Supporting Information

Fused Tetrahydroquinolines are Interfering with Your Assay

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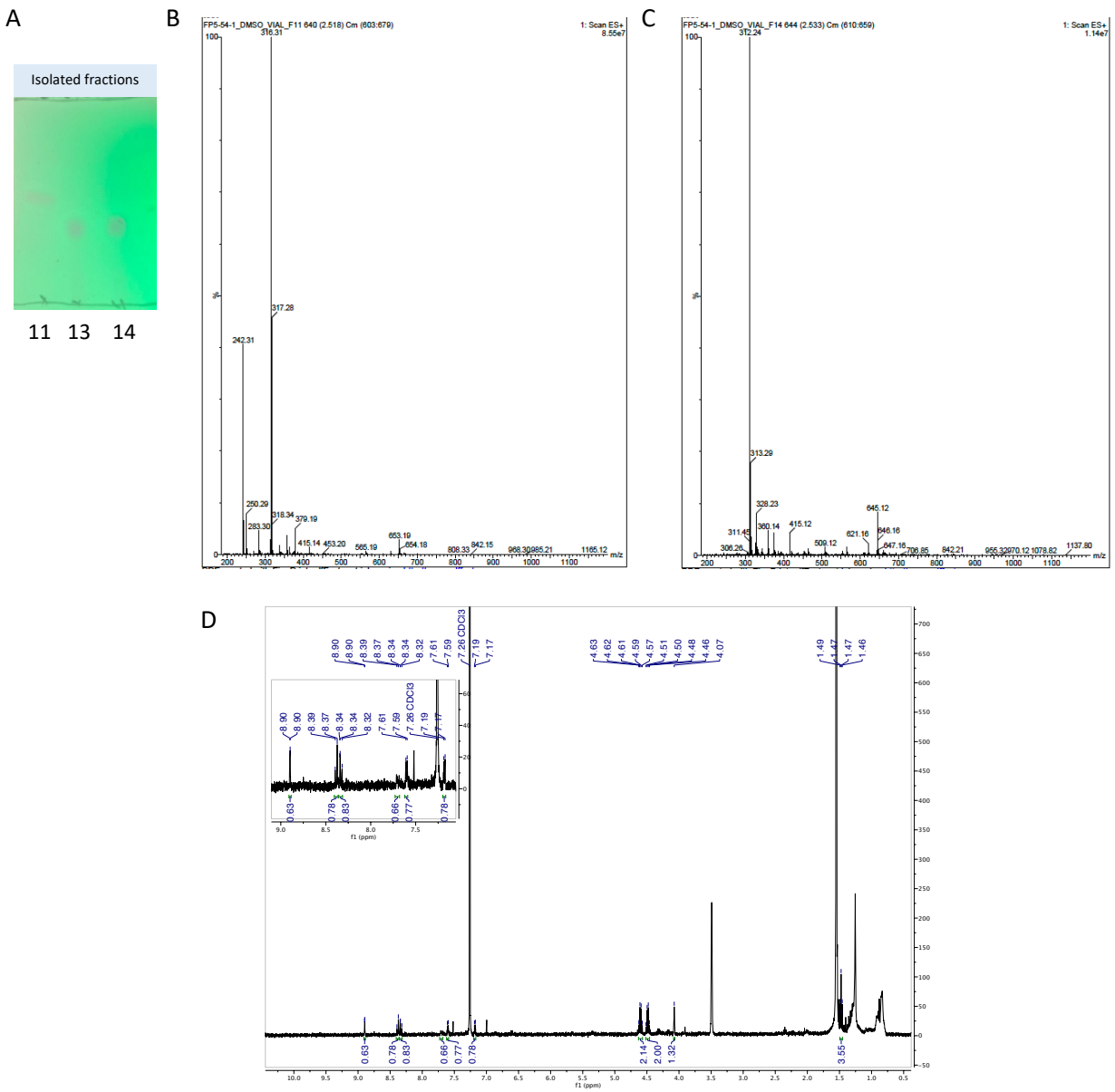


Figure S1. Isolated decomposition products from fused THQs kept in solution exposed to light. (A) Thin layer chromatography analysis (eluent: 20% ethyl acetate/80% hexane) of separated fractions following purification of decomposed THQ (5 mg) via silica column chromatography. LCMS data collected from (B) fraction 11 corresponding to fused THQ **1** and (C) fraction 14 corresponding to THQ decomposition product **1b**. (D) ^{1}H NMR of combined fractions 13 and 14 from panel A in $CDCl_3$, corresponding to the major decomposition product of **1**.

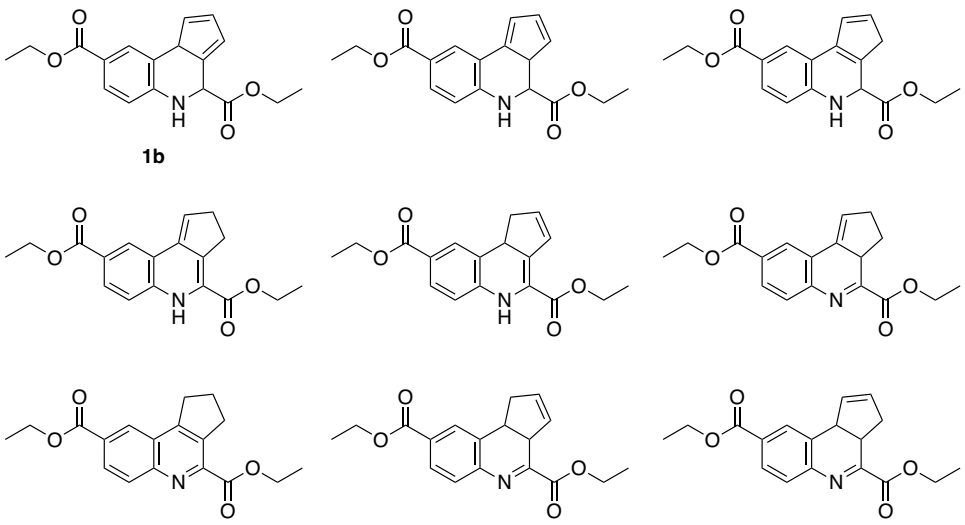


Figure S2. Potential alternative structures of **1b**.

Biology Experimental

MSN TR-FRET assay

A time-resolved fluorescence resonance energy transfer (TR-FRET) assay was established to measure the binding of CD44 peptide to MSN. Taking advantage of the interaction of 6His-tagged MSN and biotinylated CD44 peptide, a europium-labeled anti-His antibody and streptavidin-XL665 antibody enabled FRET (Figure S3). This was a slight modification of the previously developed TR-FRET assay for the protein pair.[1] The assay buffer was composed of 25 mM HEPES [pH 7.5], 200 mM NaCl, 0.05% Tween-20, and 1mM DTT, and then filtered. The donor fluorophore was LANCE Eu-W1024 Anti-6xHis at 1 nM and the acceptor fluorophore was streptavidin Alexa 647 at 10 nM.

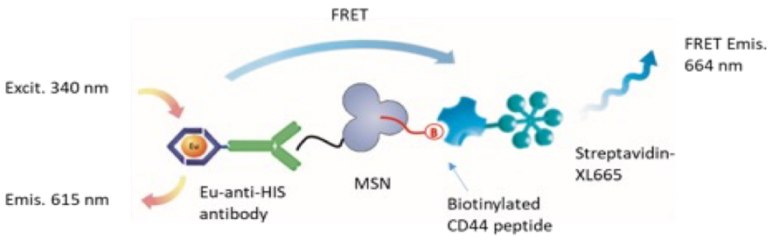


Figure S3. TR-FRET assay scheme.

Chemistry Experimental

*Diethyl 3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-4,8-dicarboxylate (**1**).* To a 10 mL round-bottom flask equipped with a reflux condenser was added ethyl 4-aminobenzoate (0.36 g, 2.15 mmol), ethyl glyoxalate (50% solution in toluene, 426 µL, 4.30 mmol) and sodium sulfate (0.46 g, 3.23 mmol) in toluene (3 mL), and the reaction was heated to 110 °C for 30 min. The reaction was cooled, filtered and the solvent was concentrated *in vacuo*. The oily residue, ethyl (*E*/*Z*)-4-((2-ethoxy-2-oxoethylidene)amino)benzoate, was used in the next step without further purification.

Ethyl (*E*/*Z*)-4-((2-ethoxy-2-oxoethylidene)amino)benzoate (1.50 g, 6.02 mmol) and acetonitrile (2.5 mL) were charged to a 50 mL round-bottom flask. The flask was fitted with a

septum and purged with nitrogen balloon. Boron trifluoride etherate (149 µL, 1.20 mmol) was added via syringe, followed by the addition of freshly distilled cyclopentadiene (988 µL, 12.04 mmol) and the reaction was stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and the solvent was concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2, 0–100% EtOAc in hexane) to afford **1** as an off-white solid (950 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 – 7.69 (m, 1H), 7.67 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 5.81 (ddt, *J* = 5.8, 2.9, 1.5 Hz, 1H), 5.66 (dd, *J* = 5.8, 2.4 Hz, 1H), 4.38 – 4.19 (m, 5H), 4.16 (d, *J* = 3.6 Hz, 1H), 4.12 – 4.08 (m, 1H), 3.36 (tt, *J* = 9.0, 4.4 Hz, 1H), 2.48 – 2.40 (m, 1H), 2.33 (ddt, *J* = 11.9, 9.0, 3.4 Hz, 1H), 1.37 – 1.34 (m, 3H), 1.34 – 1.31 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.42, 166.69, 147.89, 134.15, 130.72, 129.88, 128.51, 124.77, 120.68, 114.79, 61.46, 60.31, 55.61, 45.78, 40.61, 32.49, 14.44, 14.25. HPLC Purity: >95 %. LCMS (ESI) for $[M+H]^+$ ($C_{18}H_{22}NO_4$): 316.15, found: 316.44.

*Diethyl 2,3,3a,4,5,9b-hexahydro-1H-cyclopenta[c]quinoline-4,8-dicarboxylate (**2**).* To a 10 mL round-bottom flask was added the tetrahydroquinoline ester derivative (**1**, 50 mg) and ethanol (2.5 mL). The flask was fitted with a septum and purged with nitrogen balloon. Palladium on carbon (10% w/w, 5 mg, 0.02 mmol) was added and the mixture was run under hydrogen at room temperature for 24 h. Filtration of the dark solid through celite gave a liquid residue which was concentrated *in vacuo* to afford the desired reduced product (**2**) as an off-white solid (49 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 4.69 (s, 1H), 4.36 – 4.18 (m, 5H), 4.13 (d, *J* = 3.4 Hz, 1H), 3.39 (s, 1H), 2.81 (d, *J* = 9.0 Hz, 1H), 2.13 – 2.04 (m, 1H), 1.92 (s, 1H), 1.53 – 1.44 (m, 3H), 1.33 (dt, *J* = 14.1, 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 166.9, 147.2, 130.9, 128.6, 124.3, 120.1, 113.9, 61.4, 60.3, 54.9, 40.9, 40.1, 34.6, 24.5, 23.3, 14.4, 14.2. HPLC Purity: >95%. LCMS (ESI) for $[M+H]^+$ ($C_{18}H_{24}NO_4$): 318.16, found: 318.31.

General Procedure for Saponification: To a 50 mL round-bottom flask was suspended the tetrahydroquinoline ester derivative (**1** or **2**, 50 mg) in 1M LiOH (5 mL). THF or MeOH was added until a clear solution remained. The reaction mixture was stirred for 4 h at room temperature and then neutralized with 1N HCl (10 mL). Extraction with EtOAc (3 x 20 mL) gave the combined organic layers which were washed with brine, dried with Na_2SO_4, and concentrated *in vacuo* to yield the desired product.

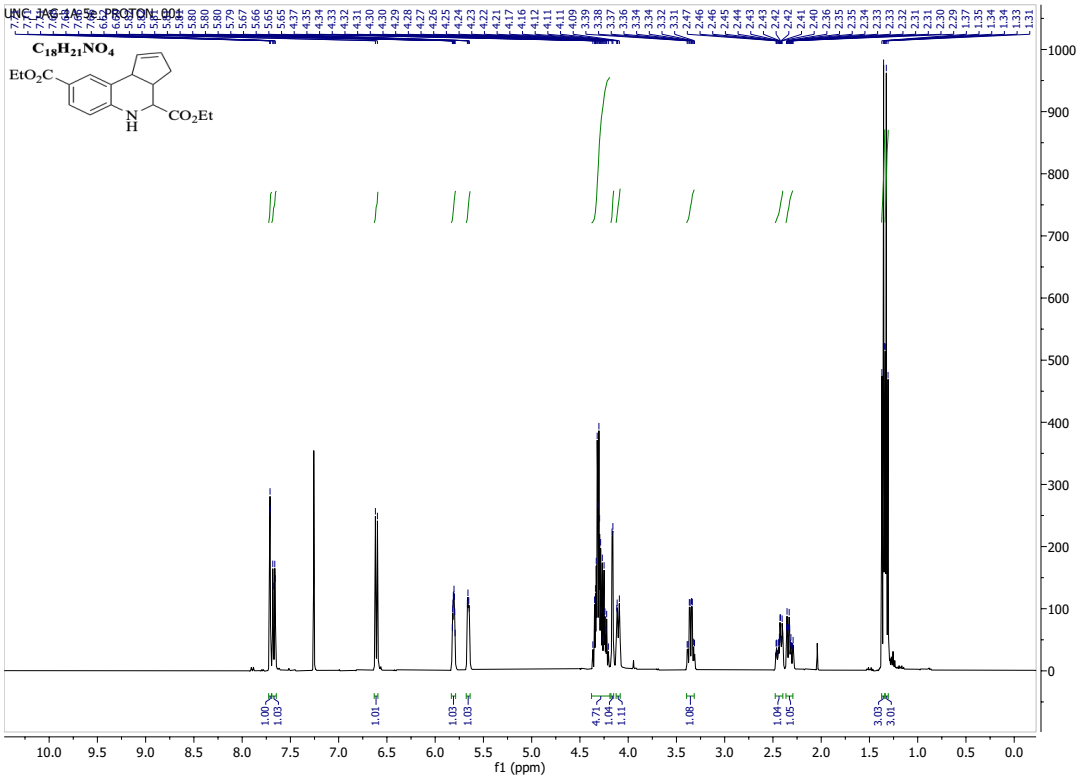
*3a,4,5,9b-Tetrahydro-3H-cyclopenta[c]quinoline-4,8-dicarboxylic acid (**5661118**).* Compound **1** (50 mg, 0.16 mmol) was dissolved in MeOH and saponified as described in the general procedure above to yield **5661118** as a light brown solid (39 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 2H), 7.52 (s, 1H), 7.43 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.09 (s, 1H), 5.83 – 5.78 (m, 1H), 5.61 (d, *J* = 6.7 Hz, 1H), 4.06 (d, *J* = 3.3 Hz, 1H), 4.01 (d, *J* = 8.3 Hz, 1H), 3.17 – 3.09 (m, 1H), 2.33 – 2.16 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.68, 167.43, 149.08, 134.85, 130.37, 129.39, 127.86, 123.65, 119.03, 114.70, 54.45, 44.93, 40.28, 32.10. HPLC Purity: >95%. LCMS (ESI) for $[M+H]^+$ ($C_{14}H_{14}NO_4$): 260.08, found: 260.14.

*2,3,3a,4,5,9b-Hexahydro-1H-cyclopenta[c]quinoline-4,8-dicarboxylic acid (**3**).* Compound **2** (50 mg, 0.16 mmol) was dissolved in MeOH and saponified as described above to yield **3** as a tan solid (34 mg, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 2H), 7.57 (s, 1H), 7.43 (dd, *J* = 8.4, 2.0

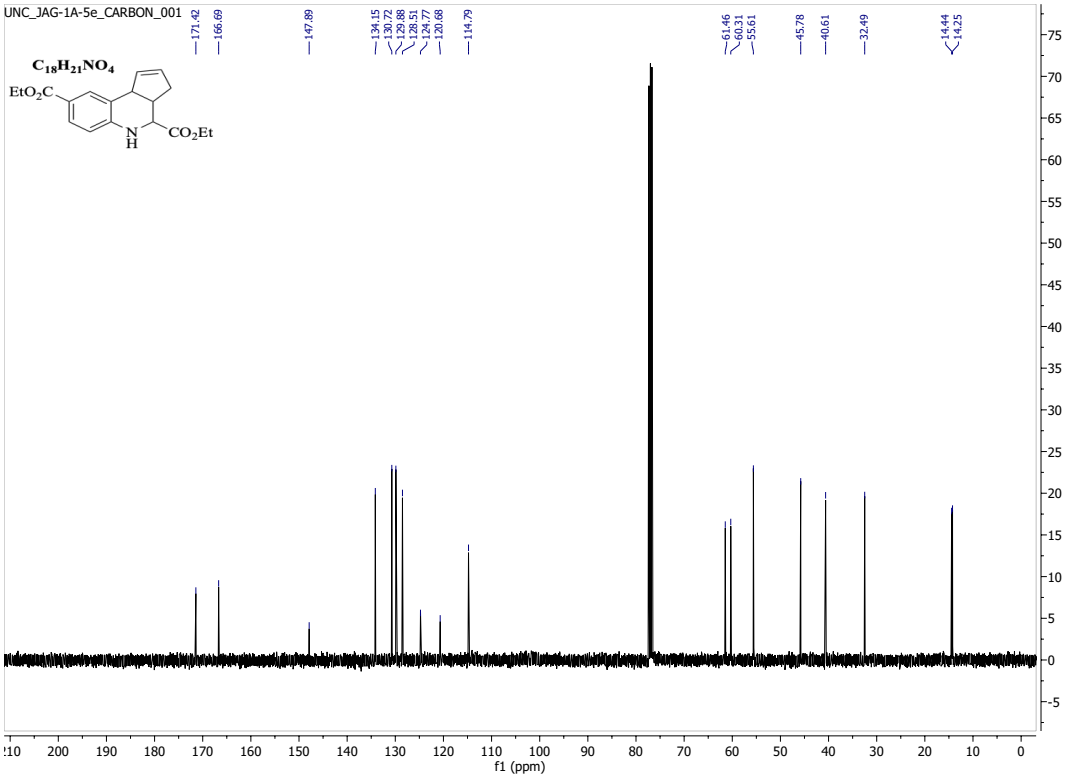
Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.23 (s, 1H), 4.04 (d, J = 3.4 Hz, 1H), 2.67 – 2.59 (m, 1H), 2.03 (dq, J = 15.0, 7.9 Hz, 1H), 1.86 – 1.21 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.41, 167.98, 148.87, 130.61, 128.48, 123.25, 118.78, 114.07, 54.15, 40.78, 34.26, 24.57, 23.09. HPLC Purity: >95 %. LCMS (ESI) for $[M+H]^+$ ($C_{14}H_{16}NO_4$): 262.10, found: 262.15.

*Diethyl 3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-4,8-dicarboxylate (**1**)*

^{1}H NMR Analysis



^{13}C NMR Analysis



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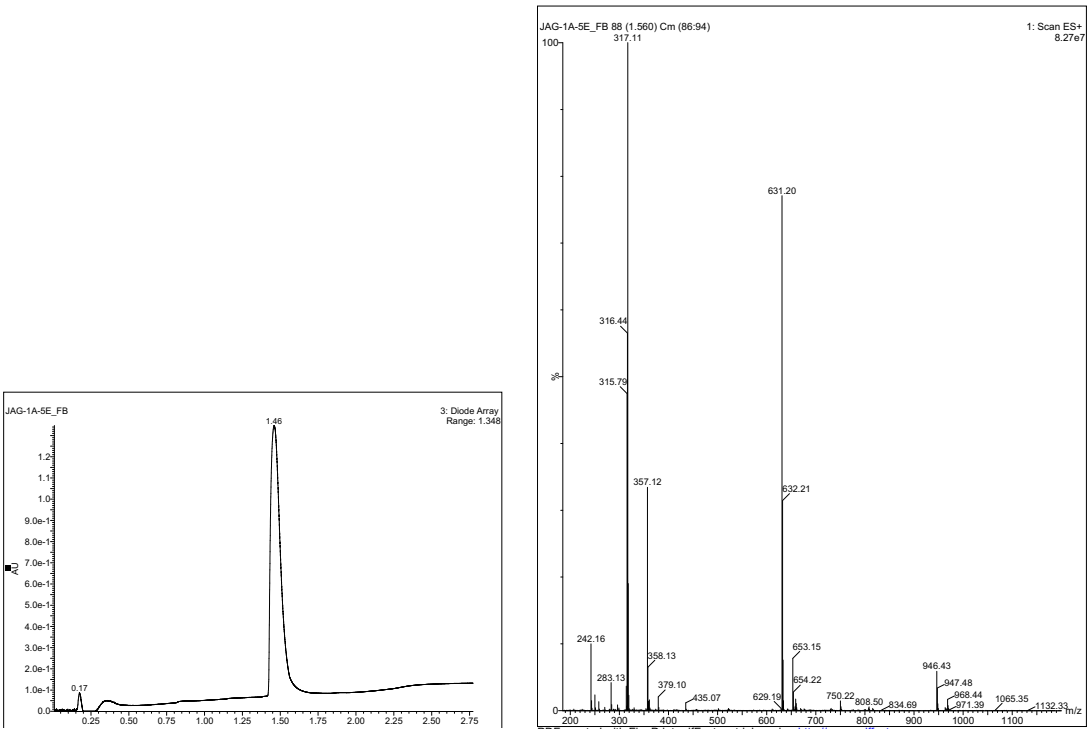
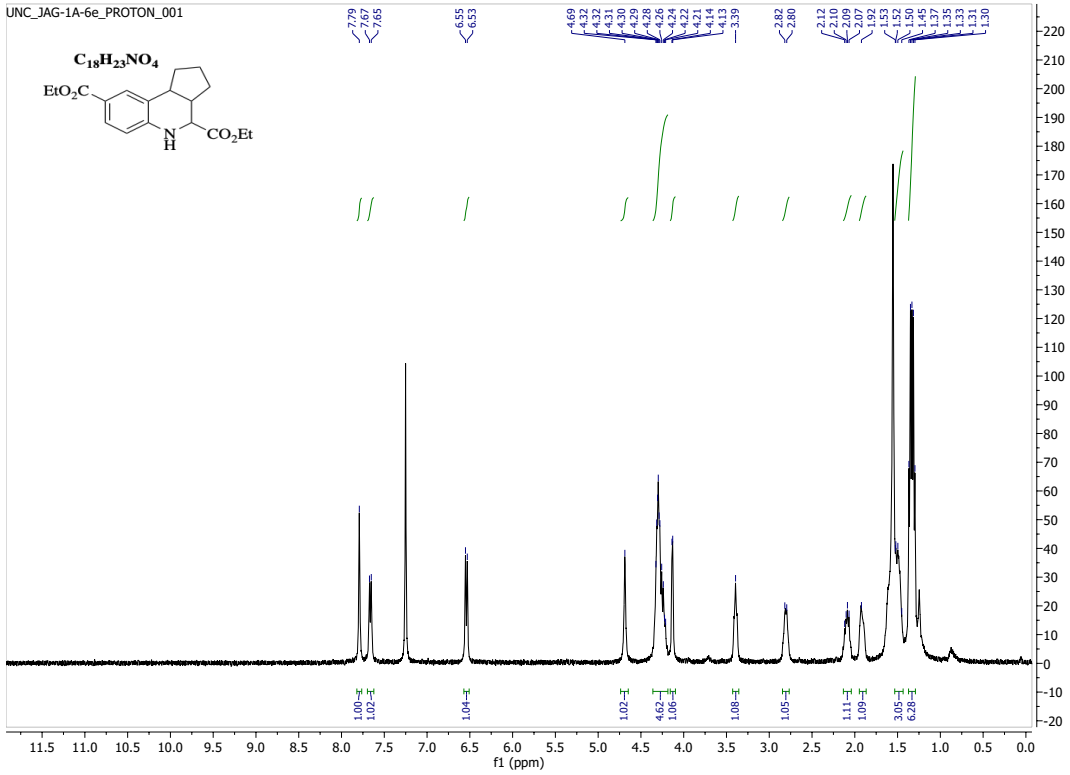


*Diethyl 2,3,3a,4,5,9b-hexahydro-1H-cyclopenta[c]quinoline-4,8-dicarboxylate (**2**).*

^{1}H NMR Analysis



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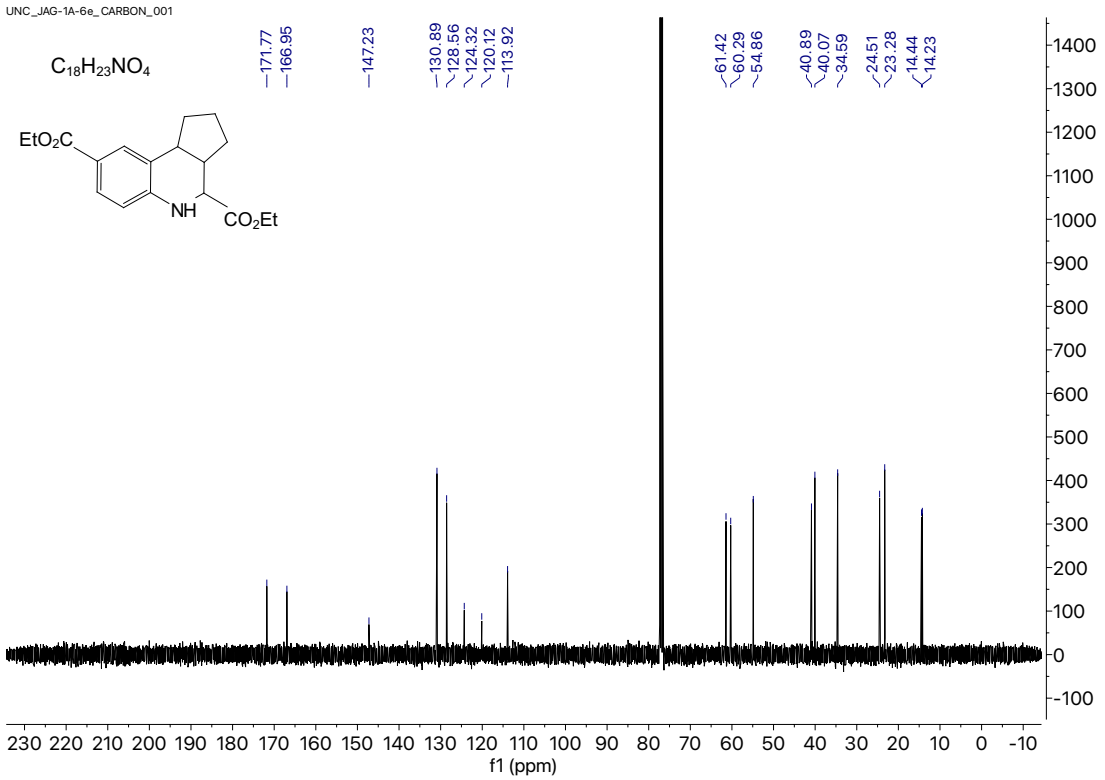

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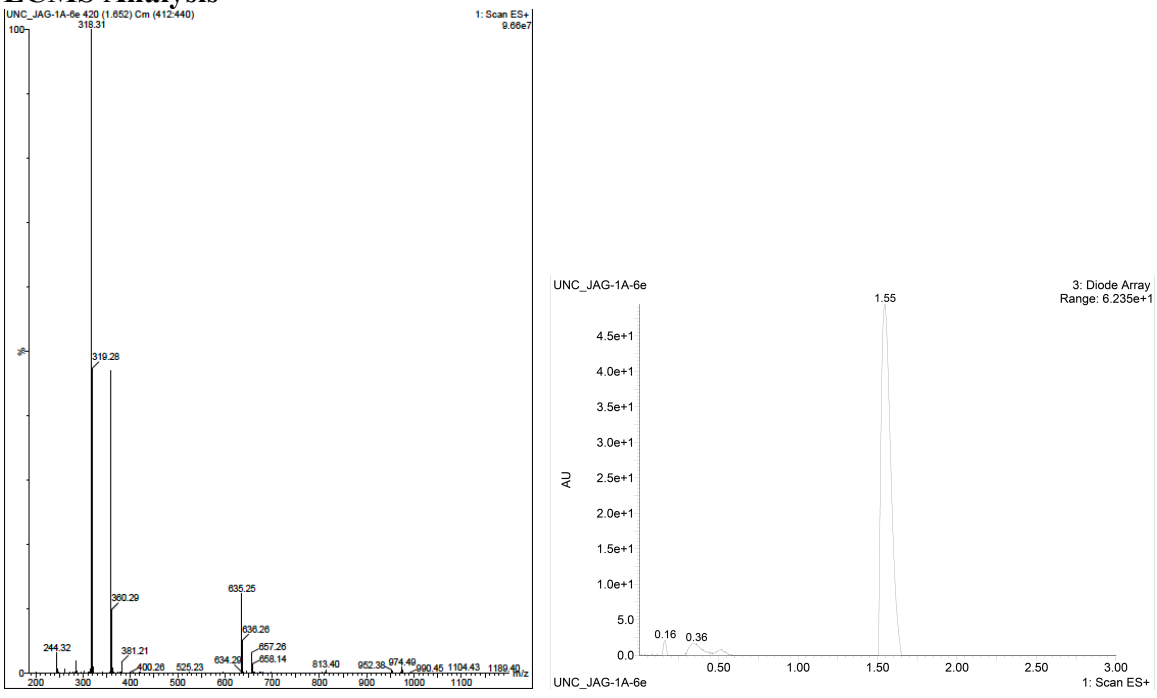
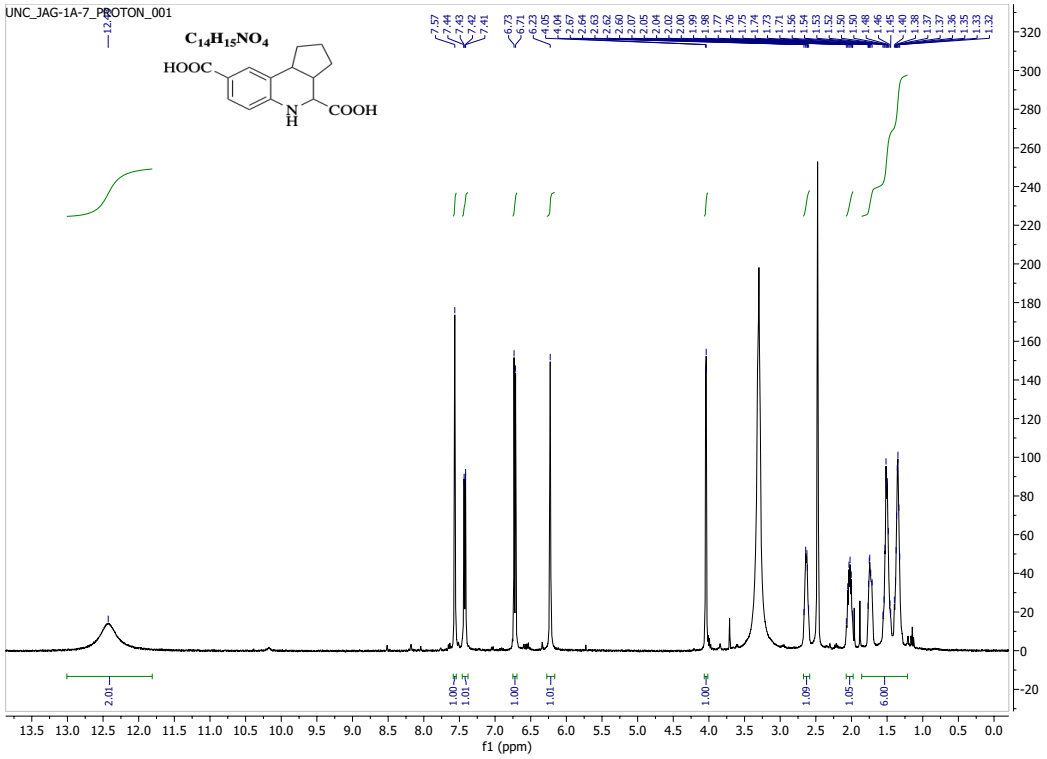


*2,3,3a,4,5,9b-Hexahydro-1H-cyclopenta[c]quinoline-4,8-dicarboxylic acid (**3**).*

^{1}H NMR Analysis



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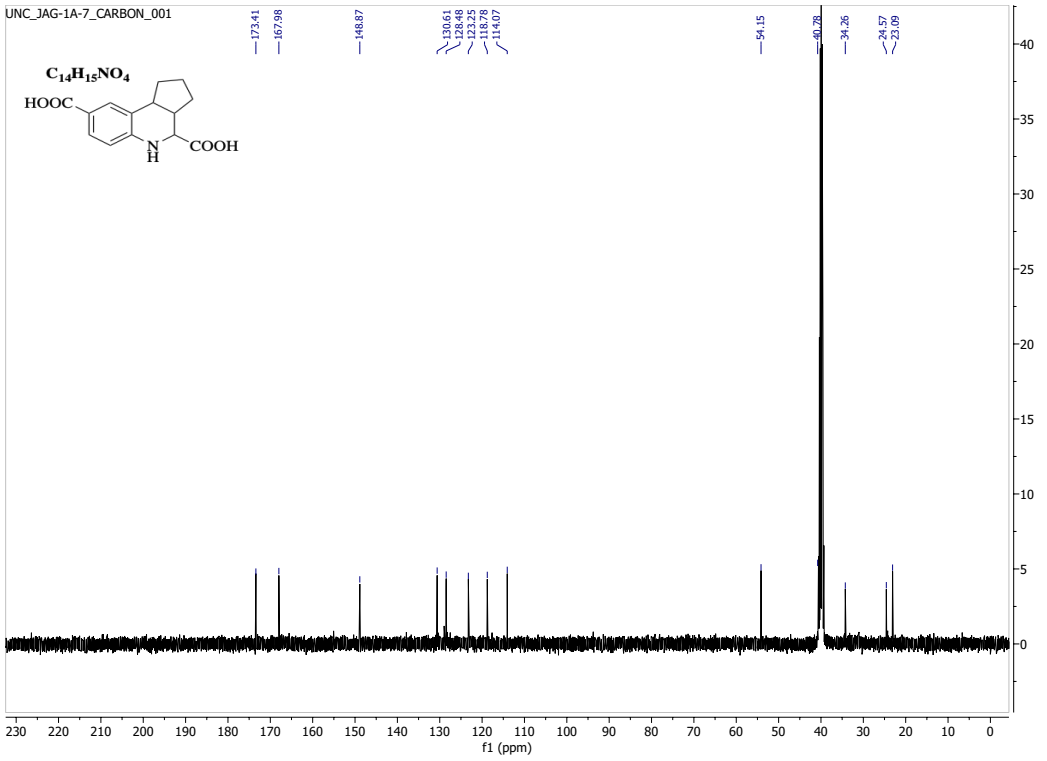

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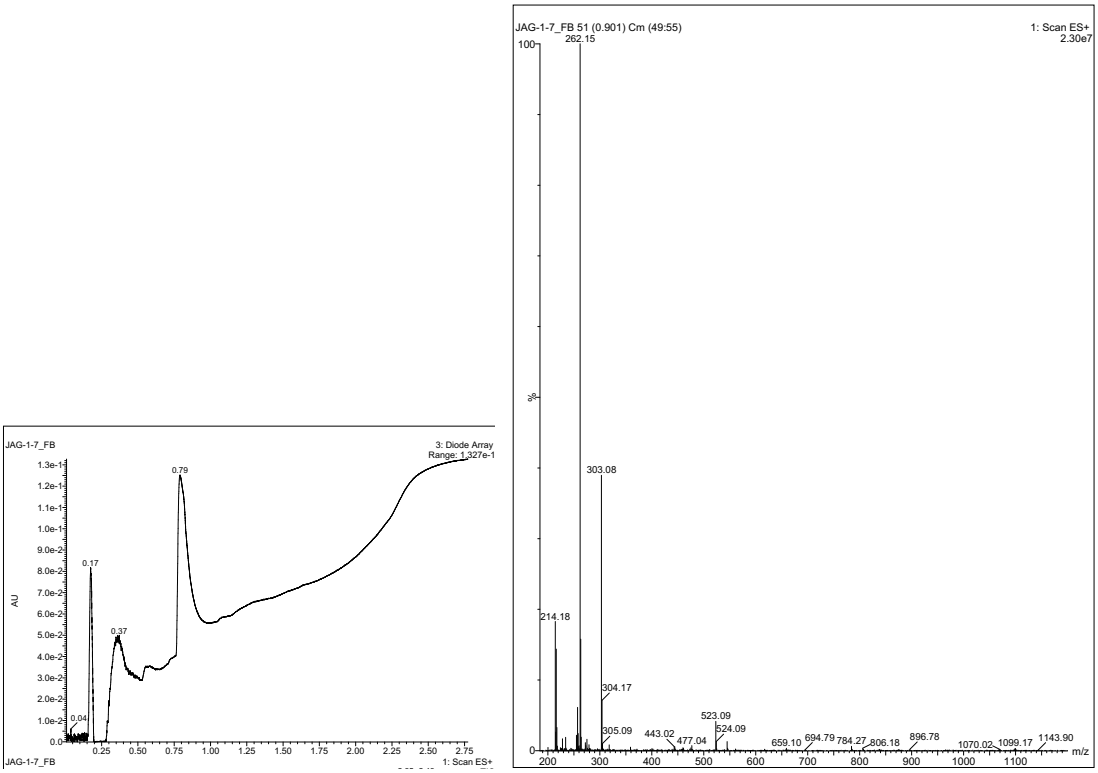
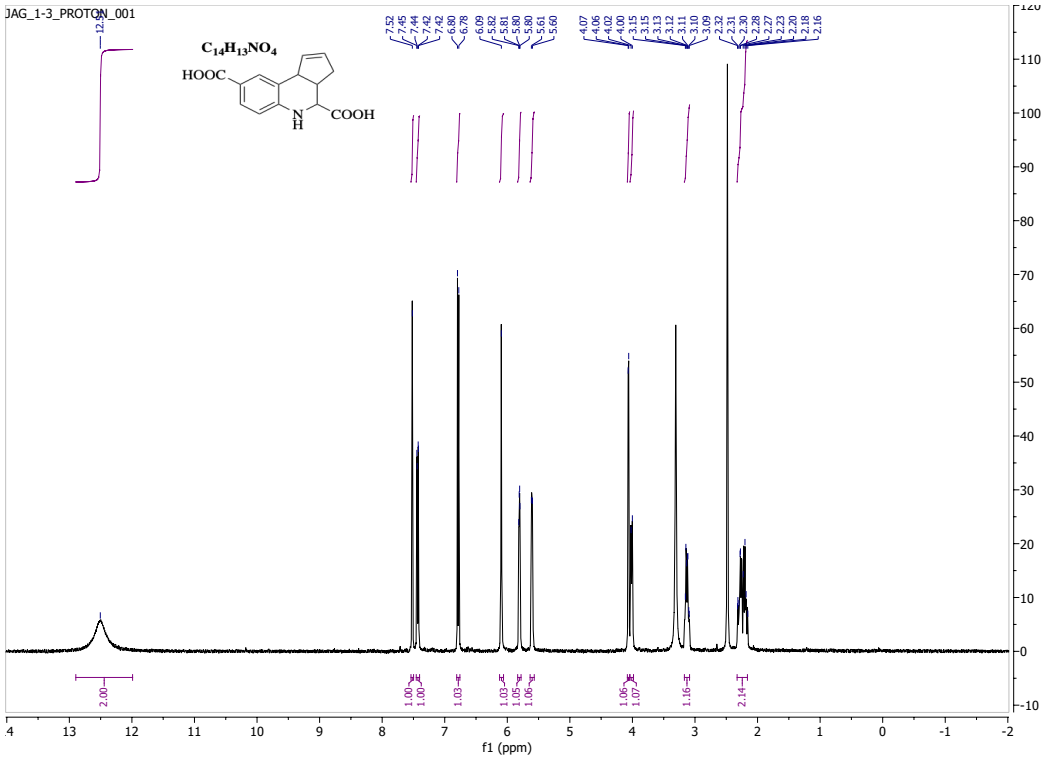


*3a,4,5,9b-Tetrahydro-3H-cyclopenta[c]quinoline-4,8-dicarboxylic acid (**5661118**).*

^{1}H NMR Analysis



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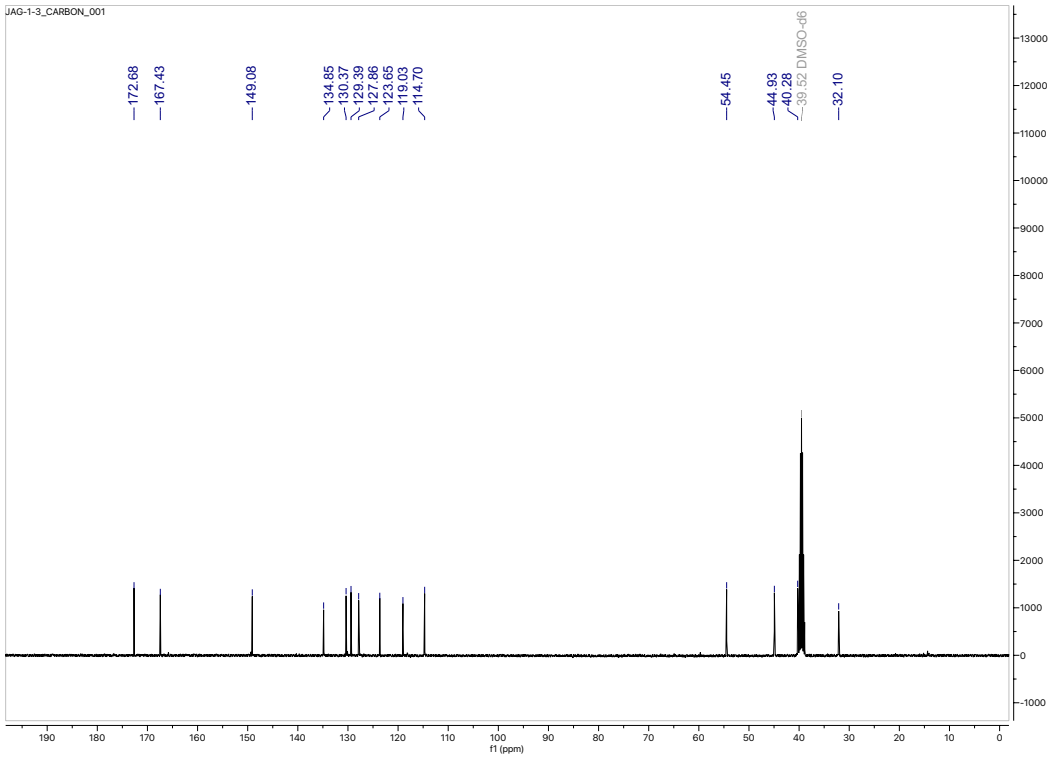

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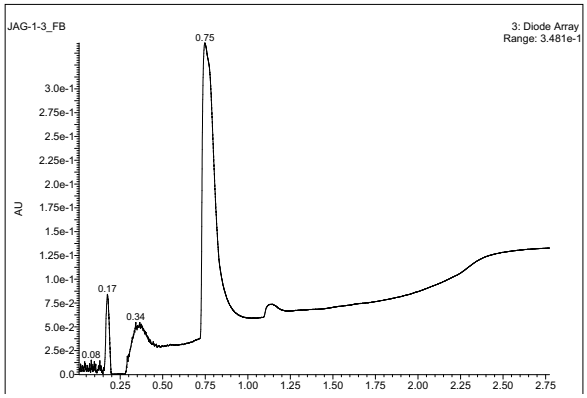

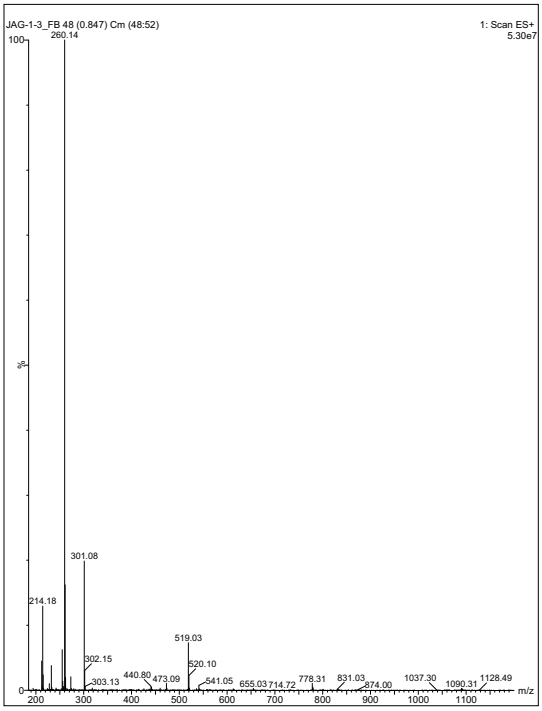

Reference

1. Du, Y.; Bradshaw, W. J.; Leisner, T. M.; Annor-Gyamfi, J. K.; Qian, K.; Bashore, F. M.; Sikdar, A.; Nwogbo, F. O.; Ivanov, A. A.; Frye, S. V.; Gileadi, O.; Brennan, P. E.; Levey, A. I.; Emory-Sage-SCG TREAT-AD Center; Axtman, A. D.; Pearce, K. H.; Fu, H.; Katis, V. L. Development of FERM domain protein-protein interaction inhibitors for MSN and CD44 as a potential therapeutic strategy for Alzheimer's disease. *BioRxiv* **2023**, 2023.2005.2022.541727. DOI: 10.1101/2023.05.22.541727.