Supporting Information

GNE-371, a potent and selective chemical probe for the second bromodomains of human transcription initiation factor TFIID subunit 1 (TAF1) and transcription initiation factor TFIID subunit 1-like (TAF1L)

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Protein expression and purification methods

Production of bromodomain proteins was carried out as previously described[1]. TR-FRET assay conditions have been reported previously.[2, 3]

Synthesis of biotinylated probes for TR-FRET assays. The synthesis and characterization of biotinylated probe molecules has been previously reported.[2]

Cell-based assays. Target engagement in cells was assessed by displacement of a fluorescent tagged ligand from nanoLuc (nLuc; Promega) luciferase-bromodomain fusion proteins. For the BRD4 assay, 293T cells were grown in DMEM medium (low glucose) supplemented with 2 mM glutamine and 10% fetal bovine serum. Cells were trypsinized, counted and resuspended at 2 x 10^5 cells/mL. Cells were transfected transiently using FuGENE HD Transfection Reagent (Promega) with 9:1 carrier DNA: nLuc-full-length BRD4 fusion construct (10 µg total DNA; 20 mL cells). Cells were plated and grown for 24 h at 37 °C, 5% CO_2. Cells were isolated and resuspended in assay medium (OptiMem I reduced serum medium lacking phenol red; Gibco) at 2 x 10^5 cells/mL and plated in white 96-well plates with tracer (0.5 µM final concentration) according to the supplier's instructions (90 µL total volume). Serially diluted compound (10 µL; top final concentration of 20 µM) was added to wells (0.2% DMSO final concentration). After incubation as described, plates were processed by addition of substrate and read according to the supplier's instructions on a GloMax GM3000 reader (Promega). The ratio of acceptor emmission (610 nm) to donor emission (450 nm) was corrected by subtracting the value for the no-tracer control and multiplied by 1000 to yield a final value in milliBRET units (mBu). The TAF1(2) assay was carried out in a similar manner, using instead an N-terminal nLuc-TAF1(2) aa 1494–1698 fusion construct and the tracer probe.
To assess the effect of inhibition of TAF1(2) on BRD4 inhibition, H23 viability was determined using CellTiter-Glo 2.0 (Promega). Cells were cultured in RPMI-1640 supplemented with 2 mM glutamine and 10% fetal bovine serum. One day preceding compound addition, cells were plated at 5000 cells/well in white 96-well culture plates. Compound dilutions and mixtures were prepared at 500x final concentration in 100% DMSO then diluted with fresh medium. Wells were aspirated to remove medium, and compound medium (100 µL) was added to triplicate wells. Cells were grown for an additional 48 h (37 °C, 5% CO_2). Plates were equilibrated to room temperature before adding CellTiter-Glo reagent and incubating briefly to develop signal. Luminescence was read on a Wallac Victor^{3}V 1420 Multilabel Counter (Perkin Elmer). Average luminescence values were converted to percent inhibition according to the equation:

% inhibition = (1-((value-MIN)/(MAX-MIN))*100,

where MAX is the average of luminescence from vehicle treated wells and MIN is the fitted lower baseline of the JQ1 inhibition curve at the highest concentration of GNE-371. Percent inhibition values from two independent experiments were averaged and provided as input to the web implementation of the program SynergyFinder[5], using the Bliss model with optional baseline correction.

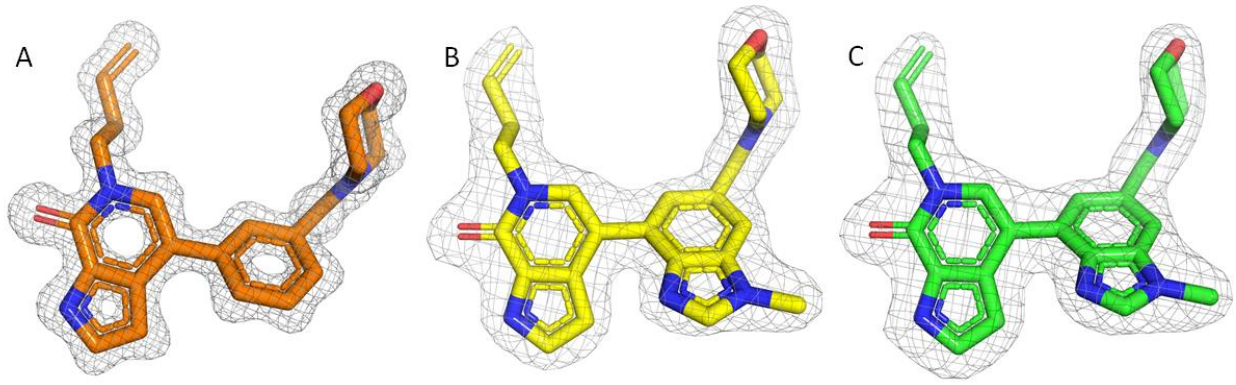
Crystallography methods. Crystallographic methods, analysis, and refinement information for Compound **1** bound to BRD4(1) and compound **2** bound to TAF1(2) has been previous reported.[2]

The co-crystal structures of TAF1-BD2 with **8 & 27** were obtained by incubating 3.3 mM of each compound with protein at a concentration of 19.9 mg/mL (1.2 mM) in 20mM HEPES, pH 7.5, 150mM NaCl, 1mM TCEP. Crystals were then grown at 4°C using the sitting drop vapor diffusion technique by equilibrating the protein:ligand complexes against a solutions containing 25% PEG1500 (**8**) or 0.1 M BIS-TRIS pH 6.5, 28% w/v PEG MME 2,000 (**27**). TAF1-BD2-**8** crystals were flash frozen in liquid nitrogen using a 1:1 mix of Paratone-N and Mineral oil and data was collected at APS beamline 21ID-G. The TAF1-BD2-**27** complex crystals were cryo-protected by the addition of 20% ethylene glycol to the well solution and data was collected at SSRF beamline 17U1.

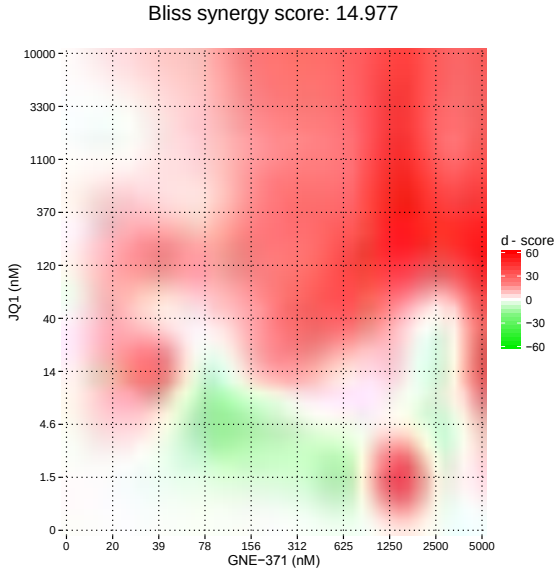
Table S1. Data collection and refinement statistics for TAF1-BD2 in complex with **8** & **27**

	TAF1-BD2 Cpd8	***TAF1-BD2 Cpd27***
PDB-ID	6DF4	6DF7
Beamline	LS-CAT 21ID-G	SSRF 17U1
Wavelength	0.9786	0.97923
Resolution range	12.88 - 1.30 (1.35 - 1.30)	41.9 - 2.003 (2.075 - 2.003)
Space group	P21221	P212121
Unit cell	47.81 59.65 60.58 90 90 90	52.42 79.70 83.83 90 90 90
Total reflections	307,048	157,471
Unique reflections	43,100 (4,240)	22,773 (1,166)
Multiplicity	7.1 (7.0)	6.9 (7.4)
Completeness (%)	99.65 (99.24)	93.8 (100)
Mean I/sigma(I)	32.2 (3.2)	12.9 (0.9)
Wilson B-factor	14.48	43.04
R-pim		0.035 (0.792)
R-merge	0.051 (0.701)	0.068 (0.57)
Refinement		
Reflections used in refinement	43011 (4192)	22749 (1790)
Reflections used for R-free	2166 (229)	1131 (79)
R-work	0.169 (0.229)	0.192 (0.314)
R-free	0.187 (0.224)	0.235 (0.287)
Number of non-hydrogen atoms	1446	2345
macromolecules	1110	2095
ligands	28	64
solvent	308	186

Protein residues	134	256
RMS(bonds)	0.013	0.014
RMS(angles)	1.49	1.58
Ramachandran favored (%)	99.24	99.6
Ramachandran allowed (%)	0.76	0.4
Ramachandran outliers (%)	0	0
Rotamer outliers (%)	0	2.9
Clashscore	0.9	1.43
Average B-factor	19.91	50.37
macromolecules	16.72	49.77
ligands	12.07	39.06
solvent	32.15	61.04



Supplementary Figure S1. (**A**) 1.3Å resolution Sigma-A weighted 2mFo-DFc* electron density map of compound 8 contoured at 1sigma. (**B**) & (**C**) 2.0Å resolution Sigma-A weighted 2mFo-DFc* electron density map of compound **27** contoured at 1sigma (from 2 molecules of the TAF1(2)-Cpd**27** complex in the assymetric unit, B & C represent **27** bound to each of these TAF molecules. (*where m is the figure of merit, and D is the Sigma-A weighting factor).



Supplementary Figure S2. Bliss synergy calculation for H23 cells co-treated with **JQ1** and **GNE-371**. The overall synergy score is 15, with a maximum Bliss score of 43.

Table S2. TR-FRET assay data for compounds Tables 1 and 2 including standard deviations.

	IC50 (uM)					
Compound	**TAF1(2)**	**TAF1(1)**	**BRD4(1)**	**BRD4(2)**	**BRD9**	**CECR2**
1	0.059 ± 0.02	3.6 ± 0.8	0.09 ± 0.02	0.65 ± 0.01	0.2 ± 0.1	0.24 ± 0.3
2	0.046 ± 0.005	9.9 ± 1	2.5 ± 0.3	5.5 ± 0.7	1.4 ± 0.3	4.8 ± 0.1
3	0.046 ± 0.01	17 ± 1	0.69 ± 0.07	1.5 ± 0.0	1.8 ± 0.0	2.9 ± 1
4	0.035 ± 0.005	16 ± 2	0.76 ± 0.05	1.9 ± 0.1	1.9 ± 0.1	4.7 ± 1
5	0.067 ± 0.009	13 ± 2	0.75 ± 0.1	2.4 ± 0.1	1.7 ± 0.1	4.0 ± 0.7
6	0.089 ± 0.02	9.5 ± 1	1.5 ± 0.1	5.0 ± 0.0	0.90 ± 0.06	0.55 ± 0.1
7	0.083 ± 0.009	14 ± 3	3.2 ± 0.0	5.0 ± 0.0	0.89 ± 0.01	1.1 ± 0.1
8	0.023 ± 0.006	11 ± 1	1.0 ± 0.4	6.9 ± 0.3	1.5 ± 0.5	3.3 ± 0.6
9	0.017 ± 0.003	15 ± 2	1.2 ± 0.1	5.0 ± 0.2	1.6 ± 0.1	2.2 ± 0.4
10	0.026 ± 0.004	4.3 ± 0.3	0.48 ± 0.01	4.0 ± 0.2	0.88 ± 0.03	2.3 ± 0.4
11	0.006 ± 0.003	6.1 ± 0.7	0.60 ± 0.1	9.0 ± 0.5	0.96 ± 0.02	0.69 ± 0.1
12	0.015 ± 0.004	8.1 ± 0.0	1.8 ± 0.1	5.0 ± 0.0	3.0 ± 0.4	0.35 ± 0.09
13	0.010 ± 0.001	9.6 ± 1	5.1 ± 0.6	11 ± 0	1.4 ± 0.1	1.8 ± 0.3
14	0.006 ± 0.000	9.7 ± 2	5.0 ± 0.0	5.0 ± 0.0	0.86 ± 0.3	2.0 ± 0.2
15	0.035 ± 0.01	15 ± 3	5.0 ± 0.0	5.0 ± 0.0	1.0 ± 0.0	5.0 ± 1
16	0.011 ± 0.004	11 ± 1	7.9 ± 1	17 ± 1	2.2 ± 0.3	2.1 ± 0.5
17	0.016 ± 0.004	11 ± 4	3.2 ± 0.3	5.0 ± 0	2.1 ± 0.4	0.83 ± 0.2
18	0.013 ± 0.004	9.3 ± 2	16 ± 2	13 ± 1	2.0 ± 0.6	1.7 ± 0.3
19	0.007 ± 0.002	6.9 ± 0.6	8.6 ± 0.9	10 ± 1	1.7 ± 0.5	1.0 ± 0.1
20	0.004 ± 0.003	5.3 ± 0.4	6.3 ± 1	7.6 ± 0.3	1.6 ± 0.1	0.59 ± 0.1
21	0.016 ± 0.003	5.9 ± 0.4	2.3 ± 0.1	4.4 ± 0.0	2.2 ± 0.2	0.82 ± 0.01
22	0.010 ± 0.001	7.0 ± 2	3.6 ± 0.2	4.4 ± 0.2	1.4 ± 0.4	0.23 ± 0.2
23	0.016 ± 0.004	8.4 ± 6	8.2 ± 0.9	12.4 ± 0.6	0.75 ± 0.1	2.6 ± 0.2
24	0.016 ± 0.04	>20	2.0 ± 0.1	1.0 ± 0.0	2.4 ± 2	2.6 ± 1.1
25	0.006 ± 0.005	6.8 ± 1	3.4 ± 0.4	6.8 ± 0.2	0.62 ± 0.03	0.59 ± 0.2
26	0.028 ± 0.01	>20	>20	>20	18 ± 1	1.1 ± 2
27	0.010 ± 0.002	>20	>20	>20	9.5 ± 2	1.2 ± 0.1

Table S3. BROMO*scan*® bromodomain selectivity data for compound **27** (GNE-371) provided by DiscoveRx Corp., Fremont, CA, USA, http://www.discoverx.com. This screen measured binding competition against immobilized ligands for 40 DNA-tagged bromodomains. Compound K_D values are averages of 2 independent experiments.

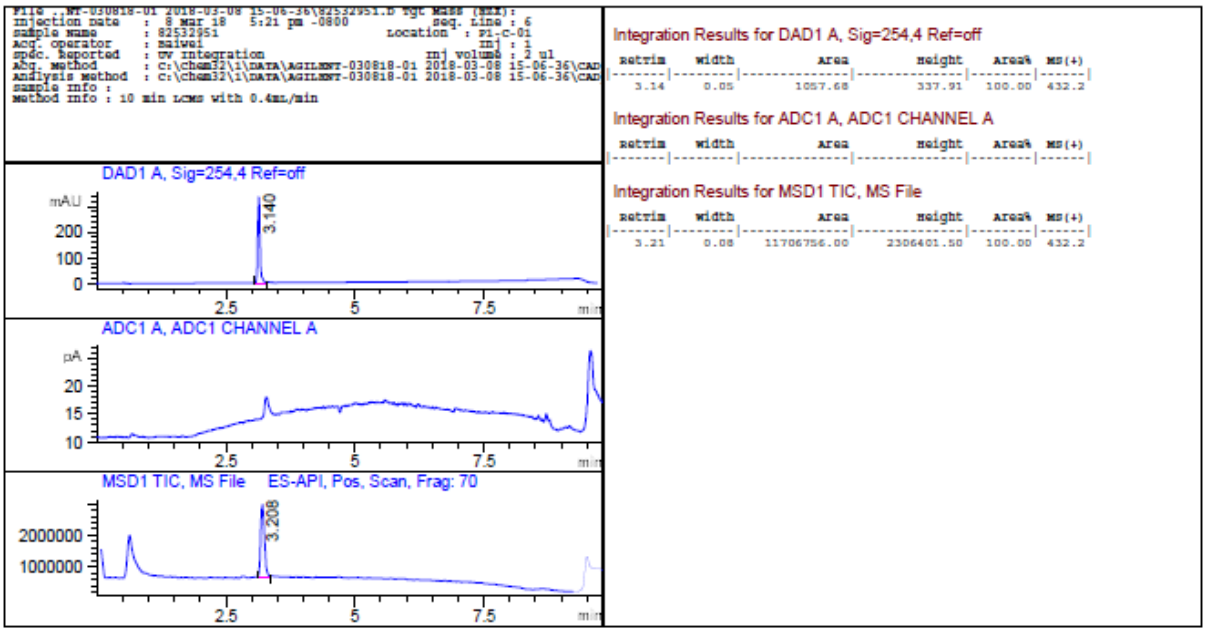
Target	GNE-371 K_D (nM)	Target	GNE-371 K_D (nM)	Target	GNE-371 K_D (nM)
ATAD2A	>10000	BRD4(2)	>10000	EP300	>10000
ATAD2B	>10000	BRD4 (full-length, Short-iso.)	8900	FALZ	>10000
BAZ2A	>10000	BRD7	>10000	GCN5L2	>10000
BAZ2B	>10000	BRD8(1)	>10000	PBRM1(2)	>10000
BRD1	>10000	BRD8(2)	>10000	PBRM1(5)	>10000
BRD2(1)	>10000	BRD9	3400	PCAF	>10000
BRD2(1,2)	>10000	BRDT(1)	>10000	SMARCA2	>10000
BRD2(2)	>10000	BRDT(1,2)	>10000	SMARCA4	>10000
BRD3(1)	>10000	BRDT(2)	>10000	TAF1(2)	1.2
BRD3(1,2)	>10000	BRPF1	>10000	TAF1L(2)	5.2
BRD3(2)	>10000	BRPF3	>10000	TRIM24(Bromo.)	>10000
BRD4(1)	>10000	CECR2	1200	TRIM24(PHD,Bromo.)	>10000
BRD4(1,2)	>10000	CREBBP	>10000	TRIM33(PHD,Bromo.)	>10000
				WDR9(2)	>10000

Table S4. Kinase selectivity data for Compound **27** (GNE-371). Invitrogen panel of 35 kinases, percent inhibition at 1.0 μM.

Kinase	**GNE-371 @ 1.0 μM**	**Kinase**	**GNE-371 @ 1.0 μM**
AKT1	2.5	MAP4K4	5.5
Abl	11	MEK1	-1
Aurora_B	-1	MST3	3
CDK2/cyclinA	1.5	MYLK3(caMLCK)	1.5
CDK5/p25	-1	Mink1	6
CHK1	6.5	MuSK	0
CLK2	5.5	PIM1	-2.5
CSF1R	3.5	PKA	2
DMPK	0	PLK1	-5
EphA1	3.5	RIPK2	-3
Flt3	2.5	RSK3	4
GSK3_beta	3	Ret	1.5
IRAK4	5.5	SIK2	-4
InsR	8	Src	4.5
JAK1	-2	TGFBR1	-1.5
JNK1_alpha1	4	TrkA	16.5
Lck	16.5	Yes	5.5
		p38_alpha(direct)	2

LCMS trace of compound **27**

MS Report



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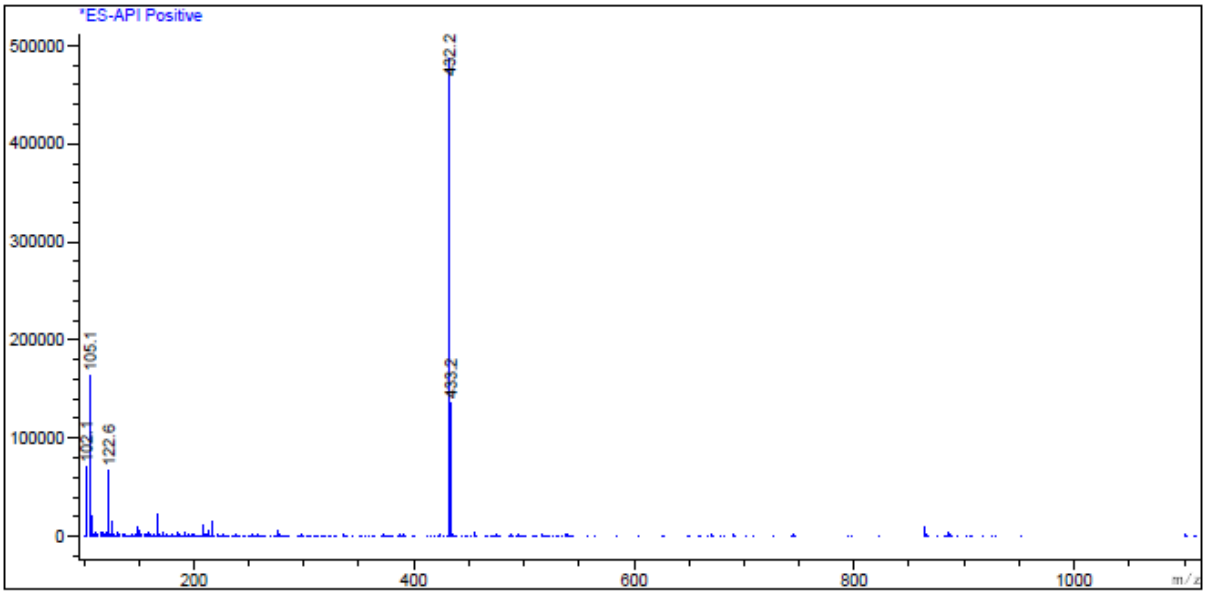

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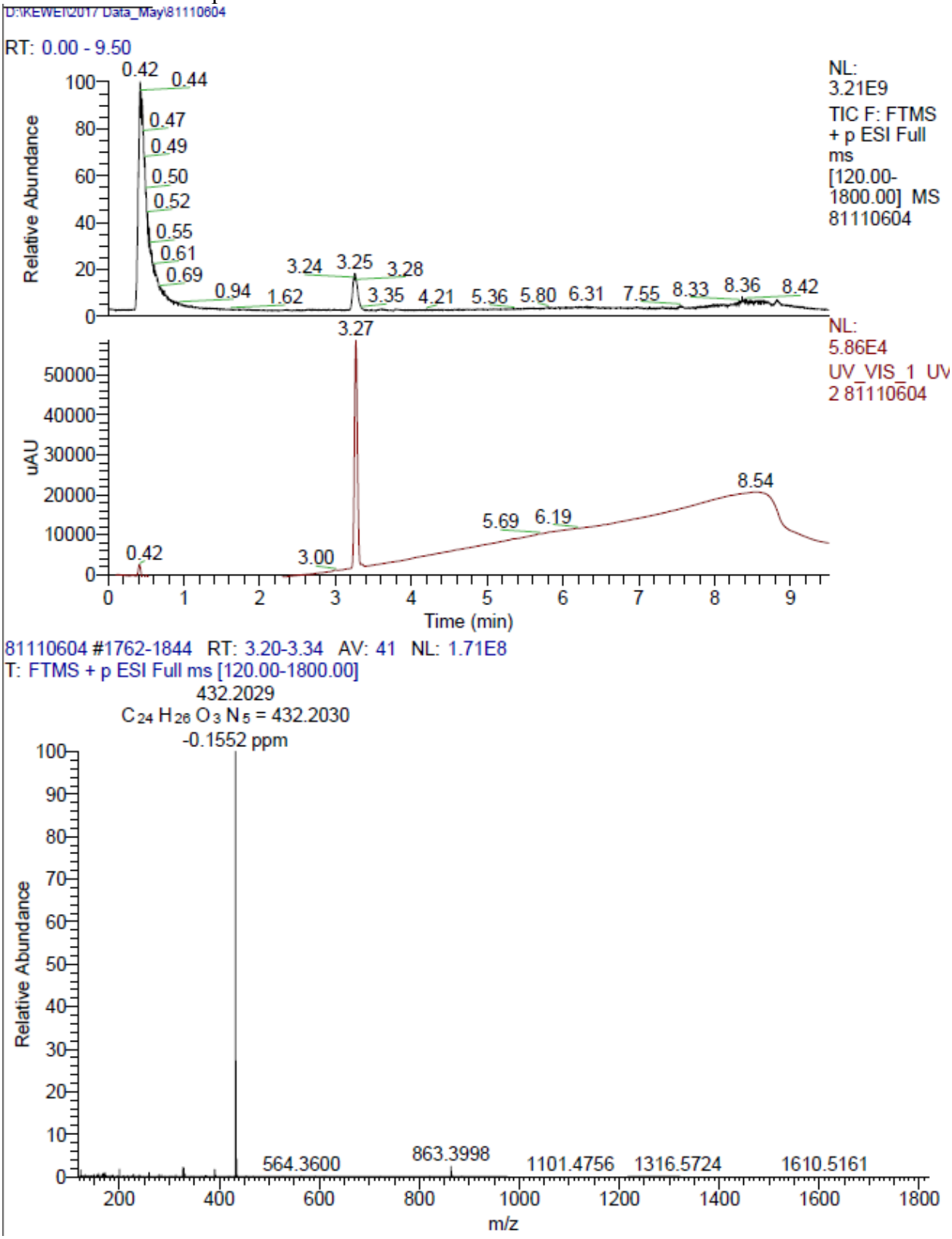
HRMS trace for Compound **27:**



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