Supporting Information for

Expansion of cat-ELCCA for the Discovery of Small Molecule Inhibitors of the pre-let-7–Lin28 RNA-Protein Interaction

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A. General Materials and Methods

General chemistry methods. All solvents and reagents were used without further purification. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm SiliCycle silica gel plates (60F-254) using UV-light (254 nm). Flash chromatography was performed using SiliaFlash P60 silica gel. Analytical RP-HPLC was performed using an Agilent 1260 Infinity HPLC equipped with a ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm; 5 μm) at a flow rate of 1 mL/min, with detection at 214 and 254 nm. NMR spectra were performed on a 300 MHz Bruker instrument and calibrated using a solvent peak as an internal reference. Spectra were processed using MestReNova software. Mass spectrometry (HRMS) was performed using an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer using ESI ionization with less than 5-ppm error for all HRMS analyses.

General assay and biology methods. Chemiluminescence data was collected on either a BioTek Cytation3 or PHERAstar FS plate reader. Gels were imaged on a ProteinSimple Fluorchem M Gel Imager. BL21DE3 *E. coli* were used for protein expression.

Data analysis. All data was analyzed using GraphPad Prism version 6.0c for Mac OS X (GraphPad Software, www.graphpad.com).

Materials. Chemically synthesized pre-let-7d (deprotected, desalted and HPLC purified) containing a pentylamine modification off of the 5'-phosphate (N5) was purchased from Dharmacon. Murine Lin28A was amplified from a pETDuet vector generously provided by Prof. Richard Gregory (Harvard). pFN29K and pFC30K His6HaloTag® T7 Flexi® vectors and the Flexi® System cloning kit were purchased from Promega. Methyltetrazine (mTet)-NHS and *trans*-cyclooctene (TCO)-PEG4-NHS were purchased from Click Chemistry Tools and used as received. Biotin-PEG7-HaloTag ligand was synthesized as described.[1] Horseradish peroxidase (HRP), streptavidin-coated 384-well plates (white, high binding capacity; cat #15505), and SuperSignal West Pico Chemiluminescent substrate kit were purchased from Pierce. mTet-HRP was prepared as described.[2] SYBR Gold was purchased from Life Technologies.

B. Protein Expression and Purification

HaloTag-mLin28A cloning. Murine Lin28A was amplified from a pETDuet vector and ligated into pFN29k using standard cloning techniques with SgfI and PmeI restriction enzymes.

Primers:

5' AGTCAGCGATCGCTTCAGGCTCGGTGTCCAACCAGC
5' AAACCTTTTCAATTCTGGGCTTCTGGGAGC

HaloTag-mLin28A expression and purification. E. coli transformed with pFN29K HaloTag-mLin28A were grown at 37 °C to an OD_{600} of 0.6 and induced with IPTG (500 μM) overnight at 37 °C. Cells were pelleted for 15 min at 3,000×g, suspended in phosphate-buffered saline (100 mM phosphate buffer (pH 7), 150 mM NaCl), centrifuged again for 15 min at 3000×g, and stored as pellets at -80 °C until needed. Pellets were thawed at 25 °C on ice before being lysed via sonication at 4 °C in lysis buffer containing 20 mM imidazole (pH 8), 10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, 0.1% PMSF and 1 mM DTT. Lysates were then centrifuged at 3000×g for 30 min at 4 °C. The supernatant was subsequently applied to Ni-NTA resin and washed twice (1× in lysis buffer; 1× in 50:50 lysis buffer/wash buffer (10 mM Tris (pH 8), 50 mM imidazole, 500 mM NaCl, 0.1% PMSF, 1 mM DTT). HaloTag-mLin28A was eluted in 10 mM Tris (pH 8), 500 mM imidazole, 500 mM NaCl, 0.1% PMSF, 1 mM DTT, before being dialyzed overnight into 20 mM Tris (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol (v/v). Protein concentrations were determined by the Bradford assay using BSA standards.

HaloTag-Lin28B cloning. Human Lin28B was amplified from A549 cDNA and ligated into pFC30K vector using standard cloning techniques with SgfI and EcoIRCI restriction enzymes. cDNA was prepared using Superscript III first strand synthesis kit (Invitrogen).

Primers:

5' AGCTGCGATCGCAATGGCCGAAGGCGGGGC
5' AGCTGAGCTCTTGTCTTTTTCCTTTTTTGAACTGAAGG

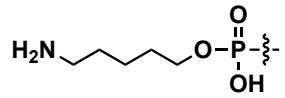
HaloTag-Lin28B expression and purification. E. coli transformed with pFN30K HaloTag-Lin28B were grown at 37 °C to an OD_{600} of 0.8 and induced with IPTG (500 μM) overnight at 37 °C. Cells were pelleted at 4 °C for 15 min at 3,000×g, suspended in phosphate-buffered saline (100 mM phosphate buffer (pH 7), 150 mM NaCl), centrifuged again for 15 min at 3,000×g, and stored as pellets at -80 °C until needed. Pellets were thawed at 25 °C on ice before being lysed via sonication at 4 °C in lysis buffer containing 20 mM imidazole (pH 8), 10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, 0.1% PMSF and 1 mM DTT. Lysates were then centrifuged at 3,000×g for 30 min at 4 °C. The supernatant was subsequently applied to Ni-NTA resin and washed twice (1× in lysis buffer; 1× in 50:50 lysis buffer/wash buffer (10 mM Tris (pH 8), 50 mM imidazole, 500 mM NaCl, 0.1% PMSF, 1 mM DTT). HaloTag-Lin28B was eluted in 10 mM Tris (pH 8), 500 mM imidazole, 500 mM NaCl, 0.1% PMSF, 1 mM DTT, before being dialyzed overnight into 20 mM Tris (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol (v/v). Lin28B was concentrated using an Amicon Ultra-15 centrifugal filter unit (10 kDa cut-off), and the protein was stored in 20 mM Tris (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol (v/v) and 2 mM of DTT. Protein concentrations were determined by the Bradford assay using BSA standards.

C. Bioconjugation Methods

Biotinylation of Lin28. HaloTag-Lin28 was biotinylated by addition of Biotin-PEG7-HaloTag ligand (10 equiv) in storage buffer (20 mM Tris (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol (v/v)) and incubation overnight at 4 °C. The resulting mixture was buffer exchanged, concentrated in storage buffer, quantified via the Bradford assay, and samples were stored at -80 °C. Biotinylation was confirmed via Western blot with streptavidin-HRP.[1]

TCO-labeling of pre-let-7d. The following RNA sequence was purchased from Dharmacon:

5'-**N5**-AGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUUGCCCACAAGGAGGUAA CUAUACGACCUGCUGCCUUUCU-3'

N5 = 

5'-amino-modified pre-let-7d (1.0 mM in 100 mM phosphate buffer, pH 8.0) was mixed with an equivalent volume of TCO-PEG4-NHS (10 mM in DMSO). The reaction was then allowed to proceed at 25 °C for 1 h. pre-let-7d-TCO was precipitated by the addition of 1.1× volume of 3.0 M sodium acetate (pH 5.2) and 40 volume equivalents of cold ethanol, and pelleted at 20,000 × g for 40 min at 4 °C. The pellet was then suspended in 100 mM phosphate buffer (pH 8.0) at a concentration of 1.0 mM and stored at -80 °C.

Confirmation of pre-let-7d–Lin28 binding via EMSA.
^{32}P: A microcentrifuge tube was charged with the following in this order: 4 μL H_2O, 1 μL 10× T4 polynucleotide kinase (PNK) buffer, 5 μL of a 100 μM stock of pre-let-7d (5'-AGAGGUAGUAGGU UGCAUAGUUUUAGGGCAGGGAUUUUGCCCACAAGGAGGUAACUAUACGACCUGCUGCC UUUCU-3'), 1 μL T4 PNK, and 5 μL of 3.33 μM stock of ^{32}P-γ-ATP (approximately 8.6 mCi/mL). The labeling reaction was allowed to progress at 37 °C for 30 min before 4 μL of 121 μM cold ATP was added. The mixture was subsequently incubated for an additional 30 min at 37 °C. PNK was then inactivated by heat denaturation at 70 °C for 15 min. The RNA was diluted to 4 nM in binding buffer (50 mM Tris (pH 7.6), 150 mM NaCl, 5% glycerol, 0.05% Tween-20; freshly dissolved and added 1 mM $ZnCl_2$ and 10 mM β-mercaptoethanol) containing various concentrations of biotinylated HaloTag-mLin28A in 4 μL-reaction volumes. After incubation at 25 °C for 30 min, the reaction was mixed with 4 μL of loading buffer (binding buffer supplemented with additional 10% glycerol and trace bromophenol blue and xylene cyanol for color) and run on an 8% native TBE gel. ^{32}P was exposed to a phosphorimaging plate and quantified by a Typhoon Phosphoimager.

SYBR Gold: TCO-labeled pre-let-7d (10 μL of a 500 nM stock) and HaloTag-mLin28A were mixed with DMSO or compound in binding buffer (50 mM Tris (pH 7.6), 150 mM NaCl, 5% glycerol (v/v), 0.05% Tween-20, and fresh 1 mM $ZnCl_2$ and 10 mM β-mercaptoethanol). All samples were subsequently adjusted to 5% DMSO final concentration. The mixture was then incubated at 25 °C for 30 min before 10 μL of loading dye. The samples were then loaded on 8% native TBE gels and run in TBE buffer before being stained with SYBR Gold for 5 min. Gels were then imaged on a Protein Simple gel imager using the multiflour green setting.

D. pre-let-7d–Lin28 cat-ELCCA Protocol (384-well format)

PBS: 100 mM phosphate buffer (pH 7), 150 mM NaCl

Storage Buffer: 20 mM Tris (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol, 0.05% Tween-20

Binding Buffer: 50 mM Tris (pH 7.6), 150 mM NaCl, 5% glycerol, 0.05% Tween-20; freshly dissolved and added 1 mM $ZnCl_2$ and 10 mM β-mercaptoethanol

PBST: 100 mM phosphate buffer (pH 7), 150 mM NaCl, 0.05% Tween-20

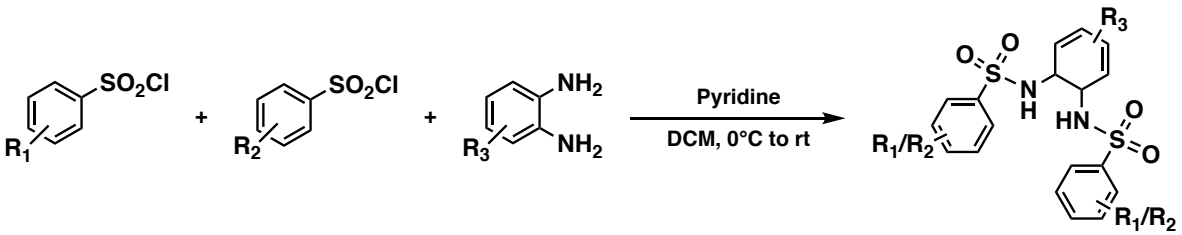
1. Wash the wells with PBS (3× 50 μL)
2. Immobilization of biotinylated HaloTag-mLin28A (10 μL of 200 nM in Storage Buffer)
    a. Centrifuge at 223×g for 1 min
    b. Cover with plate-sealing tape
    c. Overnight incubation (4 °C)
3. Wash the wells with PBS (3× 50 μL)
4. Pre-let-7d and compound incubation
    a. Pre-let-7d (10 μL of 200 nM in Binding Buffer)
    b. Compound (0.5 μL in DMSO)
    c. Adjust to 5% DMSO final concentration
    d. Centrifuge at 223×g for 1 min
    e. Incubate at 25 °C for 1 h
5. Wash the wells with PBS (3× 50 μL)
6. Click chemistry with mTet-HRP
    a. mTet-HRP (10 μL of 750 nM in PBS)
    b. Centrifuge at 223×g for 1 min
    c. Incubate at 25 °C for 1 h
7. Wash the wells with PBST (3× 150 μL)
8. Wash the wells with PBS (3× 50 μL)
9. Chemiluminescence detection
    a. Add 25 μL SuperSignal West Pico Chemiluminescent Substrate (prepared following kit instructions)

E. HTS

Compound libraries. Compounds screened were housed at the University of Michigan Center for Chemical Genomics (CCG). In total, 127,007 compounds from the following collections were used: Sigma LOPAC library of pharmacologically active compounds (1,280), Prestwick library of approved drugs (1,280), ChemDiv 100K library (100,000), Maybridge MB24K library (23,552), and University of Michigan Chemistry library (895). Compounds were tested at 25 μM in the primary and confirmation screens using 5 mM DMSO stocks. Concentration-response curves (CRC) were generated over eight points (1.67-fold serial dilution) from 3.3–120 μM using 5 mM DMSO stocks; however, compounds were first dispensed with a Mosquito X1 (TTP Labtech; Cambridge, MA) into polypropylene 384-well plates (Grenier 784201; Monroe, NC), and subsequently diluted with Binding Buffer (15 μL) before addition of diluted compound into the Lin28A-immobilized plate (5 μL). Lin28A, pre-let-7d, HRP, and SuperSignal West Pico Chemiluminescent Substrate were dispensed by a Multidrop Combi Reagent Dispenser (Thermo Fisher Scientific). All washing steps were carried out on a Biotek 405 ELX plate washer.

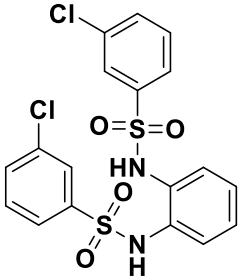
HTS data analysis. HTS data were monitored and analyzed using MScreen.[3]

F. Synthetic Methods



General synthetic method. The synthesis of *N,N'*-(1,2-phenylene)dibenzenesulfonamides was carried out by dissolving the corresponding diamine (1.0 equiv) in dry dichloromethane (2 mL), and subsequently cooling the reaction to 0 °C using an ice bath. After 5 min, pyridine (2.4 equiv) and aryl sulfonyl chloride (dropwise, 2.4 equiv) were added, and the resultant mixture was stirred at 25 °C for 12 h. The reaction was then quenched with water (~5 mL), and the organic layers were extracted with dichloromethane (3 × 20 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure *in vacuo*. The crude reaction mixture was purified by silica gel chromatography using EtOAc in hexanes to yield the corresponding sulfonamides in 55–80% yield.

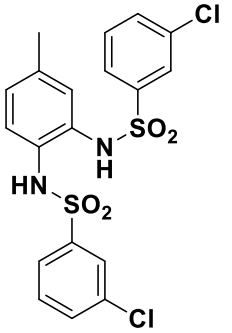
N,N'-(1,2-phenylene)bis(3-chlorobenzenesulfonamide) ***7a****.*



White solid (72%); R_f 0.45 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.69 (t, *J* = 1.9 Hz, 2H), 7.57 (ddt, *J* = 16.2, 7.9, 1.4 Hz, 4H), 7.40 (t, *J* = 7.9 Hz, 2H), 7.19–7.04 (m, 4H), 6.98 (dd,

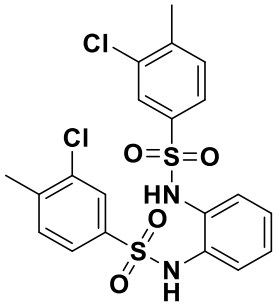
J = 6.0, 3.6 Hz, 2H); HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{14}Cl_2N_2O_4S_2$ $[M + NH_4]^+$ 474.0116, found 474.0109.

N,N'-(4-methyl-1,2-phenylene)bis(3-chlorobenzenesulfonamide) ***7b***.



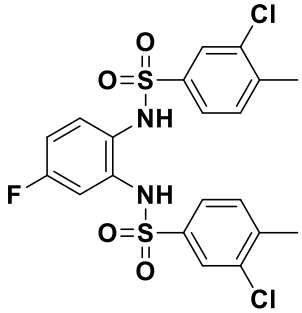
White solid (70%); R_f 0.50 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.78–7.60 (m, 4H), 7.54 (t, *J* = 8.4 Hz, 2H), 7.39 (td, *J* = 7.9, 3.9 Hz, 1H), 7.11 (td, *J* = 8.6, 2.7 Hz, 3H), 6.87 (dd, *J* = 16.1, 7.8 Hz, 2H), 6.69 (dd, *J* = 12.8, 8.1 Hz, 1H), 2.20 (d, *J* = 2.5 Hz, 3H); HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{16}Cl_2N_2O_4S_2$ $[M + H]^+$ 471.0007, found 471.0021.

N,N'-(1,2-phenylene)bis(3-chloro-4-methylbenzenesulfonamide) ***7c***.



White solid (75%); R_f 0.42 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.68 (d, *J* = 1.9 Hz, 2H), 7.49 (dd, *J* = 8.0, 1.9 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.14 (s, 2H), 7.07 (dt, *J* = 7.4, 3.7 Hz, 2H), 6.98 (dd, *J* = 5.9, 3.6 Hz, 2H), 2.41 (s, 6H); HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{18}Cl_2N_2O_4S_2$ $[M + NH_4]^+$ 502.0429, found 502.0417.

N,N'-(4-fluoro-1,2-phenylene)bis(3-chloro-4-methylbenzenesulfonamide) ***7d***.



Yellow solid (80%); R_f 0.50 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, Chloroform-d) δ 7.83 (s, 2H), 7.63 (s, 2H), 7.52–7.24 (m, 4H), 7.07 (d, *J* = 6.8 Hz, 1H), 6.79–6.60 (m, 2H), 2.44 (s, 6H); HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{17}Cl_2FN_2O_4S_2$ $[M + NH_4]^+$ 520.0335, found 520.0337.

G. Supplemental Figures

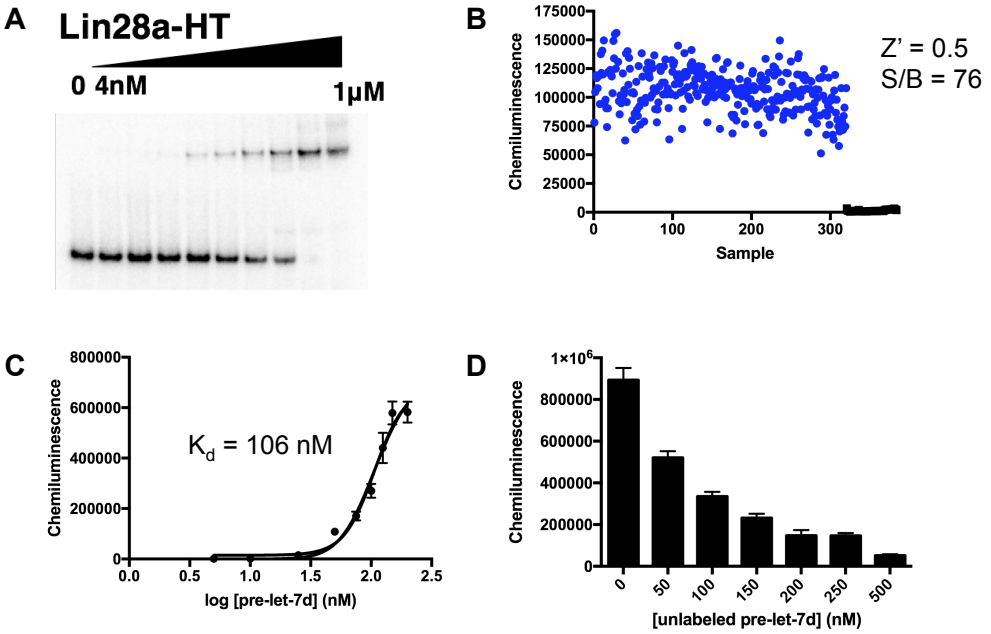


Figure S1. Characterization of pre-let-7d–Lin28 cat-ELCCA. (A) Confirmation of binding of biotinylated HaloTag-Lin28A to pre-let-7d containing a 5'-TCO modification. (B) Z' factor using automated liquid handling. (C) $K_{d,app}$ measurement. (D) Assay inhibition using unlabeled pre-let-7d.

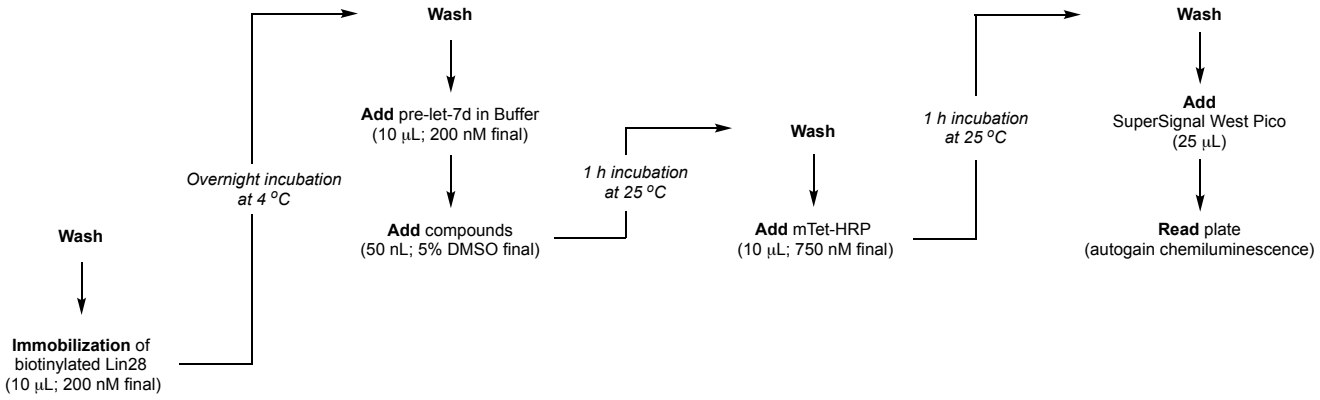
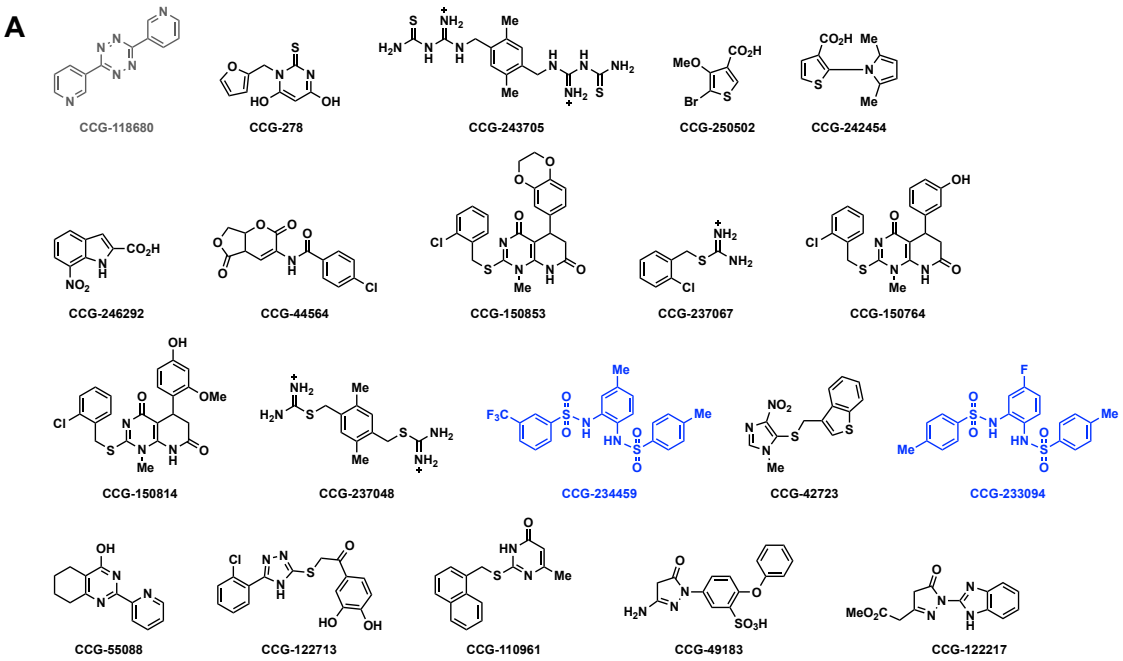
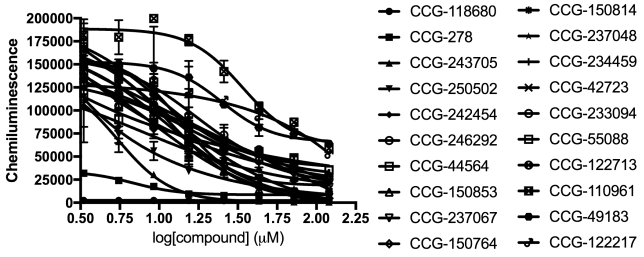


Figure S2. HTS protocol flow scheme.





C

CCG ID	IC_{50} (μM)	Hill Slope
278	<1.0	-1.3
243705	4.2	-2.3
250502	4.8	-1.4
242454	5.4	-0.8
246292	7.9	-0.9
44564	8.1	-0.6
150853	10.5	-1.7
237067	11.2	-1.6
150764	12.3	-1.8
150814	12.6	-2.1
237048	14.1	-1.9
234459	14.8	-0.8
42723	15.1	-1.1
233094	15.5	-1.1
55088	15.5	-1.1
122713	22.4	-1.1
110961	33.9	-2.6
49183	47.9	-0.9
122217	75.8	-0.7

Figure S3. Hit compounds. Note: (A) Structures. (B) IC_{50} curves from CCG. (C) Table of IC_{50} values from (B).

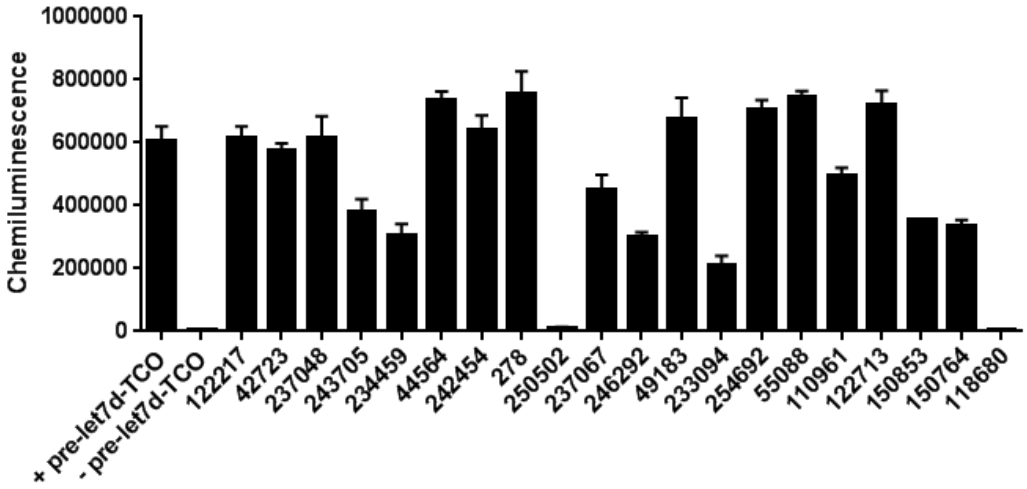


Figure S4. Single point inhibition of re-purchased hit compounds at 100 μM.

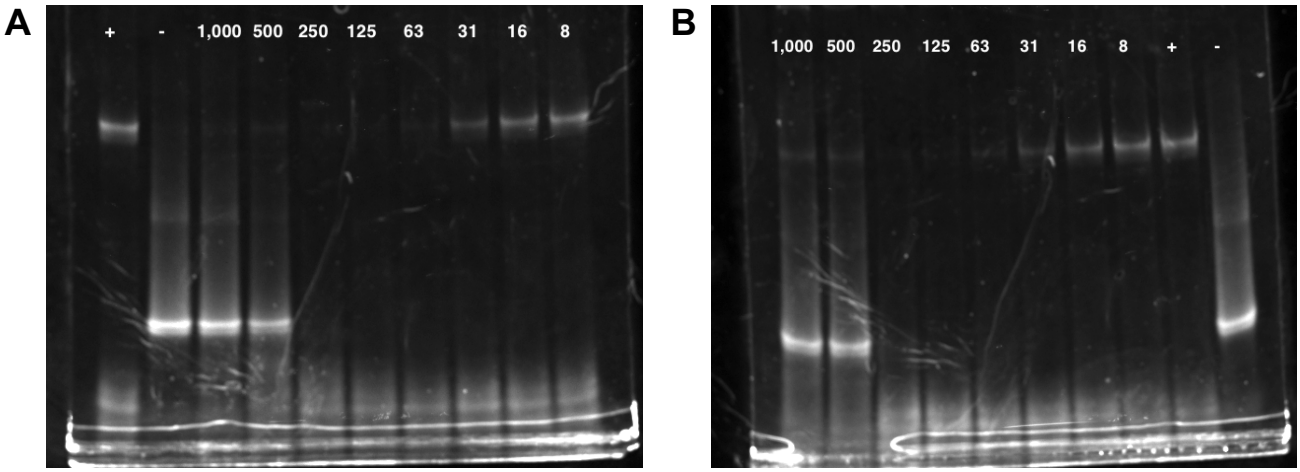


Figure S5. Dose-responsive inhibition of the pre-let-7d–Lin28 interaction of hit compounds, (A) **CCG-233094** and (B) **CCG-234459**, via EMSA. (+) = pre-let-7d with Lin28. (-) = pre-let-7d only. Concentrations are in μM.

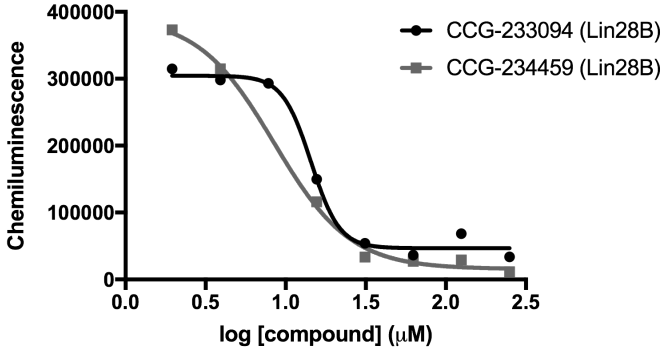


Figure S6. IC_{50} curves of **CCG-233094** (14.4 μM) and **CCG-234459** (12.9 μM) against cat-ELCCA of the pre-let-7d–Lin28B interaction.

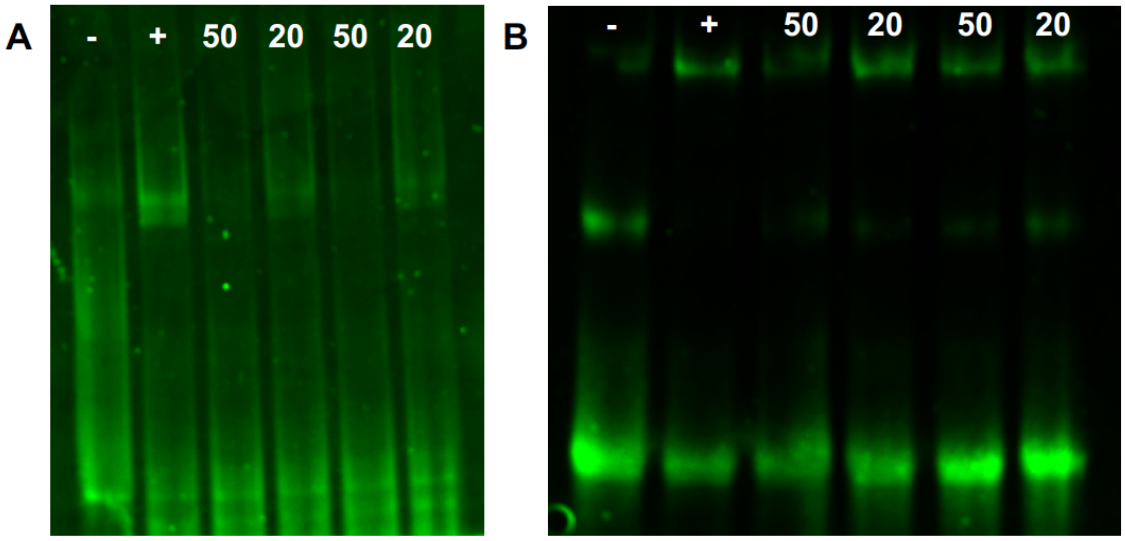


Figure S7. Inhibition of the (A) pre-let-7a– and (B) pre-let-7g–Lin28 interactions by **CCG-233094** and **CCG-234459**. Lane 1 (-) = pre-let-7 only. Lane 2 (+) = pre-let-7 with Lin28. Lanes 3,4 = **CCG-233094**. Lanes 5,6 = **CCG-234459**. Concentrations are in μM.

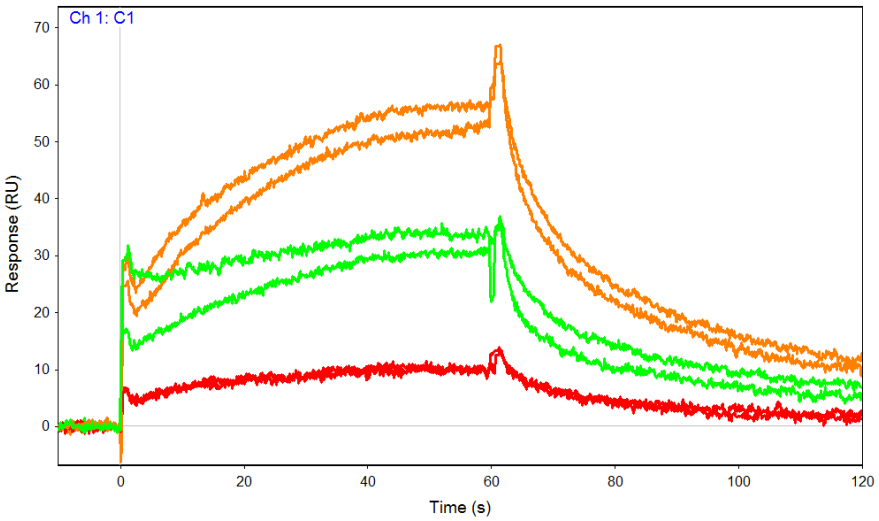


Figure S8. SPR data for **CCG-233094** binding to biotinylated HaloTag-Lin28A. In brief, SPR was performed using a SensiQ Pioneer instrument and a BioCap biosensor. The running buffer was 50 mM Tris (pH 7.6), 150 mM NaCl, 1 mM β-mercaptoethanol, 5% glycerol, 0.05% Tween-20, 1 mM $ZnCl_2$, and 5% DMSO. The chip was conditioned with 30 μL injections of 1 M sodium chloride in 50 mM sodium hydroxide, a solution of 0.5% sodium dodecyl sulfate, and the running buffer with 1 M of sodium chloride. Protein was immobilized at 2,300 RU. Compounds were tested at 75, 37.5, and 18.7 μM with injection volumes of 50 μL and a flow-rate of 50 μL/min. The chip was regenerated after each cycle with a 10 μL injection of 50 mM Tris (pH 7), 260 mM NaCl, 0.5 mM EDTA, and 0.1% Tween-20. Data was processed using QDAT analysis software.

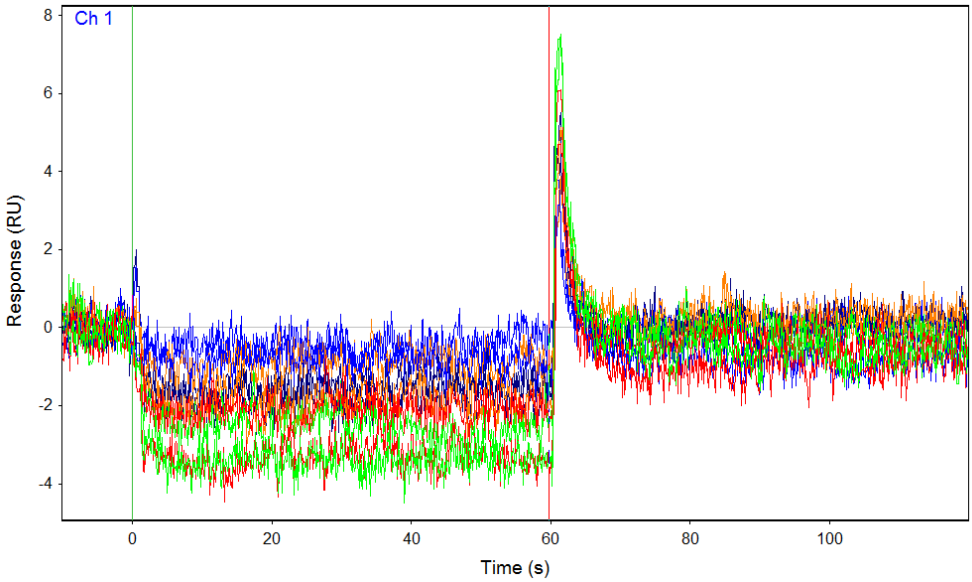


Figure S9. Representative SPR data for **CCG-233094** binding to biotinylated pre-let-7d. As can be seen in the sensorgram, no detectable binding was observed. In brief, SPR was performed using a SensiQ Pioneer instrument and a BioCap biosensor. The running buffer was 50 mM Tris (pH 7.6), 150 mM NaCl, 5% glycerol, 0.05% Tween-20, 1 mM $ZnCl_2$, and 5% DMSO. The chip was conditioned with 30 µL injections of 1 M sodium chloride in 50 mM sodium hydroxide, a solution of 0.5% sodium dodecyl sulfate, and the running buffer with 1 M of sodium chloride. Biotinylated pre-let-7d was immobilized at 2,300 RU. Compounds were tested at 100, 50, 25, 12.5, and 6.25 µM with injection volumes of 30 µL and a flow-rate of 30 µL/min. The chip was regenerated after each cycle with a 10 µL injection of 50 mM Tris (pH 7), 260 mM NaCl, 0.5 mM EDTA, and 0.1% Tween-20. Data was processed using QDAT analysis software.

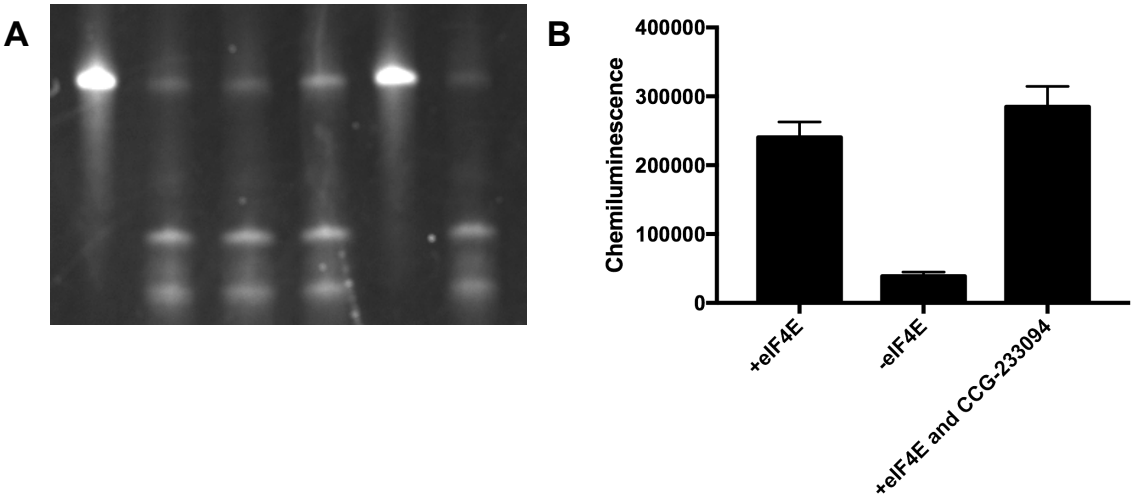


Figure S10. Selectivity profiling against (A) Dicer-mediated pre-let-7d maturation[2] (lane 1 = no Dicer, lane 2 = Dicer, lane 3 = Dicer + **CCG-233094**, lane 4 = Dicer + **CCG-234459**, lane 5 = no Dicer, lane 6 = Dicer) and (B) cat-ELCCA of the eIF4E–4E-BP1 protein-protein interaction.[1] For each assay, the concentration of compound used was 100 µM.

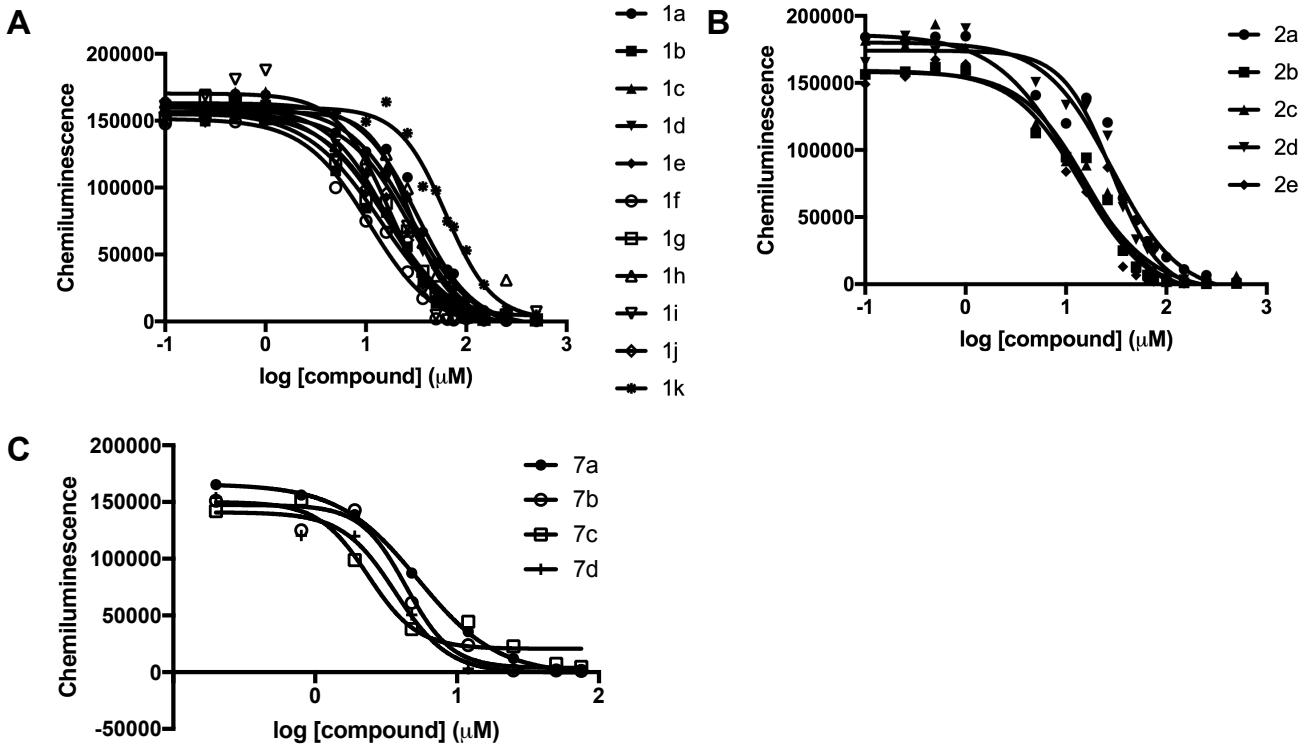
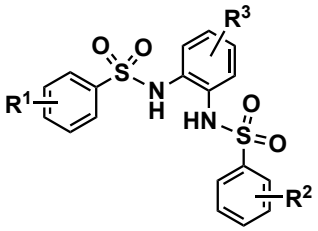


Figure S11. IC_{50} curves of compounds (A) **1a**–**1k**, (B) **2a**–**2e** and (C) **7a**–**7d** against cat-ELCCA of the pre-let-7d–Lin28A interaction.

Table S1. IC_{50} values for all compounds with 95% confidence interval (CI) values



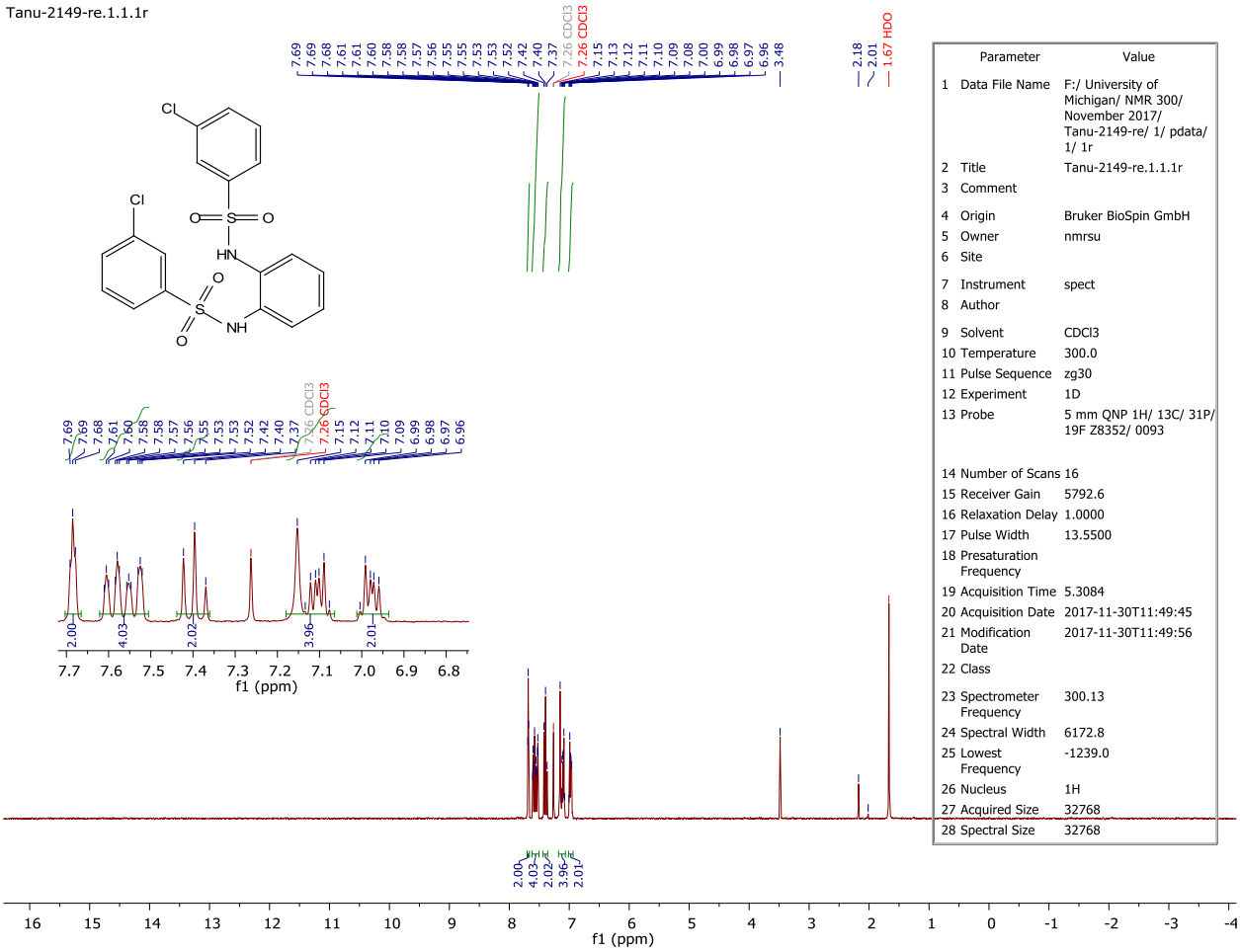
Compound	R^1	R^2	R^3	IC_{50} (μM)	95% CI (μM)
CCG-233094	p-Me	p-Me	F	8.3	6.2 - 11.2
CCG-234459	m-CF_3	p-Me	Me	10.4	7.6 - 14.0
1a	H	H	H	32.2	27.1 - 38.3
1b	p-F	H	H	13.9	11.1 - 17.4
1c	p-F	p-F	H	28.3	23.2 - 34.4
1d	p-F	p-Cl	H	27.3	22.1 - 33.6
1e	p-F	p-OMe	H	17.2	13.3 - 22.3
1f	p-F	m-Cl	H	10.8	9.3 - 12.6
1g	p-F	m-Br	H	16.2	11.9 - 22.1
1h	p-Cl	p-Cl	H	27.5	22.0 - 34.5
1i	p-Br	p-Br	H	17.5	13.9 - 22.0
1j	p-OMe	p-OMe	H	16.7	13.8 - 20.2
1k	p-NHCOMe	p-NHCOMe	H	62.5	53.2 - 73.4
2a	H	H	Me	28.8	22.0 - 37.7
2b	p-F	p-F	Me	16.2	13.1 - 20.0
2c	p-F	p-Me	Me	11.7	9.2 - 14.8
2d	p-Cl	p-Cl	Me	28.4	23.8 - 33.8
2e	p-Me	p-Me	Me	14.1	9.5 - 21.1
7a	m-Cl	m-Cl	H	5.7	3.3 - 8.5
7b	m-Cl	m-Cl	Me	4.3	3.4 - 5.6
7c	m-Cl, p-Me	m-Cl, p-Me	H	2.3	1.1 - 4.9
7d	m-Cl, p-Me	m-Cl, p-Me	F	3.7	2.8 - 4.9

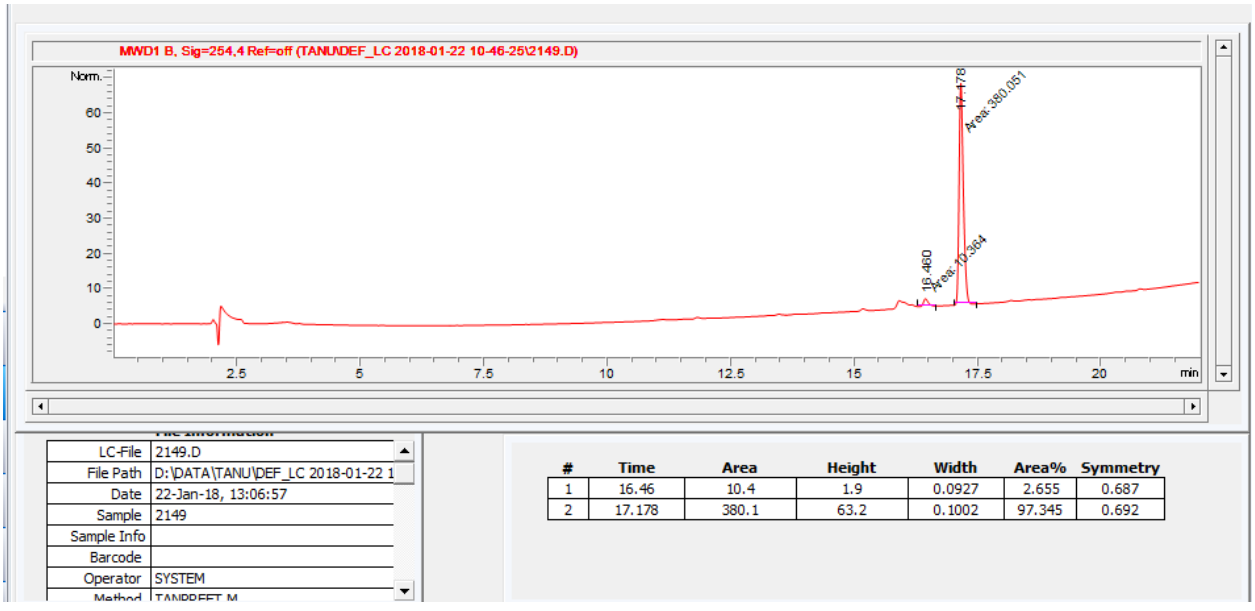
H. References

1. Song, J. M.; Menon, A.; Mitchell, D. C.; Johnson, O. T.; Garner, A. L. "High-throughput chemical probing of full-length protein-protein interactions." *ACS Comb. Sci.* **2017**, *19*, 763–769.
2. Lorenz, D. A.; Garner, A. L. "A click chemistry-based microRNA maturation assay optimized for high-throughput screening." *Chem. Commun.* **2016**, *52*, 8267–8270.
3. Jacob, R. T.; Larsen, M. J.; Larsen, S. D.; Kirchhoff, P. D.; Sherman, D. H.; Neubig, R. R. "MScreen: an integrated compound management and high-throughput screening data storage and analysis system." *J. Biomol. Screen.* **2012**, *17*, 1080–1087.

I. NMR and Purity Spectra

N,N'-(1,2-phenylene)bis(3-chlorobenzenesulfonamide) ***7a****:*





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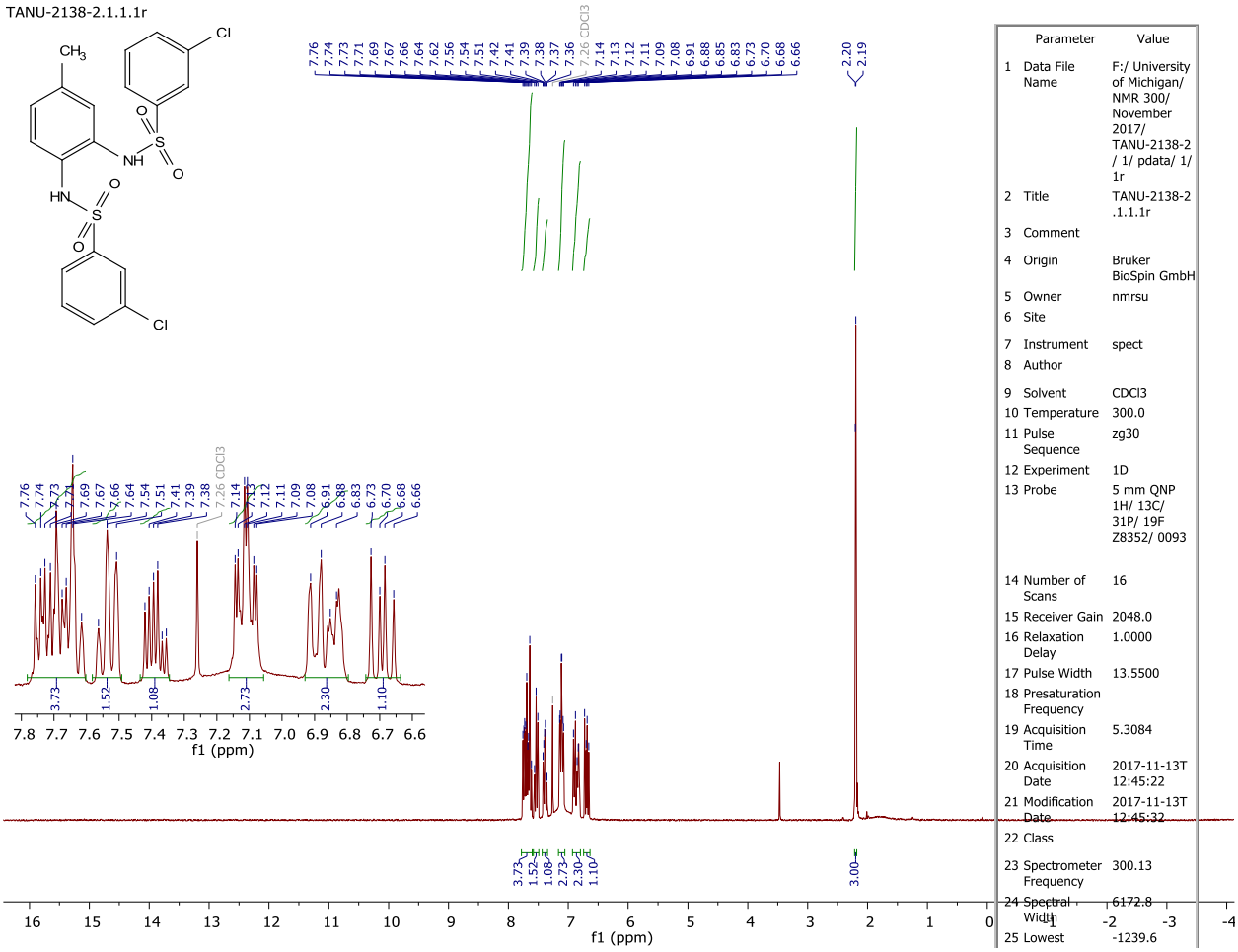

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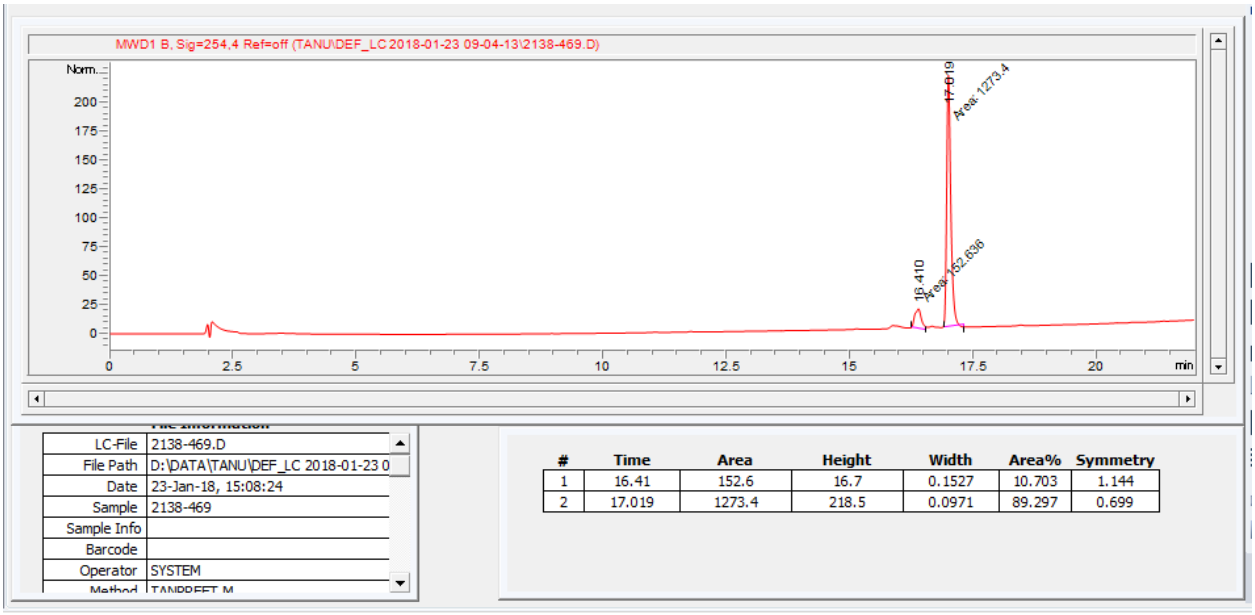

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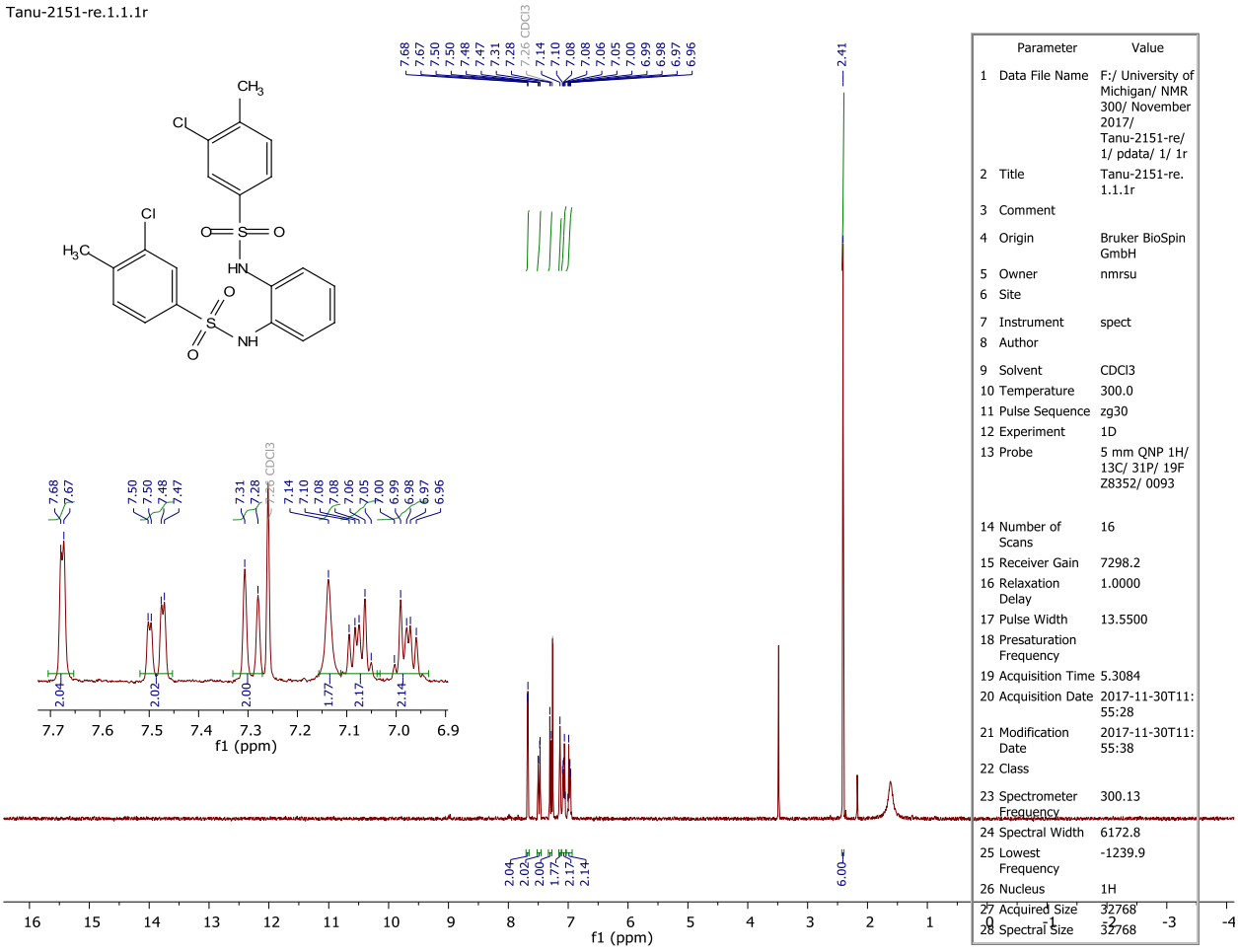

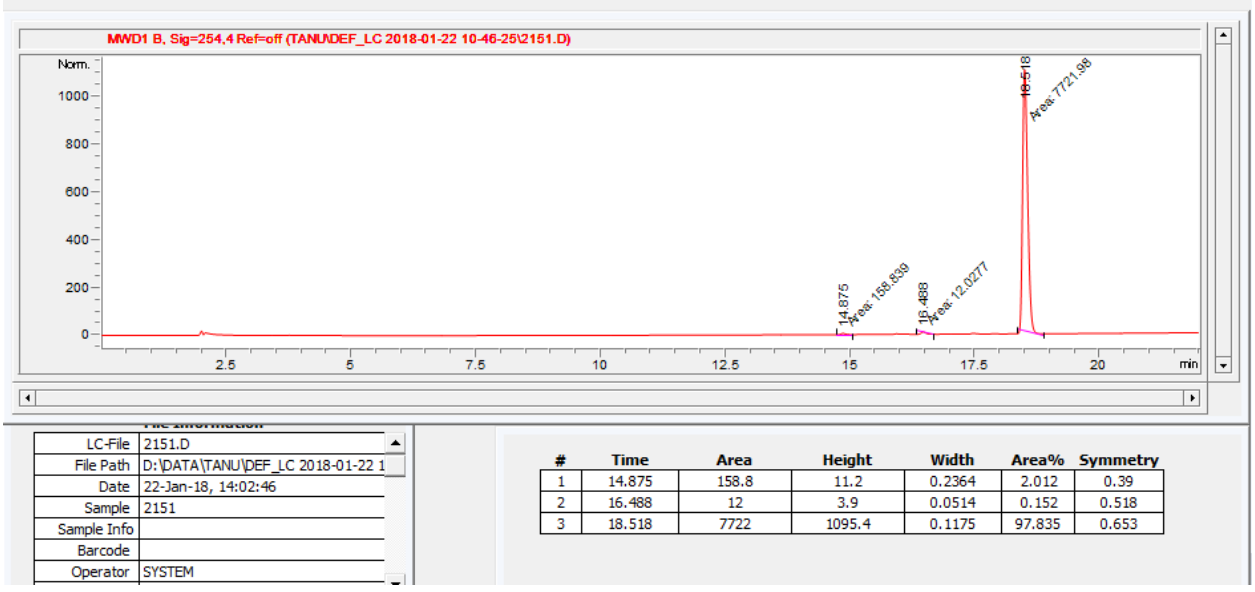

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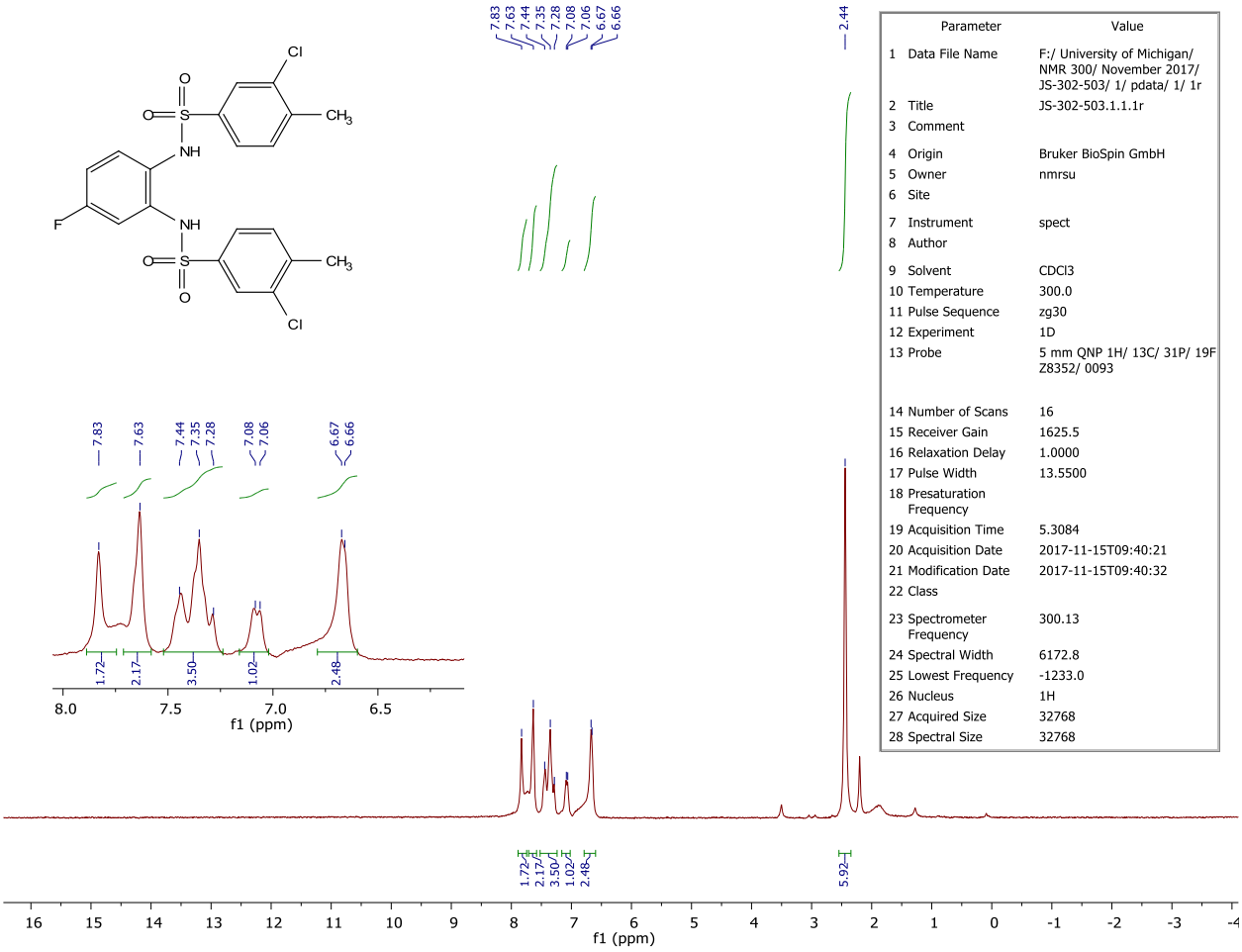
3	18.518	7722	1095.4	0.1175	97.835	0.653

N,N'-(4-fluoro-1,2-phenylene)bis(3-chloro-4-methylbenzenesulfonamide ***7d****:*



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7	Instrument	spect
8	Author	
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11	Pulse Sequence	zg30
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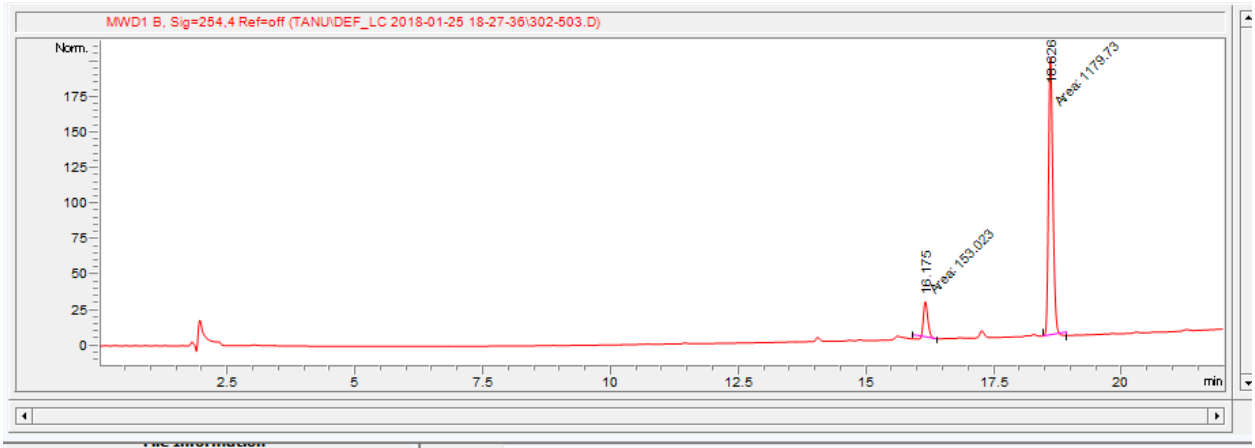

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