00 (s, 6H)

HRMS (ESI) *m/z*: calculated for $C_{26}H_{28}N_3^+$ $[M]^+$: 382.2278, found 382.2282

CHN Anal. Calcd for $C_{75}H_{70}N_6O_6 \cdot 2H_2O$: C, 75.86; H, 6.28; N, 7.08. Found C, 75.48; H, 6.21; N, 7.02.

^{1}H NMR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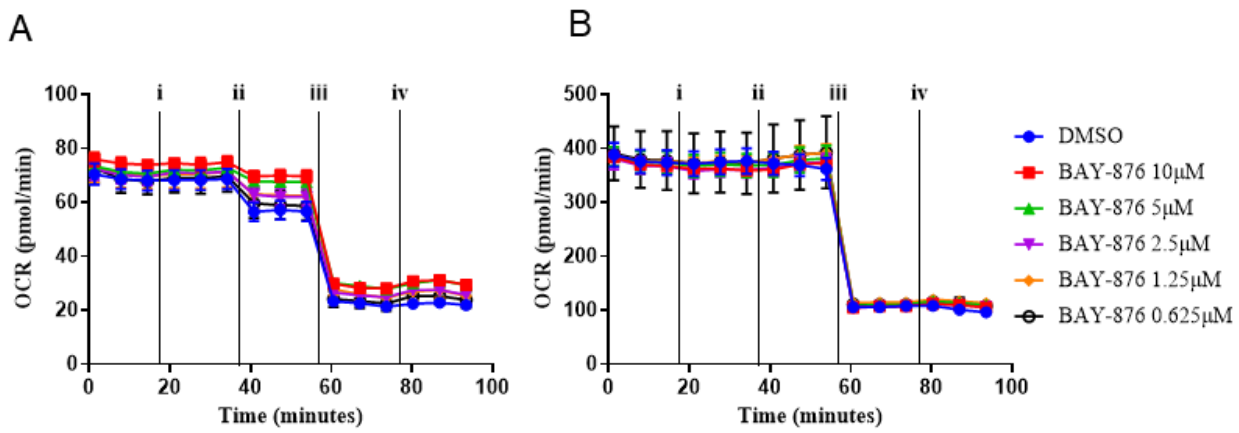

Figure S1: A. MDA-MB-231 oxygen consumption rates (OCR (pmol/min)) vs. time after injection of i) treatment or DMSO ii) glucose iii) oligomycin and iv) 2-deoxyglucose after treatment with BAY-876 (10-0.625µM). **B.** 4T1 oxygen consumption rates (OCR (pmol/min)) vs. time after injection of i) treatment or DMSO ii) glucose iii) oligomycin and iv) 2-deoxyglucose after treatment with BAY-876 (10-0.625µM).

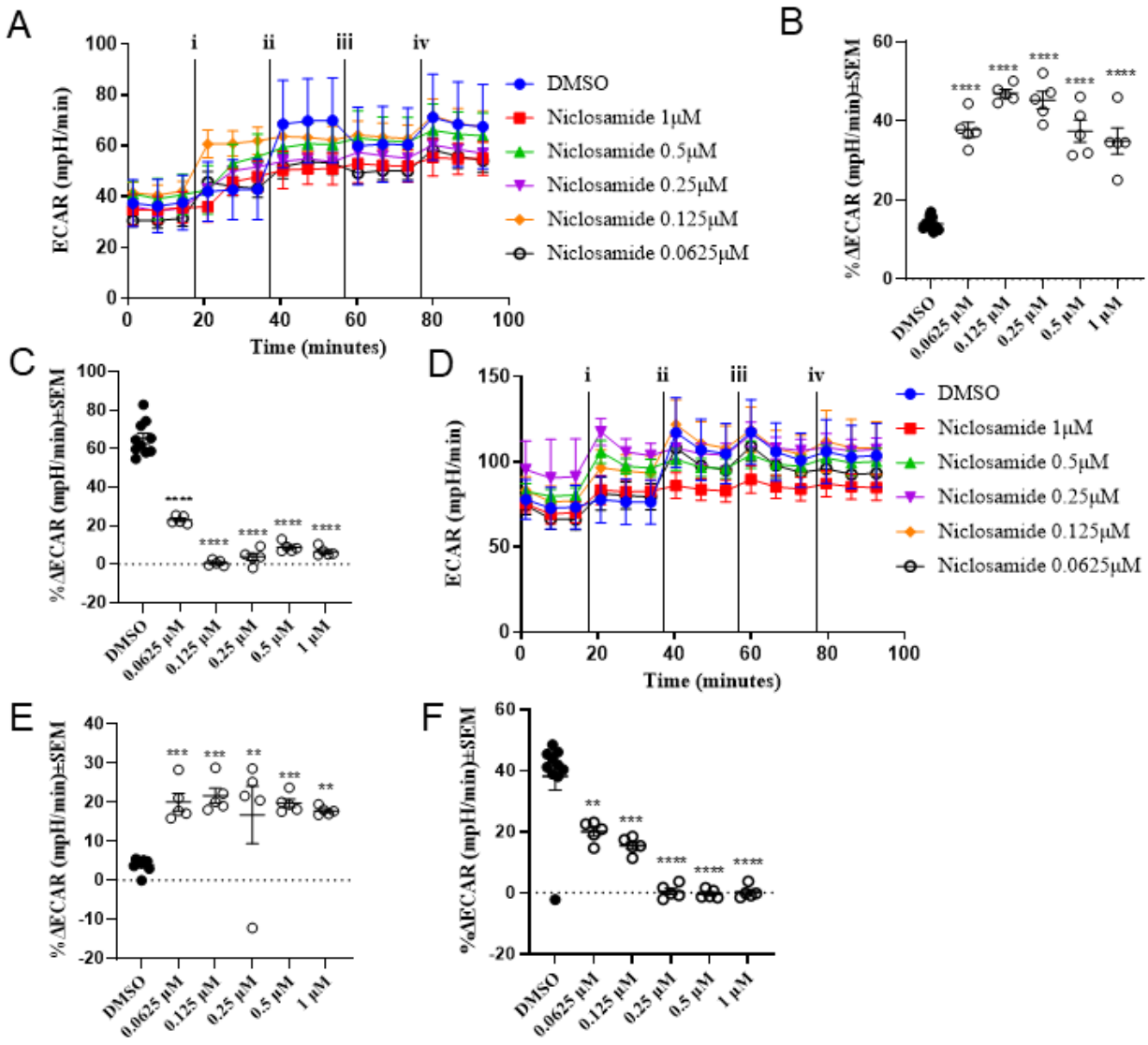

Figure S2: A. MDA-MB-231 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment with niclosamide (1-0.0625µM). **B.** Percent change in ECAR after acute injection of niclosamide (1-0.0625µM) indicating compensatory glycolysis in MDA-MB-231 cells. **C.** Percent change in ECAR after injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in MDA-MB-231 cells. **D.** 4T1 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment with niclosamide (1-0.0625µM). **E.** Percent change in ECAR after acute injection of niclosamide (1-0.0625µM) indicating compensatory glycolysis in 4T1 cells. **F.** Percent change in ECAR after injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in 4T1 cells. **B, C, E, F:** results are representative of 3 biological replicates experiments having a combined total of 15

technical replicates per group ± SEM. Statistical analysis was carried out via Dunnett's one-way ANOVA compared to DMSO control (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

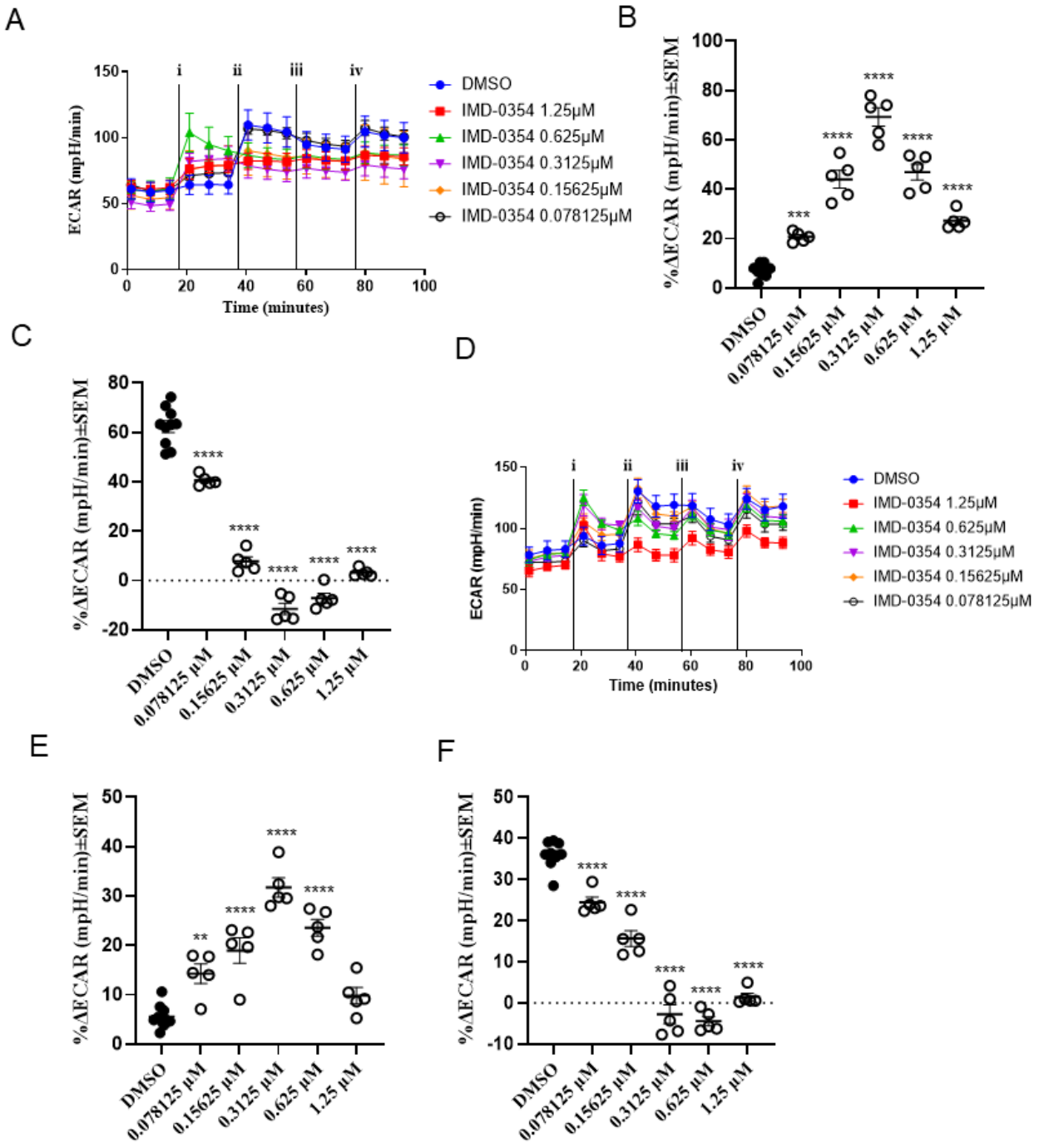

Figure S3: A. MDA-MB-231 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment IMD-0354 (1.25-0.078125μM). **B.** Percent change in ECAR after acute injection of IMD-0354 (1.25-0.078125μM) indicating compensatory glycolysis in MDA-MB-231 cells. **C.** Percent change in ECAR after

injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in MDA-MB-231 cells. **D.** 4T1 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment with IMD-0354 (1.25-0.078125μM). **E.** Percent change in ECAR after acute injection of IMD-0354 (1.25-0.078125μM) indicating compensatory glycolysis in 4T1 cells. **F.** Percent change in ECAR after injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in 4T1 cells. **B, C, E, F:** results are representative of 3 biological replicates experiments having a combined total of 15 technical replicates per group ± SEM. Statistical analysis was carried out via Dunnett's one-way ANOVA compared to DMSO control (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

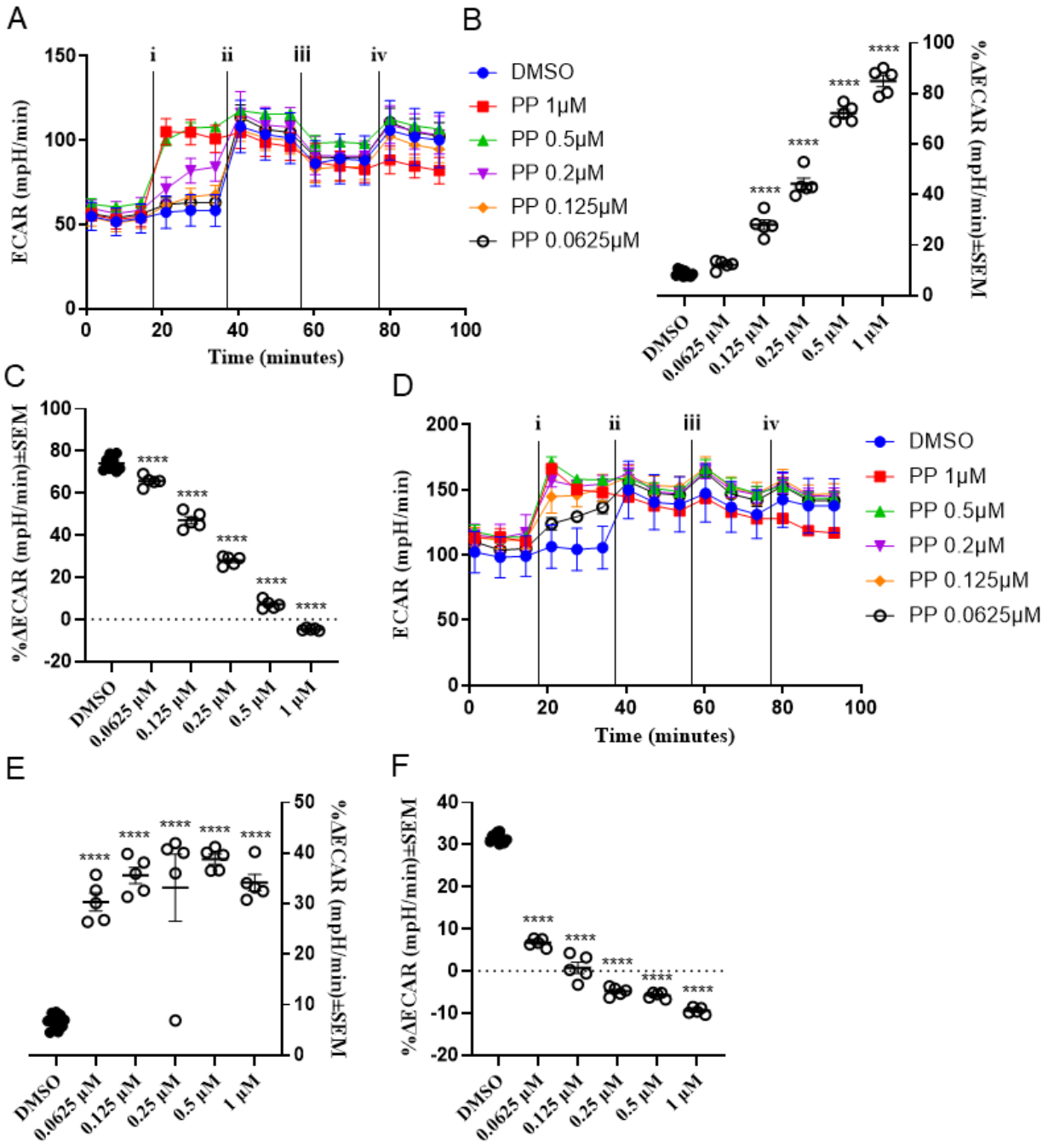

Figure S4: A. MDA-MB-231 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment with PP (1-0.0625μM). **B.** Percent change in ECAR after acute injection of PP (1-0.0625μM) indicating compensatory glycolysis in MDA-MB-231 cells. **C.** percent change in ECAR after injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in MDA-MB-231 cells. **D.** 4T1 extracellular acidification rates (ECAR (mpH/min)) vs. time after injection of i) treatment

or DMSO ii) oligomycin iii) FCCP and iv) Rotenone and Antimycin A after treatment with PP (1-0.0625μM). **B.** Percent change in ECAR after acute injection of PP (1-0.0625μM) indicating compensatory glycolysis in 4T1 cells. **C.** percent change in ECAR after injection of oligomycin indicating switch to glycolytic metabolism after uncoupling mitochondrial ATP production in 4T1 cells. **B, C, E, F:** results are representative of 3 biological replicates experiments having a combined total of 15 technical replicates per group ± SEM. Statistical analysis was carried out via Dunnett's one-way ANOVA compared to DMSO control (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).