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Supplemental information

CLK2 and CLK4 are regulators of DNA damage-induced NF-κB targeted by novel small molecule inhibitors

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Supplemental Item Legends

Figure S1: Synthesis strategies and cell permeabilities of lead compounds MW01 and MW05, related to Figure 1

(A) Synthetic scheme for MW01, as previously published in [1].

(B) Synthetic scheme for MW05, as previously published in [2].

(C) Mean apparent permeability (P_{app}) of MW01, MW05, or positive control propranolol in CaCo2 cells.

Figure S2: MW01 and MW05 inhibit critical NF-κB pathway steps leading to apoptosis, related to Figure 1

(A) MW01 and MW05 are inhibitors of DNA damage-induced p65 nuclear translocation. U2-OS cells were pre-treated with DMSO, MW01 or MW05. Etoposide was added and after 2 hours cells were fixed and nuclei stained with DAPI. p65 and γH2AX were stained by immunofluorescence and images taken at a confocal Zeiss 710 LSM microscope with a 40x oil objective.

(B) Immunoprecipitation of IKKγ from HEK293 lysates pre-treated for 1 hour with 10 μM MW01 or MW05, then irradiated (IR) and lysed.

(C) Western blot analysis, using an IKKγ S85-specific antibody, of U2-OS cells pre-treated with 10 μM MW01, MW05 or ATM inhibitor KU55933 for 1 hour and irradiated (IR). The specific IKKγ S85 band was confirmed by induction following irradiation and by sensitivity to ATMi treatment.

(D) Western blot analysis of U2-OS cells pre-treated for 1 hour with 10 μM of the indicated substances, irradiated (IR) and whole cell lysates probed for poly(ADP)-ribose.

(E) Western blot analysis of cytoplasmic and nuclear fractions from U2-OS cells after 1 hour pre-treatment with DMSO, MW01 (10 μM), or MW05 (10 μM) and 45 min following irradiation (IR). PARP1 and LDHA are used as loading controls for nuclear and cytoplasmic fractions, respectively.

(F) Western blot analysis of U2-OS cells 8 hours after 10 Gy irradiation following 1 hour pre-treatment with 10 μM DMSO, MW01, or MW05. Tubulin was used loading control.

(G) mRNA expression analysis of BIRC3 in U2-OS cells 8 hours after 10 Gy irradiation following 1 hour pre-treatment with 10 μM DMSO, MW01, or MW05 and. Values were normalized to DMSO-treated unstimulated cells.

Figure S3: MW01 and MW05 inhibit shared kinase targets, related to Figure 2

(A) IC50 (nM) of all kinases strongly inhibited by MW01 and MW05. Heatmap indicates lower IC50 by red color, higher by blue.

(B) Venn diagram depicting strongly inhibited kinases for MW01 (orange) and MW05 (green), with shared hits.

Figure S4: Inactive derivatives of MW01 and MW05 do not inhibit CLK, related to Figure 3

(A) Selected inactive structural derivatives of MW01 and MW05, as indicated. MW01-E6: (5,15-dimethoxy-1,11,14-triazapentacyclo[10.7.1.0^{2},^{7}.0^{8},^{20}.0^{13},^{18}]icosa-2(7),3,5,8(20),9,11,13(18),14,16-nonaen-19-one); and MW01-E14: (5,17-dimethoxy-1,11,16-triazapentacyclo[10.7.1.0^{2},^{7}.0^{8},^{20}.0^{13},^{18}]icosa-2(7),3,5,8(20),9,11,13(18),14,16-nonaen-19-one); MW05-E2: (1-[3-(dimethylamino)benzoyl]-6-methoxy-1H-pyrazolo[3,42uinolinelin-3-amine); MW05-E10: (1-[3-(dimethylamino)benzoyl]-7-methoxy-N,N-dimethyl-1H-pyrazolo[3,42uinolinelin-3-amine).

(B) (left) Western blot analysis of U2-OS cells following pre-treatment with DMSO, MW01 (10 μM), MW01-E6 (10 μM), or MW01-E14 (10 μM) in unstimulated, after irradiation (IR), or after TNFα conditions. (right) Western blot analysis following pre-treatment with DMSO, MW05 (10 μM), MW05-E2 (10 μM), or MW05-10 (10 μM) under the same conditions. Vinculin is used as loading control.

(C) mRNA expression analysis of NFKBIA using same experimental conditions as in (B) for MW01 and inactive derivatives (left) and MW05 and inactive derivatives (right). ns: not significant; *, **, ***: $p<0.05$, $p<0.01$, $p<0.001$.

(D) Percent inhibition of all CLK isoforms by derivatives MW01-E6 and MW01-E14, and MW05-E2 and MW05-E10, which were inactive in cell-based assays. Higher inhibition is indicated by red color, moderate by white, and low by blue.

Western blots are representative images of three independent experiments. mRNA expression analyses are expressed as mean of three independent experiments with three technical replicates each.

Table S3: Percentage of inhibition of the indicated kinases by active and inactive derivatives of MW01 or MW05, related to Figure 3

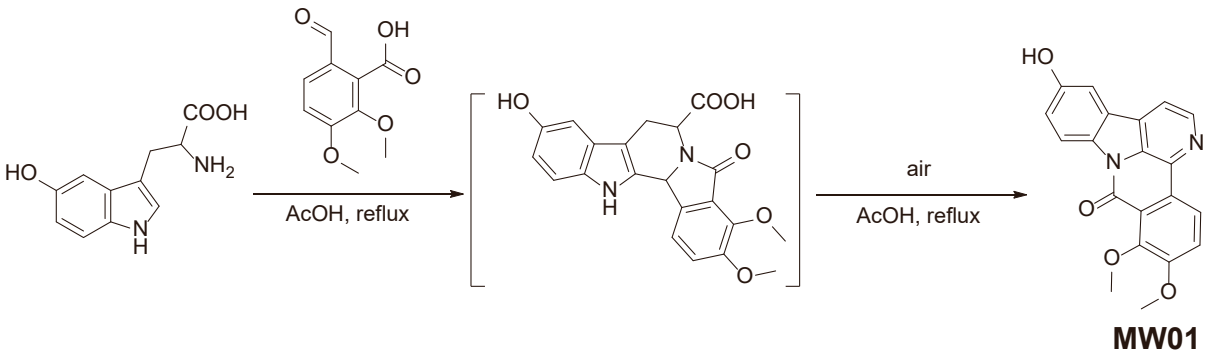
Data S1: Characterization of compounds and extended raw data, related to "Compound characterization" section in STAR Methods. Contains LCMS data and 1H NMR descriptions for the lead compounds and their chemical derivatives, as indicated.

Supplemental References

1. Sen'ko, O.A., Dybenko, A.G., Garazd, M.M., and Kartsev, V.G. (2017). Synthesis of Benzoannelated Canthin-6-One Analogs. Chemistry of Natural Compounds *53*, 523-528. 10.1007/s10600-017-2037-9.
2. Lapa, G.B., Bekker, O.B., Mirchink, E.P., Danilenko, V.N., and Preobrazhenskaya, M.N. (2013). Regioselective acylation of congeners of 3-amino-1H-pyrazolo[3,4-b]quinolines, their activity on bacterial serine/threonine protein kinases and in vitro antibacterial (including antimycobacterial) activity. J Enzyme Inhib Med Chem *28*, 1088-1093. 10.3109/14756366.2012.716056.

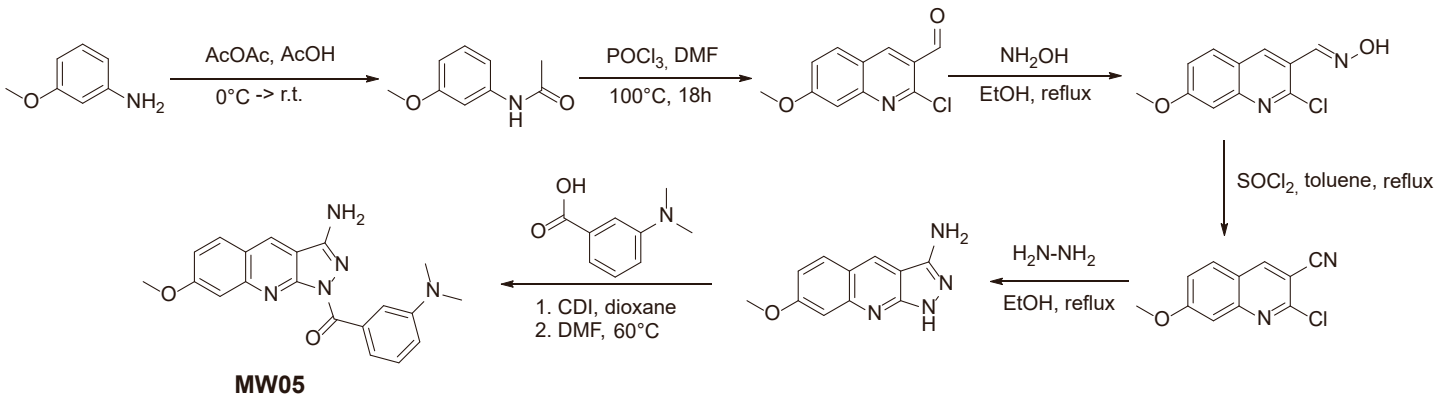
Figure S1

A



MW01

B

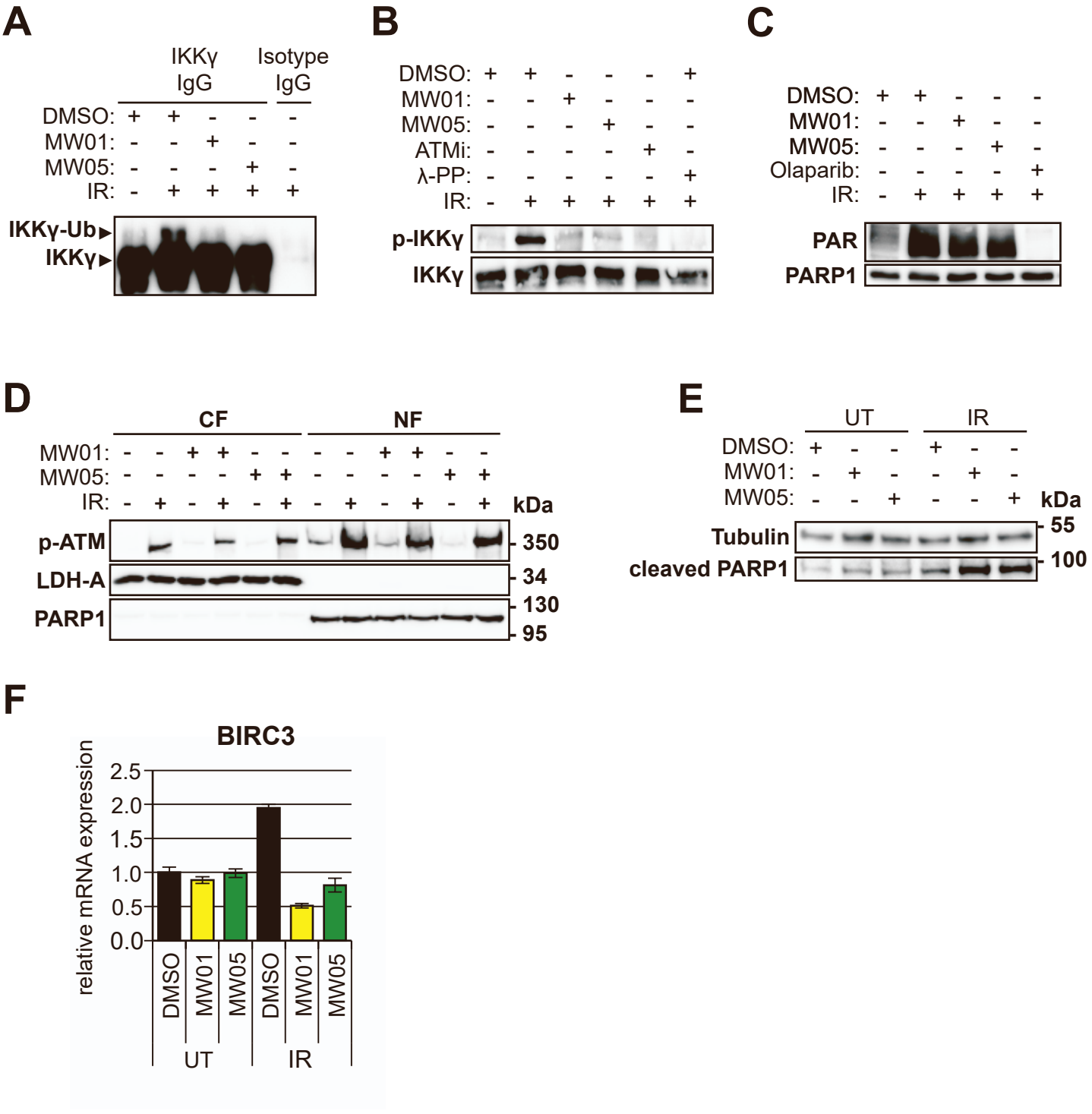


MW05

C

Compound	Mean P_{app} (× 10^{-6}cm/s)
MW01	12.60 ± 6.20
MW05	17.28 ± 2.65
Propranolol	33.43 ± 0.98

Figure S2


A
IKKγ IgG
Isotype IgG
DMSO: + + - - -
MW01: - - + - -
MW05: - - - + -
IR: - + + + +
IKKγ-Ub▶
IKKγ▶
B
DMSO: + + - - - +
MW01: - - + - - -
MW05: - - - + - -
ATMi: - - - - + -
λ-PP: - - - - - +
IR: - + + + + +
p-IKKγ
IKKγ
C
DMSO: + + - - -
MW01: - - + - -
MW05: - - - + -
Olaparib: - - - - +
IR: - + + + +
PAR
PARP1
D
CF
NF
MW01: - - + + - - - - + + - -
MW05: - - - - + + - - - - + +
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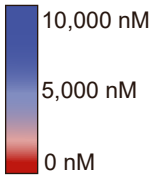
kDa
p-ATM
LDH-A
PARP1
350
34
130
95
E
UT
IR
DMSO: + - - + - -
MW01: - + - - + -
MW05: - - + - - +
kDa
Tubulin
cleaved PARP1
55
100
F
BIRC3
relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
DMSO
MW01
MW05
DMSO
MW01
MW05
UT
IR

Figure S3

A

Kinase	MW01 IC50	MW05 IC50
CLK2	360	1440
CSF1R (FMS)	1,000	10,000
DNA-PK	150	2,930
DYRK1A	949	10,000
DYRK1B	4,630	10,000
FLT3	6,010	10,000
FRAP1 (mTOR)	2,630	1,920
GSG2 (Haspin)	72.5	10,000
LRRK2 FL	49.4	10,000
MAP4K2 (GCK)	877	10,000
MAP4K4 (HGK)	1,800	10,000
MINK1	1,420	10,000
PI4KB (PI4K beta)	11.6	10,000
PIK3C2B (PI3K-C2 beta)	283	10,000
PIK3C2G (PI3K-C2 gamma)	25.6	610
PIK3CA/PIK3R1 (p110 alpha/p85)	15	9,570
PIK3CA/PIK3R3 (p110 alpha/p55)	29.5	9,670
PIK3CB/PIK3R2 (p110 beta/p85)	1,710	4,760
PIK3CD/PIK3R1 (p110 delta/p85)	62.8	2,200
PIK3CG (p110 gamma)	22.7	1,440
PIP5K1A	7,730	10,000



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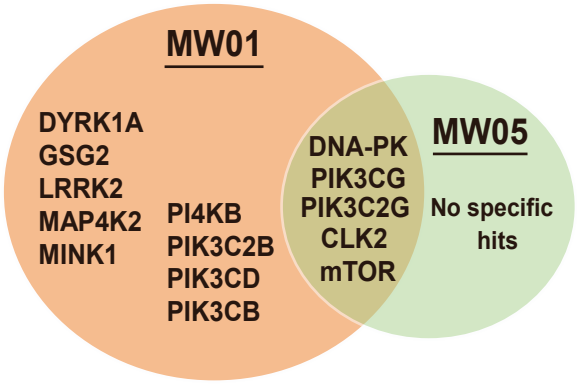
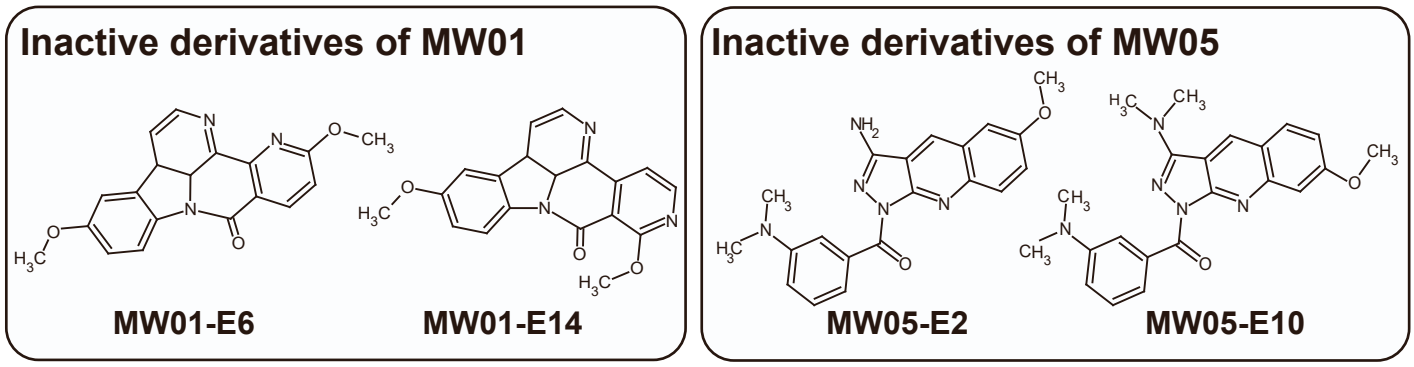


Figure S4

A



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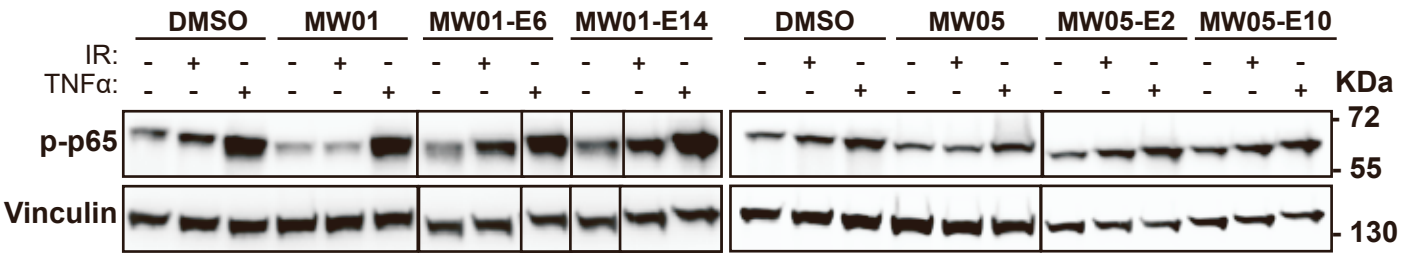

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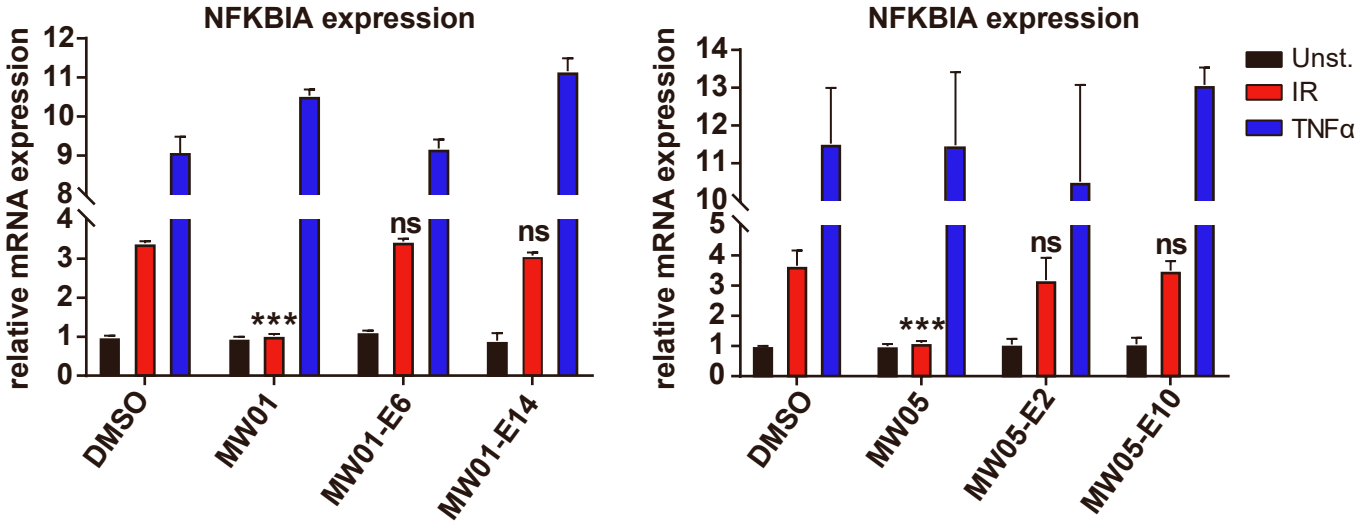

D

Lead	MW01		MW05	
Kinase	MW01-E14 (10μM)	MW01-E6 (10μM)	MW05-E10 (10μM)	MW05-E2 (10μM)
CLK1	6	10	35	16
CLK2	20	18	28	74
CLK3	7	9	2	5
CLK4	67	58	92	75

Percent inhibition
= low, 0-40%
= moderate, 40-70%
= high, 70-100%

Table S3

	% Inhibition							
Lead	**MW01**				**MW05**			
NF-kB Inhibition	**active**	**active**	**inactive**	**inactive**	**active**	**active**	**inactive**	**inactive**
Kinase	**MW01-E18 (10μM)**	**MW01-E10 (10μM)**	**MW01-E14 (10μM)**	**MW01-E6 (10μM)**	**MW05-E9 (10μM)**	**MW05-E3 (10μM)**	**MW05-E10 (10μM)**	**MW05-E2 (10μM)**
AKT1 (PKB alpha)	12	18	9	6	8	29	9	9
AKT2 (PKB beta)	3	18	14	4	5	20	7	8
AKT3 (PKB gamma)	19	24	23	12	14	33	12	11
ALK	35	75	15	19	20	19	9	24
BLK	23	42	9	23	5	30	17	10
BTK	30	69	29	30	7	37	12	7
CLK1	61	69	6	10	79	81	35	16
CLK2	70	70	20	18	87	86	28	74
CLK3	35	35	7	9	38	41	2	5
CLK4	98	99	67	58	84	99	92	75
CSF1R (FMS)	57	61	18	33	11	32	14	20
DNA-PK	94	62	43	9	64	67	8	16
DYRK1A	65	56	12	14	36	76	20	53
DYRK1B	68	81	23	20	46	58	14	84
EGFR (ErbB1)	14	15	9	11	5	12	6	33
FLT3	65	27	12	31	-4	22	9	17
FRAP1 (mTOR)	43	42	26	12	71	52	2	5
GSG2 (Haspin)	88	80	24	29	23	83	19	36
IKBKB (IKK beta)	16	31	16	10	7	23	10	12
IRAK1	69	71	9	56	6	37	-19	2
IRAK4	2	-186	-15	-9	-11	-13	-6	-3
LRRK2	75	86	-4	64	-1	37	-6	8
MAP4K2 (GCK)	93	98	81	70	0	51	5	2
MINK1	85	92	12	57	19	16	13	29
PI4K2A (PI4K2 alpha)	30	13	35	22	-7	4	-3	-4
PI4K2B (PI4K2 beta)	24	22	21	11	-6	3	1	8
PI4KB (PI4K beta)	81	90	36	12	-14	24	1	9
PIK3C2A (PI3K-C2 alpha)	44	31	23	26	-6	17	6	10
PIK3C2G (PI3K-C2 gamma)	81	81	50	35	41	72	10	27
PIK3CA/PIK3R1	90	90	80	38	21	51	-9	26
PIK3CA/PIK3R3	94	90	59	10	16	47	-3	8
PIK3CB/PIK3R1	62	34	26	13	19	40	4	1
PIK3CB/PIK3R2	63	35	12	12	22	34	2	7
PIK3CD/PIK3R1	77	87	84	34	25	55	6	28
PIK3CG	99	93	49	-1	40	56	-7	2
PIP4K2A	-1	-2	53	-4	-6	2	-14	-6
PIP5K1A	28	5	11	20	-16	-2	-8	-9
PIP5K1B	-3	5	26	14		29	-8	-12
PIP5K1C	27	33	8	14	-5	39	-4	-2
PRKCN (PKD3)	52	74	-1	-8	14	31	5	7

Data S1

MW01

==========

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Different Inj Volume from Sample Entry! Actual Inj Volume : 0.500 µl
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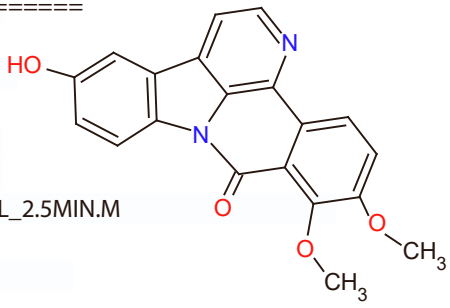

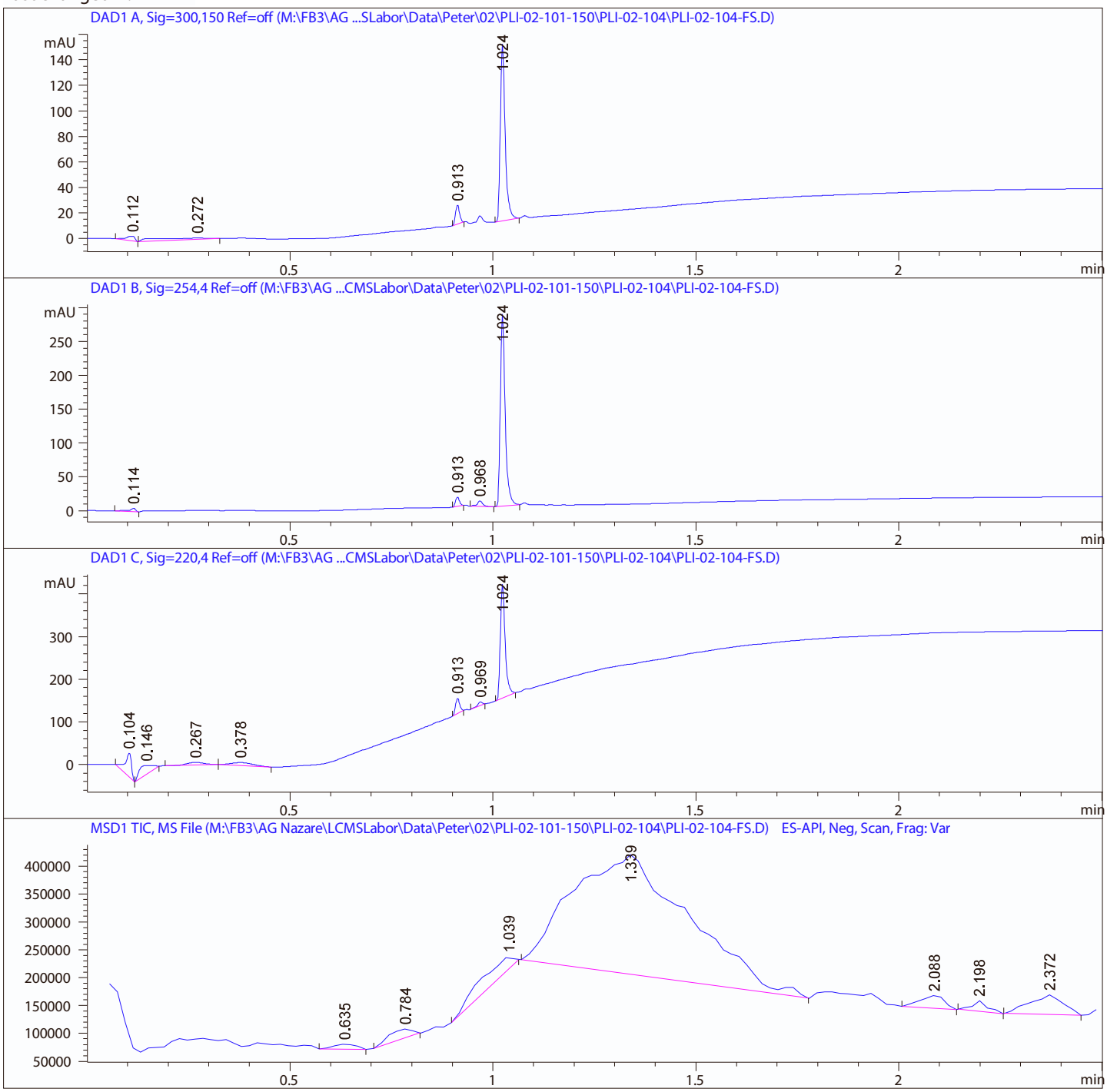

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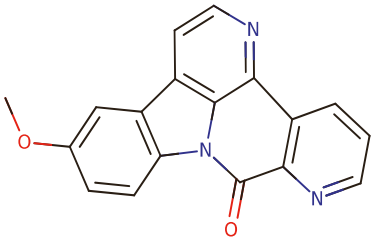

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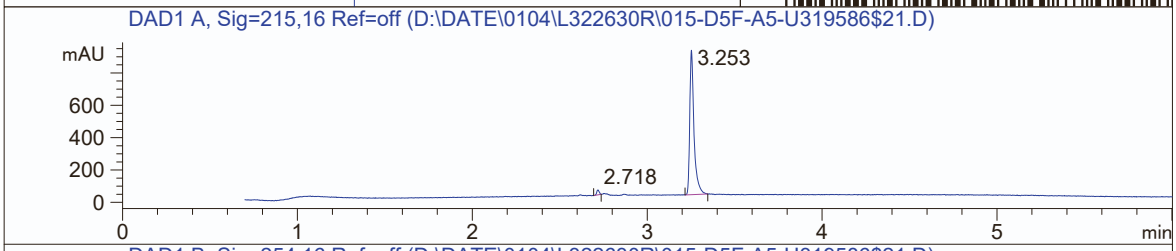
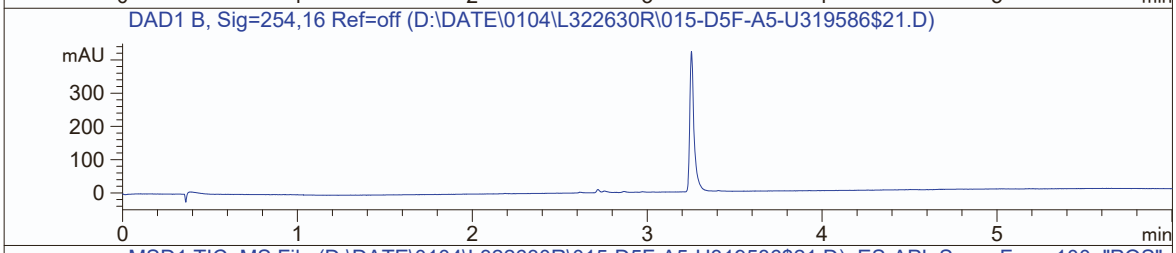
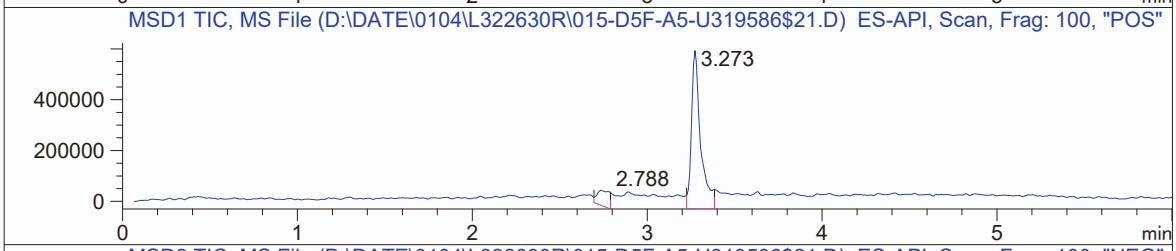
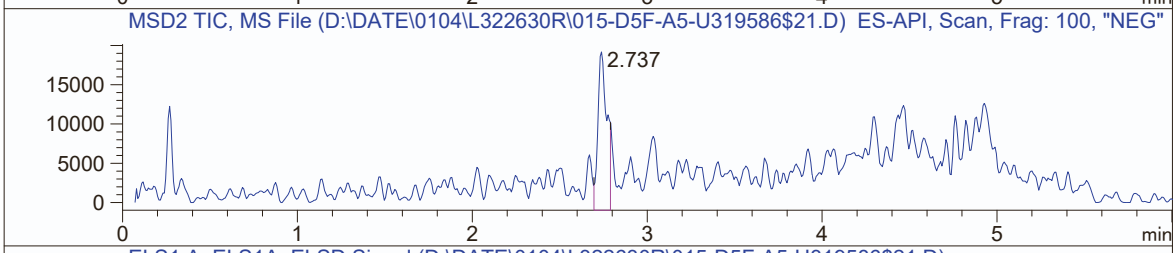
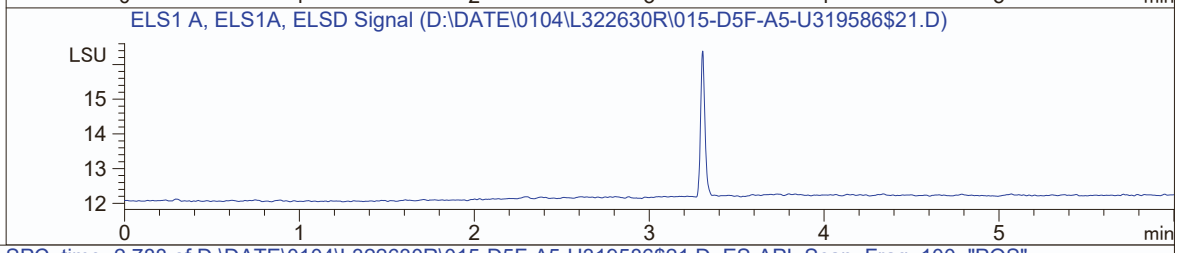


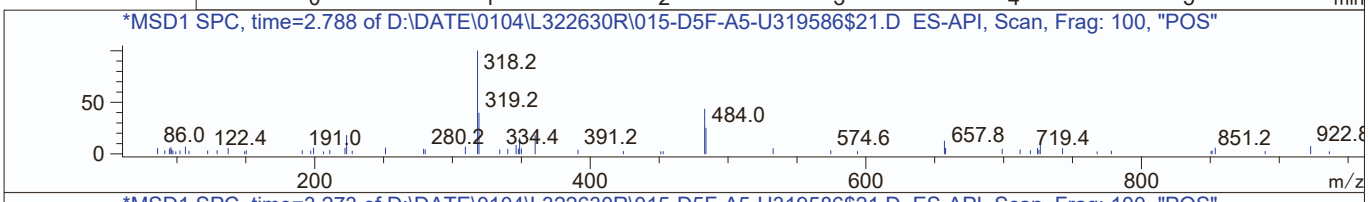








RT 2.788



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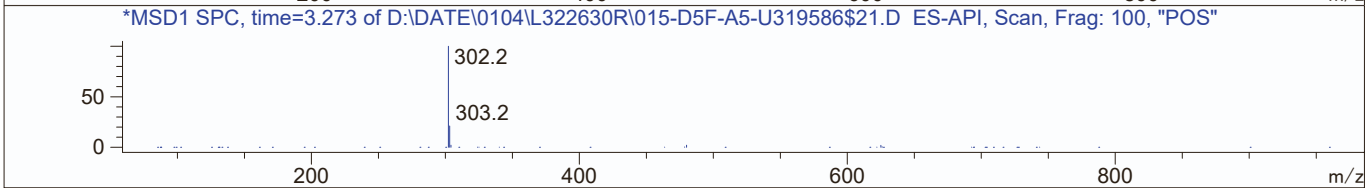

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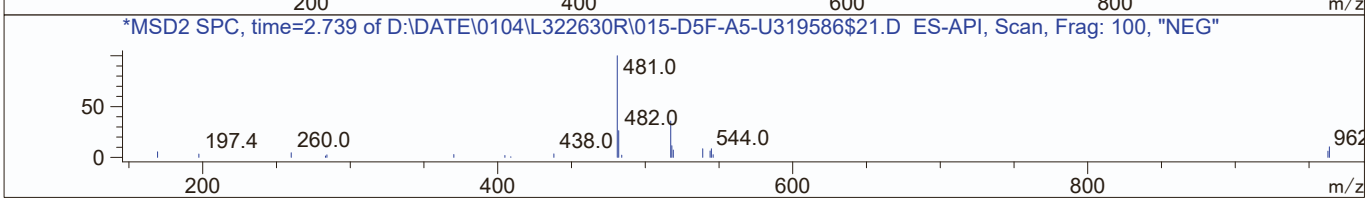

MW01-E2
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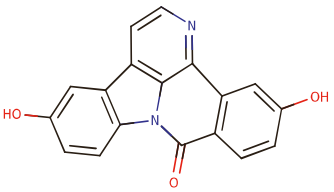

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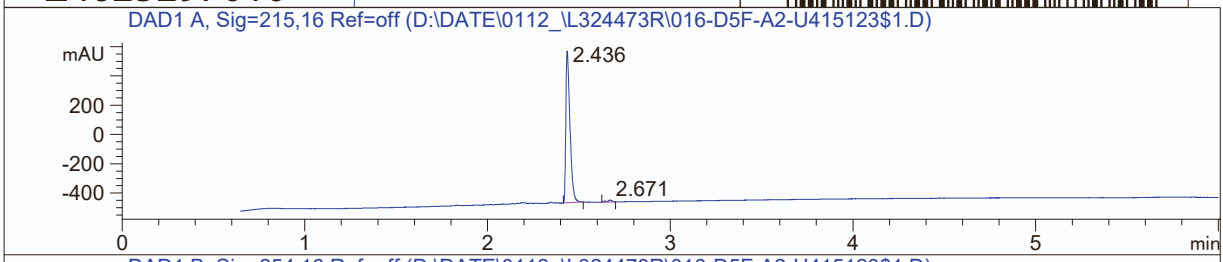

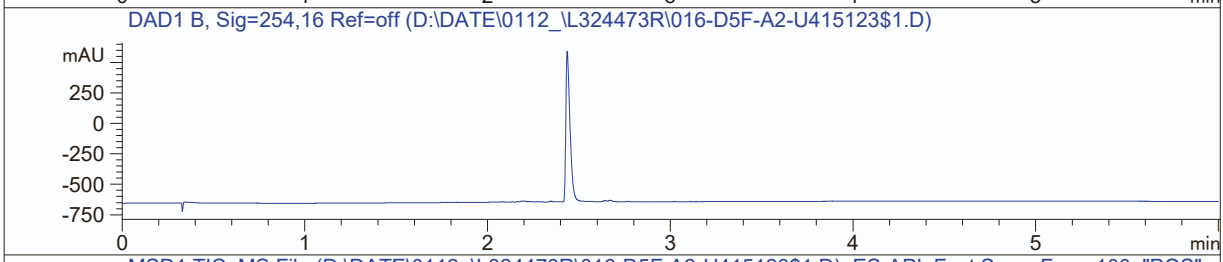

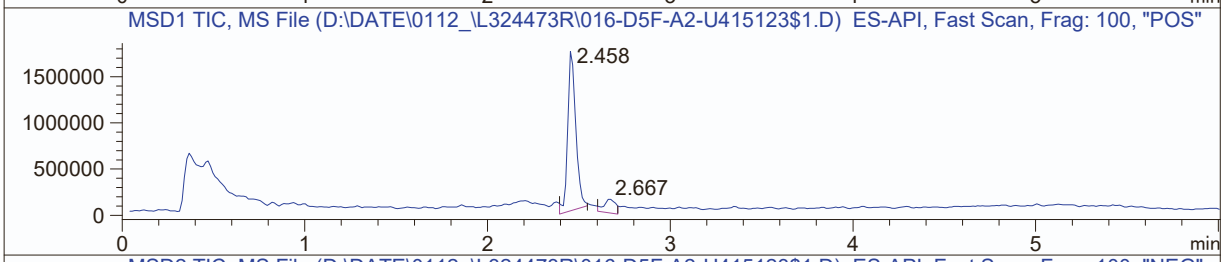

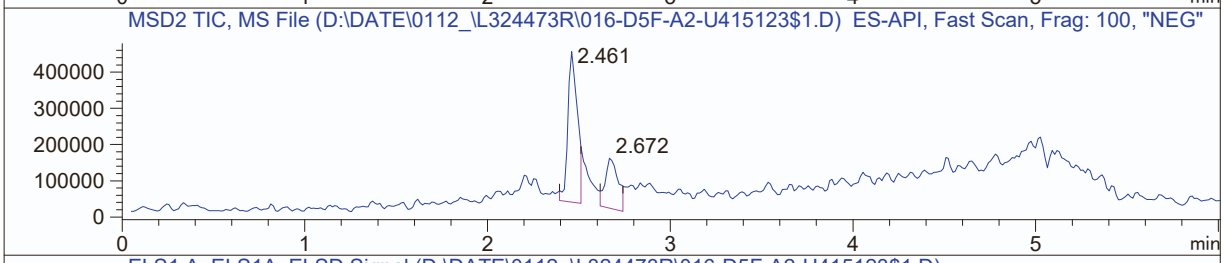

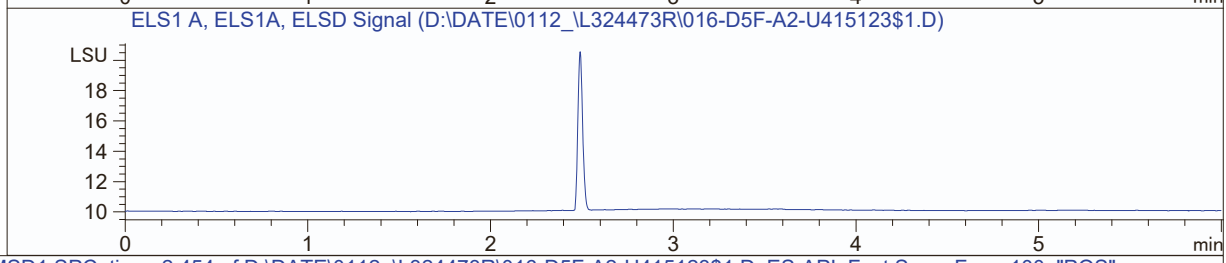

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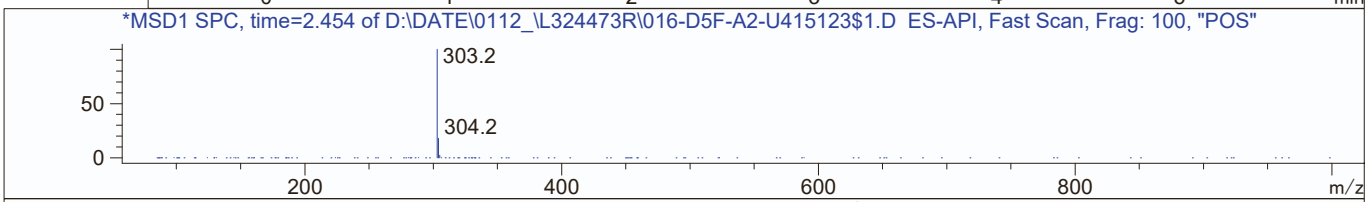

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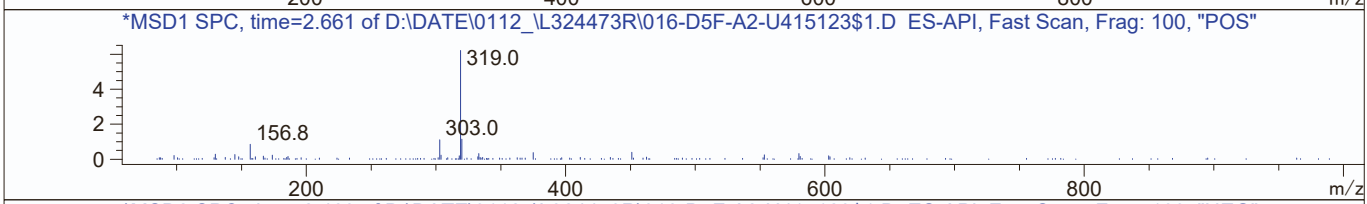
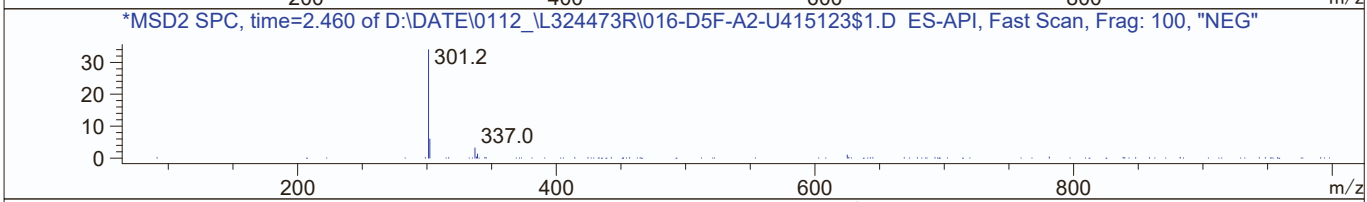
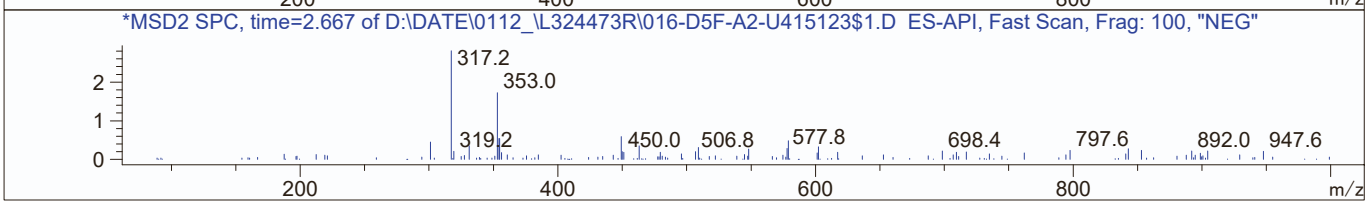


RT 2.461



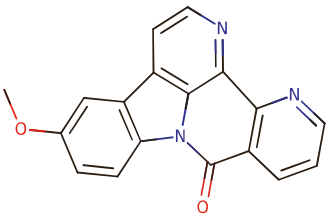
RT 2.672



Inj.Date 1/12/2021 O Acq. Method C:\Users\ -> ->

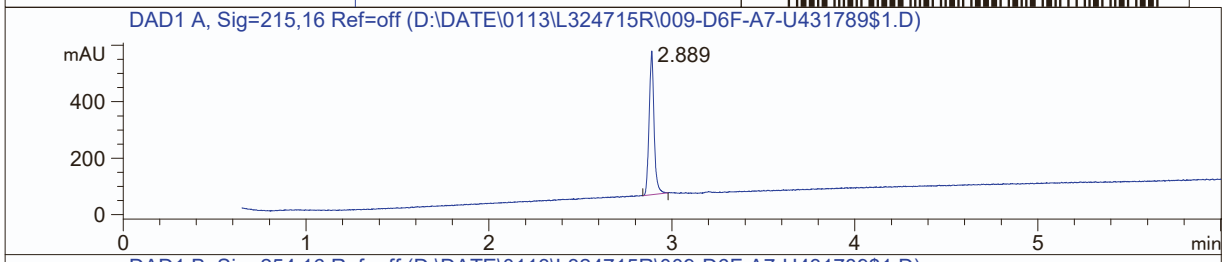
MW01-E3
Z4613515656

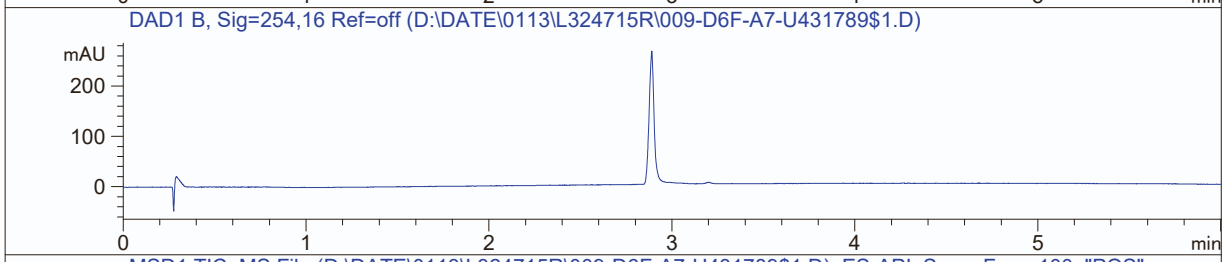
MaxPeak: 100.00%
Ret_Time: 2.889 min

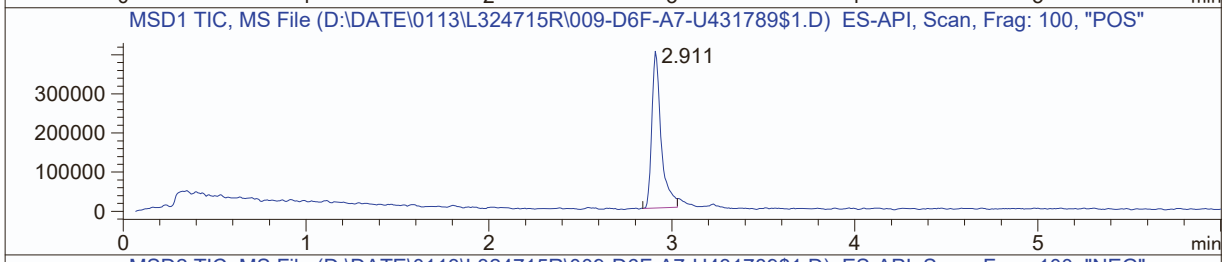


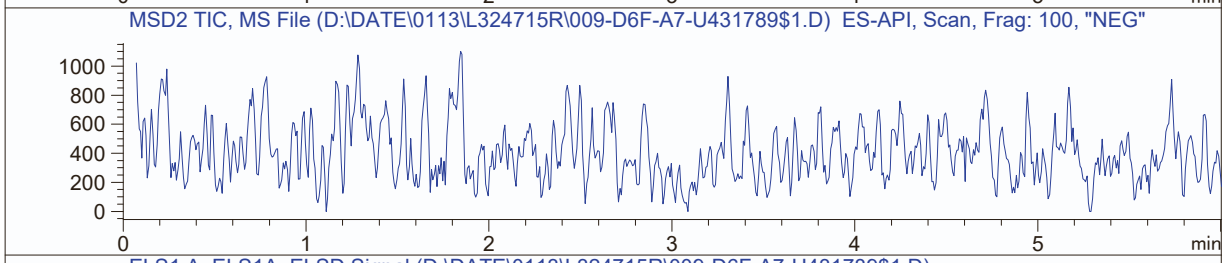
Mol Wt **301.3**
Exact Mass **301.09**

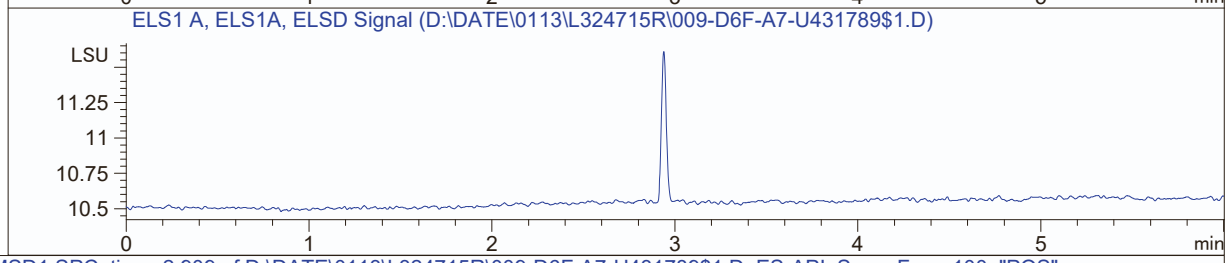
#	Time	Area%
1	2.889	100.00



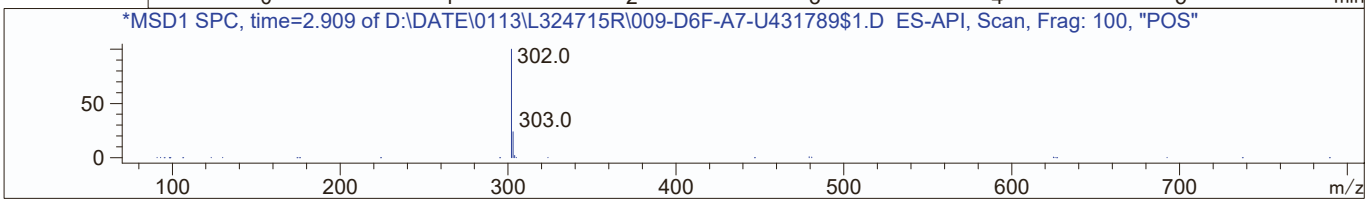






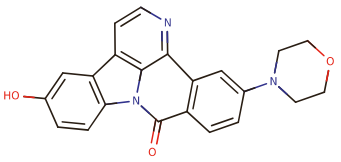


RT 2.911



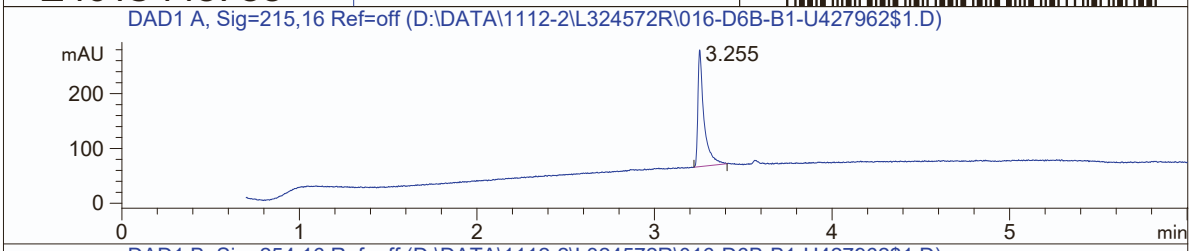
MaxPeak: 100.00%
Ret_Time: 3.255 min

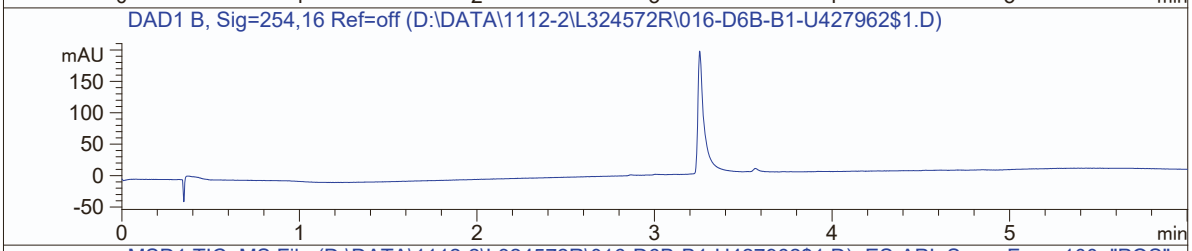
MW01-E4
Z4613448788

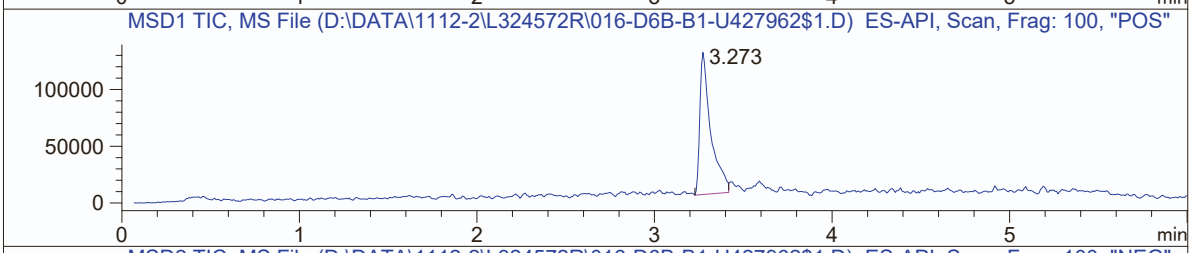


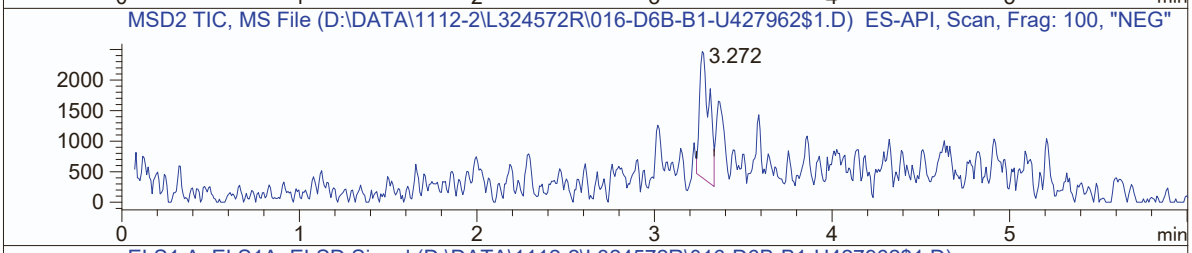
Mol Wt 371.39
Exact Mass 371.14

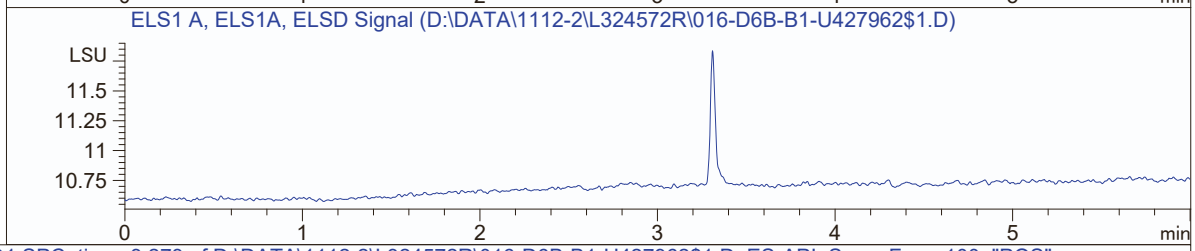
#	Time	Area%
1	3.255	100.00



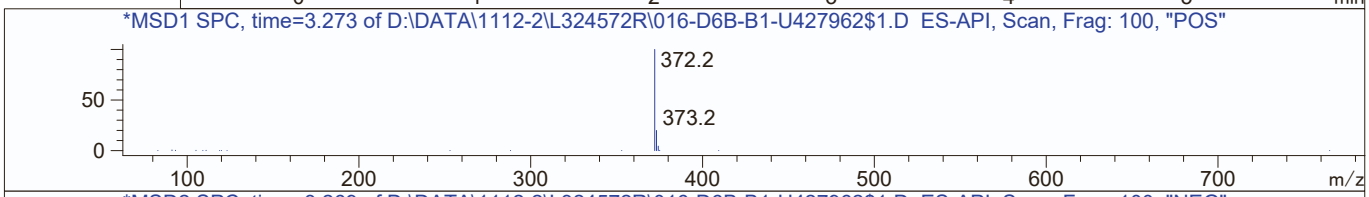




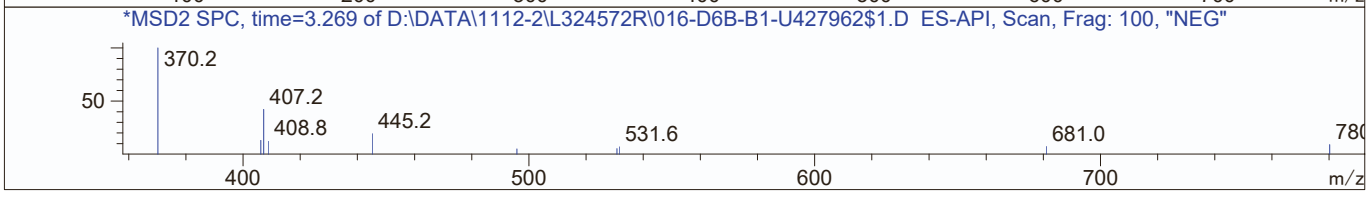




RT 3.273

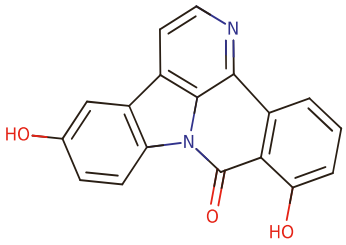


RT 3.272



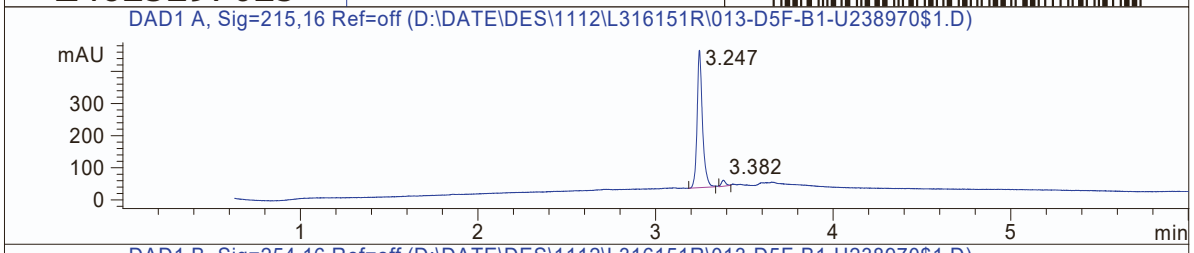
MaxPeak: 96.57%
Ret_Time: 3.247 min

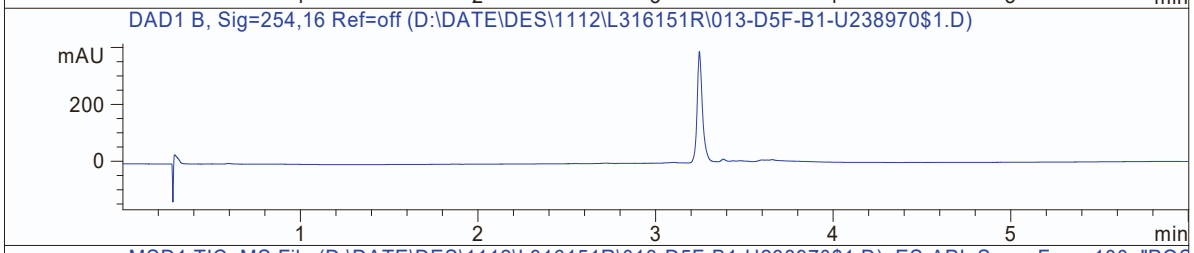
MW01-E5
Z4623297025

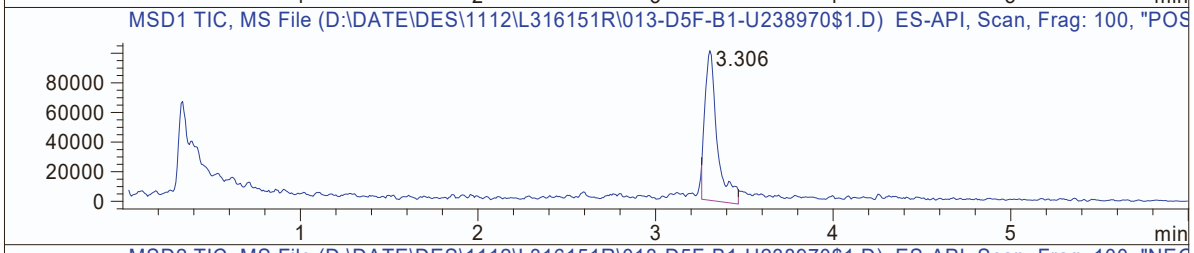


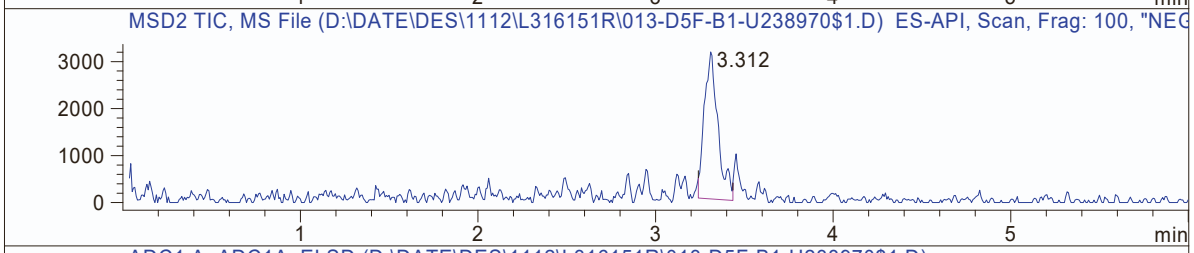
Mol Wt 302.28
Exact Mass 302.07

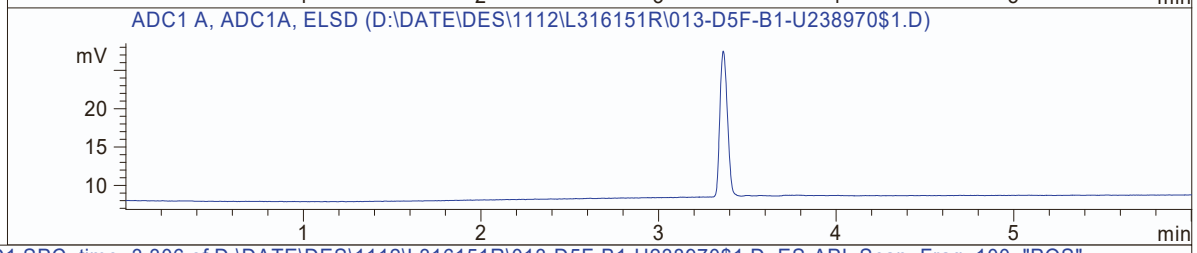
#	Time	Area%
1	3.247	96.57
2	3.382	3.43



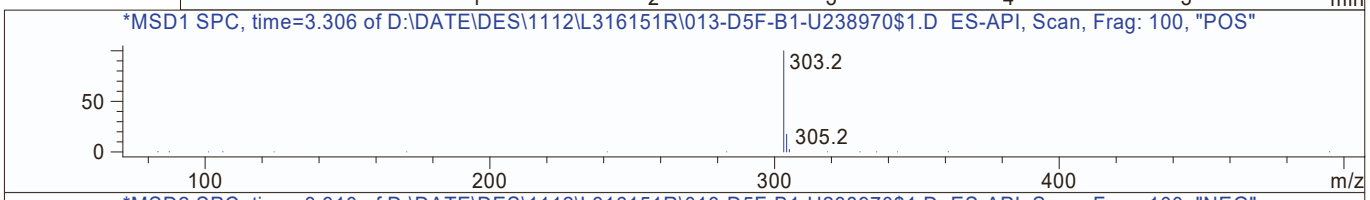




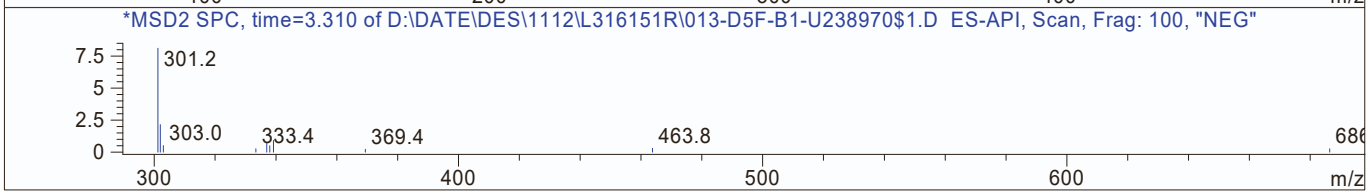




RT 3.306

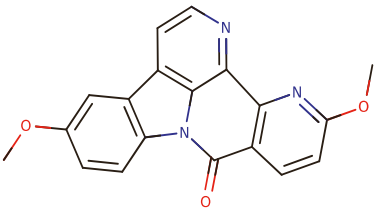


RT 3.312

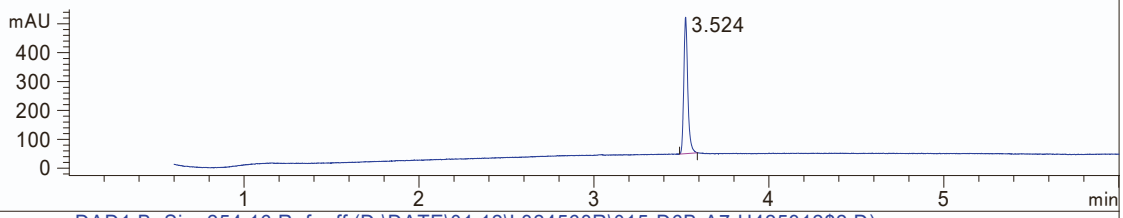


MaxPeak: 100.00%
Ret_Time: 3.524 min

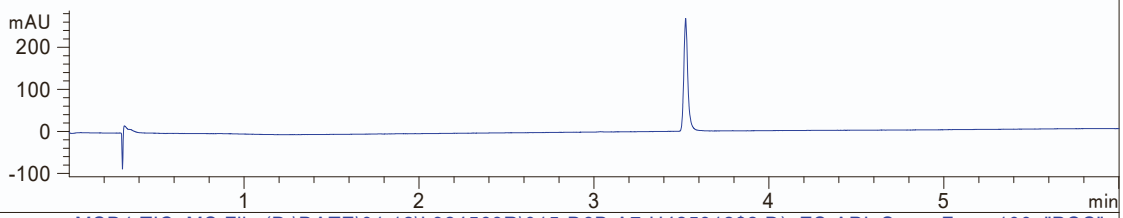
MW01-E6
Z4613838256



DAD1 A, Sig=215,16 Ref=off (D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D)



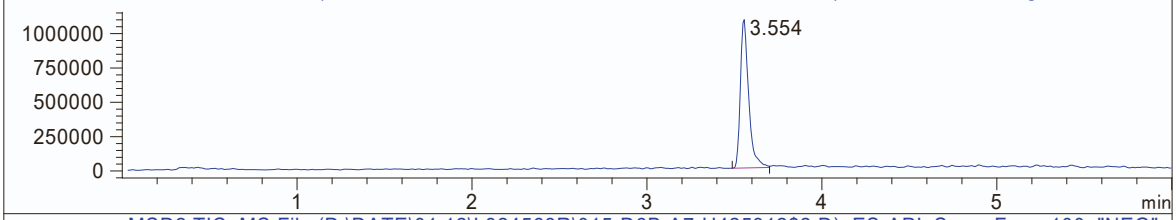
DAD1 B, Sig=254,16 Ref=off (D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D)



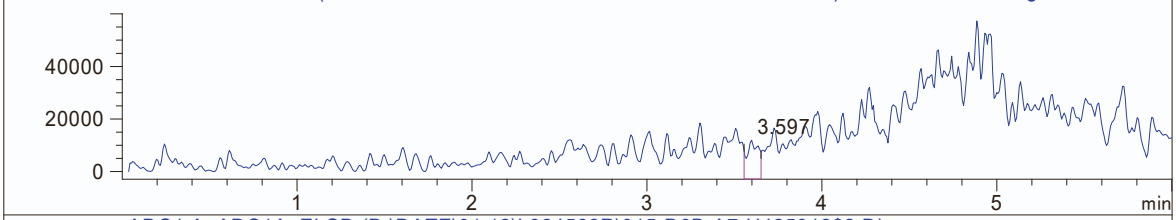
Mol Wt **331.32**
Exact Mass **331.1**

#	Time	Area%
1	3.524	100.00

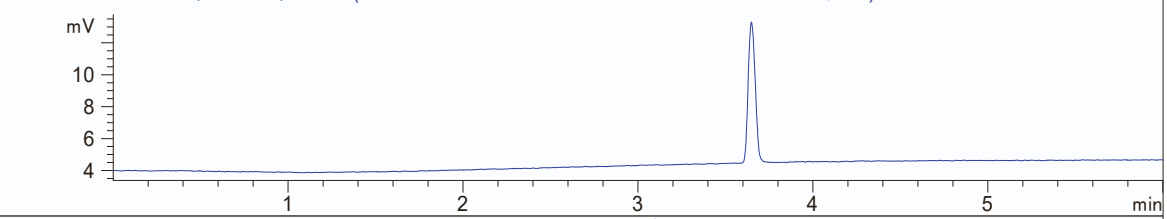
MSD1 TIC, MS File (D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D) ES-API, Scan, Frag: 100, "POS"



MSD2 TIC, MS File (D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D) ES-API, Scan, Frag: 100, "NEG"

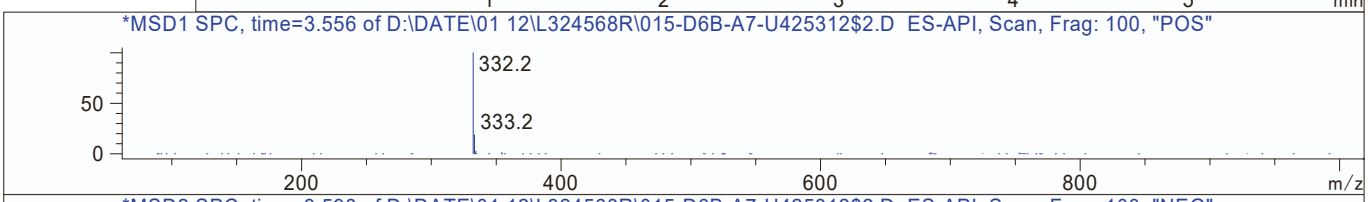


ADC1 A, ADC1A, ELSD (D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D)



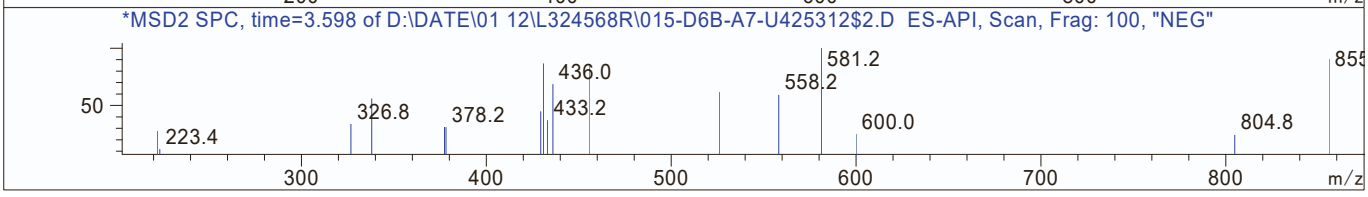
RT 3.554

*MSD1 SPC, time=3.556 of D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D ES-API, Scan, Frag: 100, "POS"



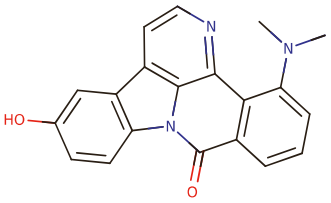
RT 3.597

*MSD2 SPC, time=3.598 of D:\DATE\01 12\L324568R\015-D6B-A7-U425312$2.D ES-API, Scan, Frag: 100, "NEG"



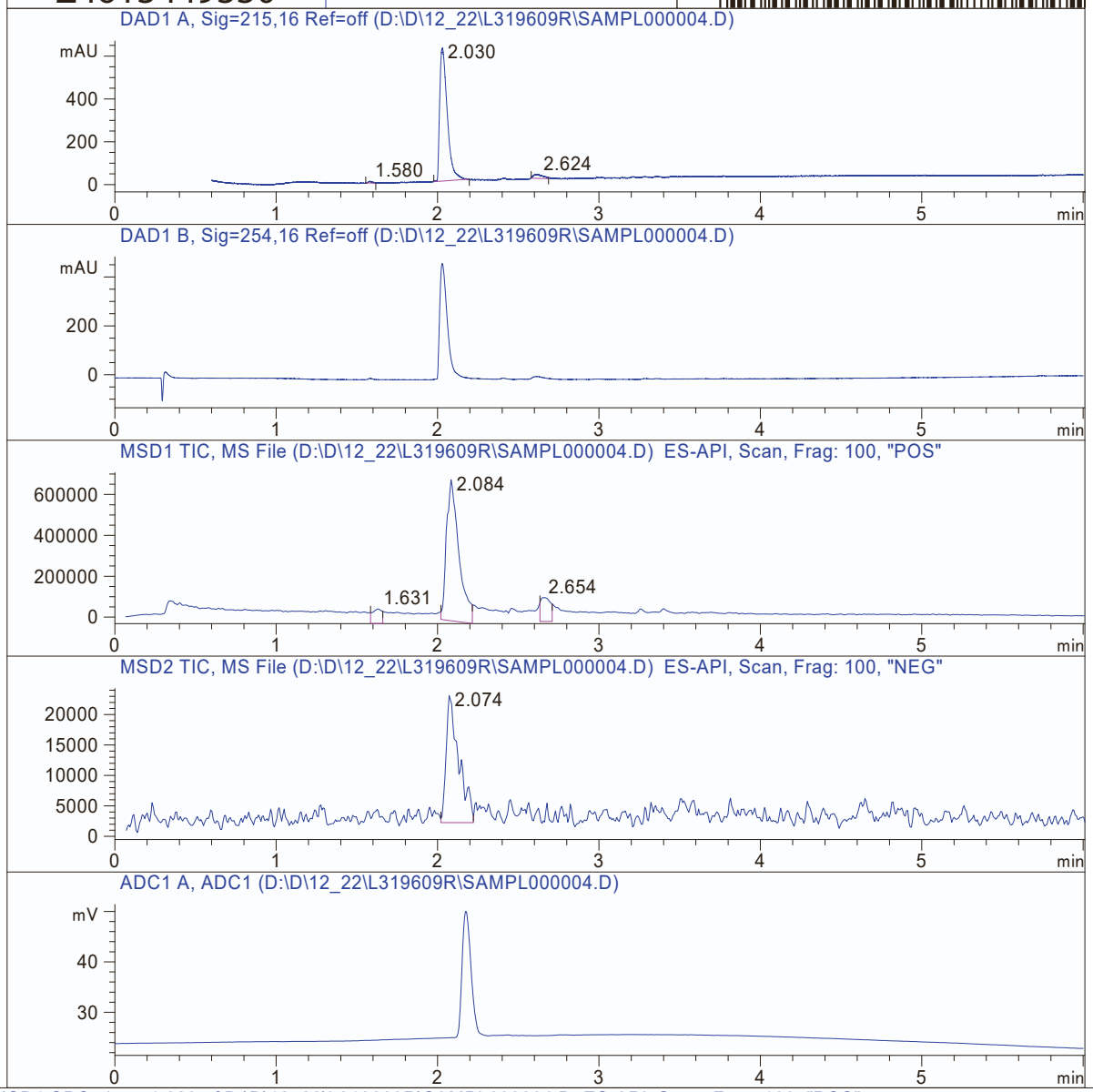
MaxPeak: 95.87%
Ret_Time: 2.030 min

MW01-E7
Z4613449330

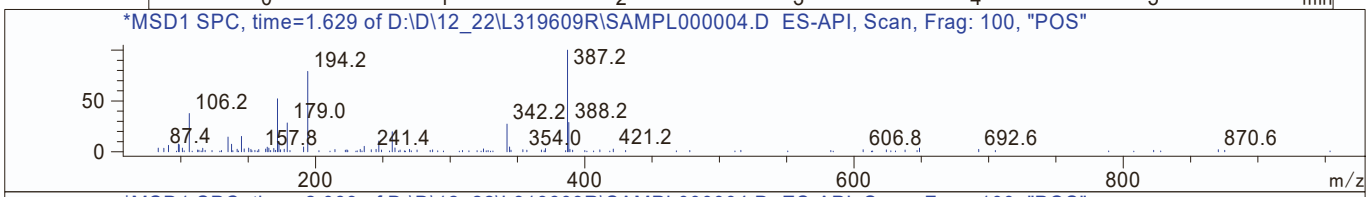


Mol Wt 329.35
Exact Mass 329.13

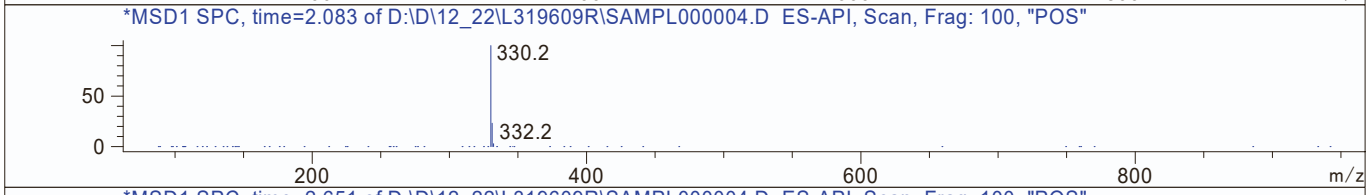
#	Time	Area%
1	1.580	0.64
2	2.030	95.87
3	2.624	3.50



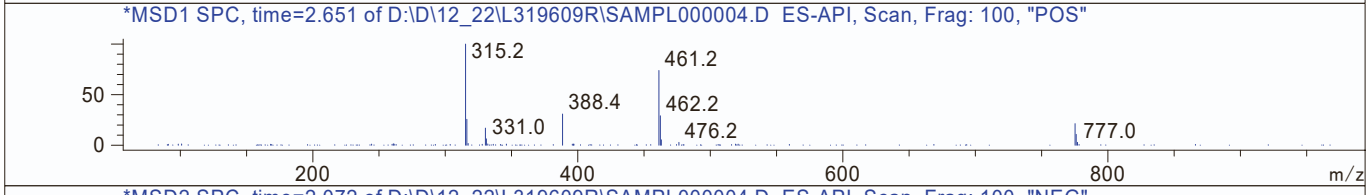
RT 1.631



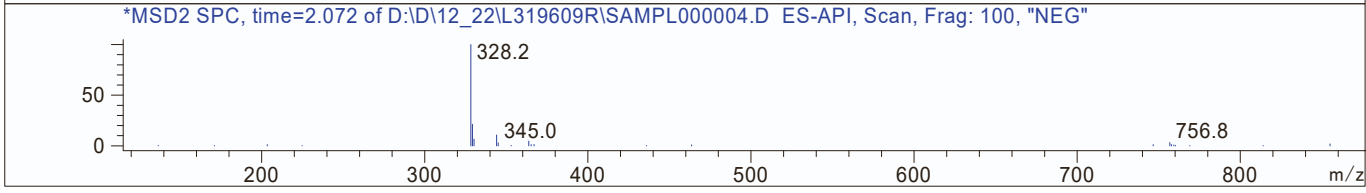
RT 2.084



RT 2.654

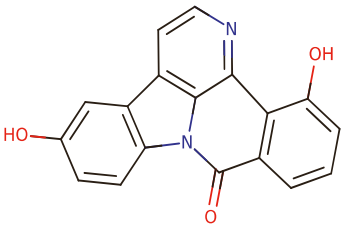


RT 2.074



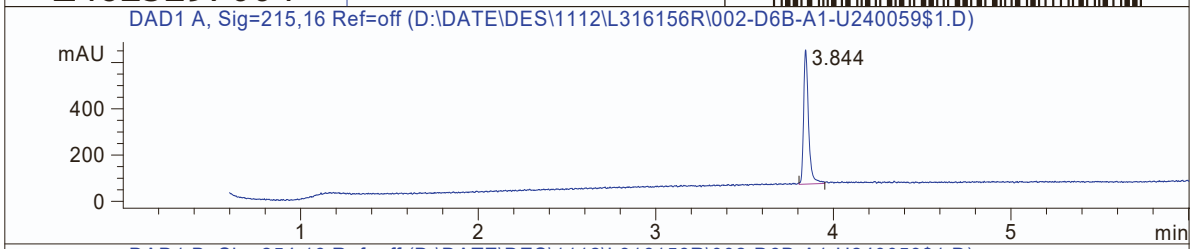
MaxPeak: 100.00%
Ret_Time: 3.844 min

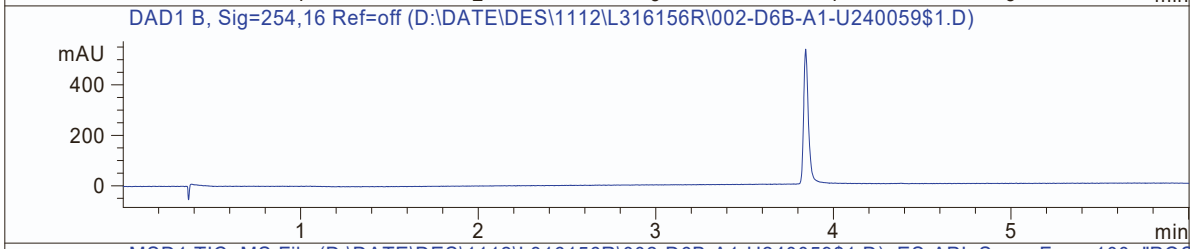
MW01-E8
Z4623297004

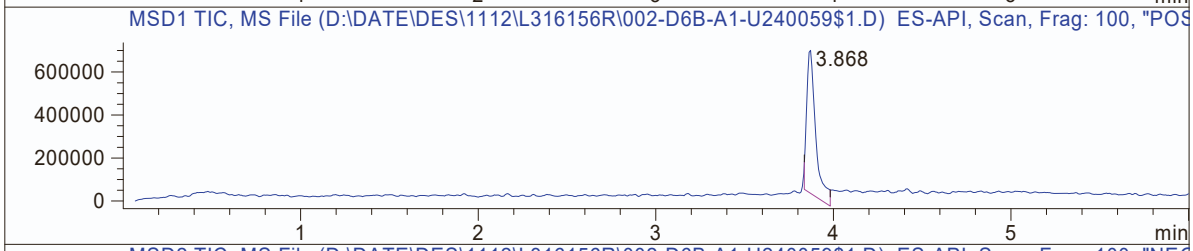


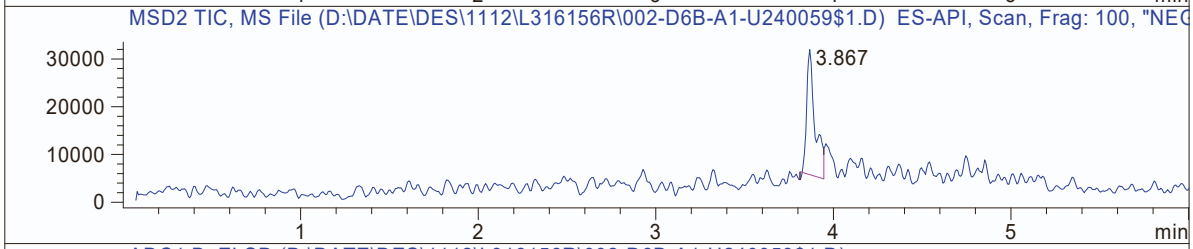
Mol Wt 302.28
Exact Mass 302.07

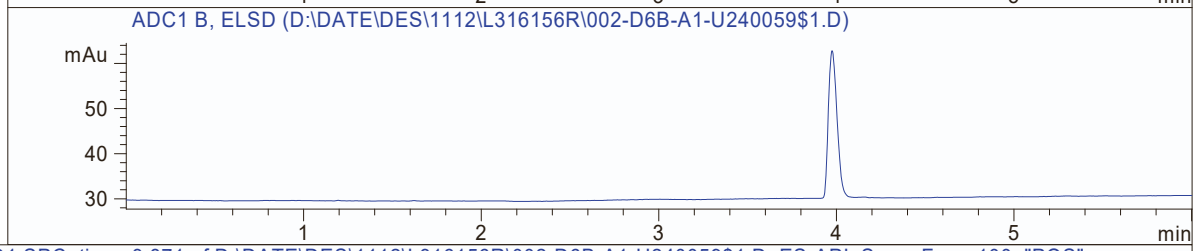
#	Time	Area%
1	3.844	100.00



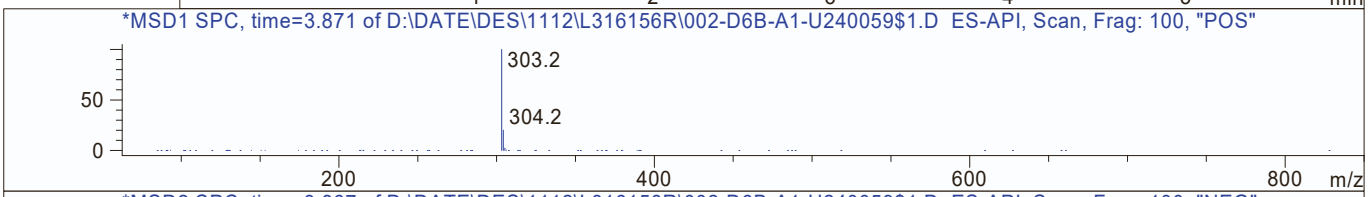




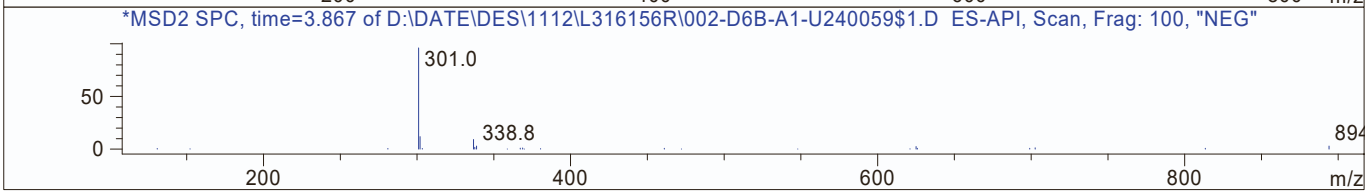




RT 3.868

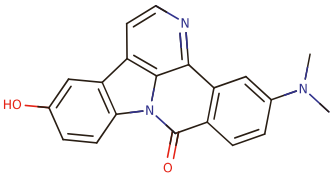


RT 3.867



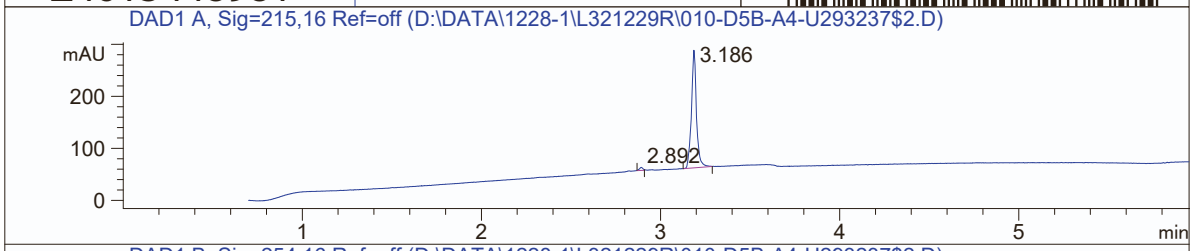
MaxPeak: 98.31%
Ret_Time: 3.186 min

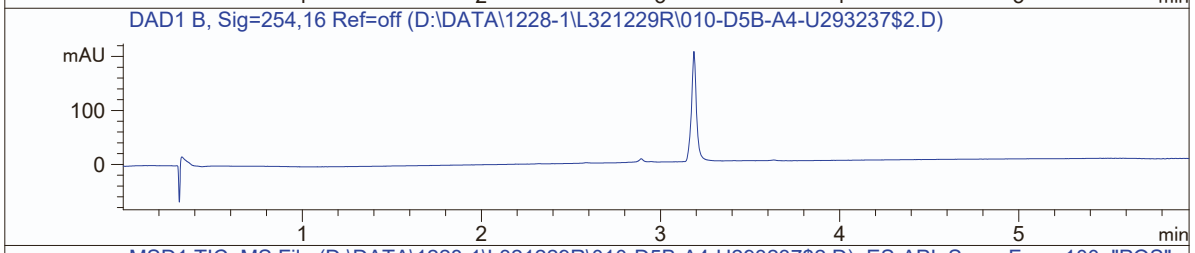
MW01-E9
Z4613448981

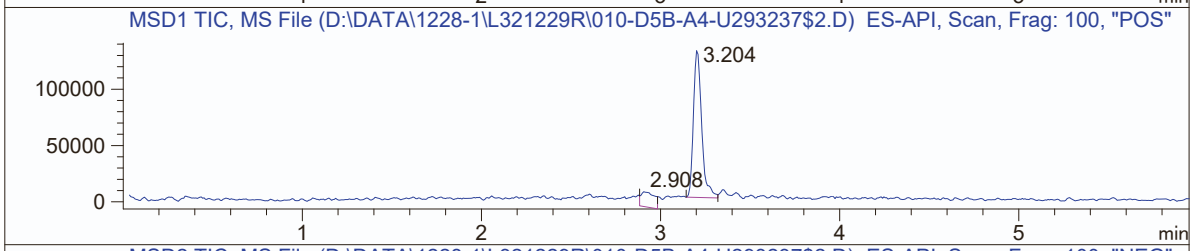


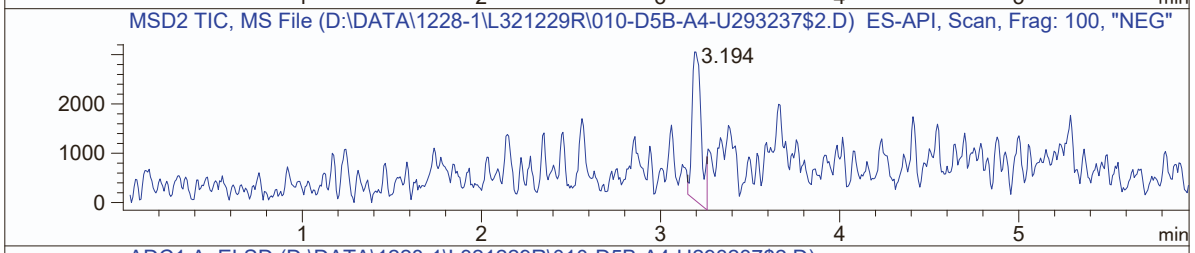
Mol Wt **329.35**
Exact Mass **329.13**

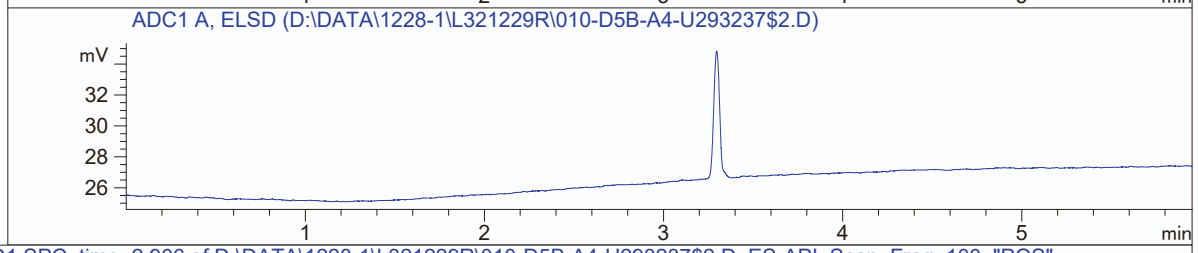
#	Time	Area%
1	2.892	1.69
2	3.186	98.31



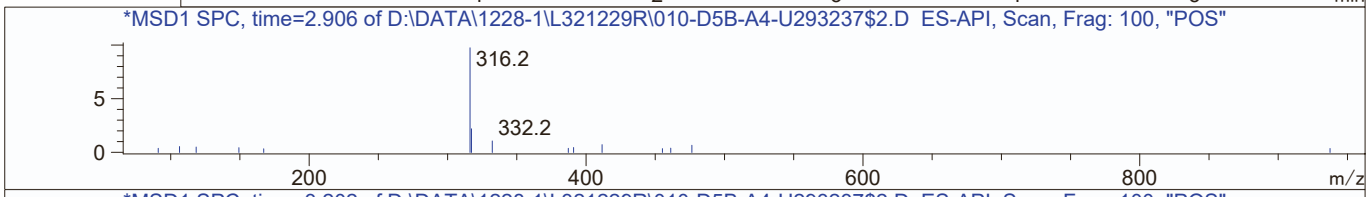




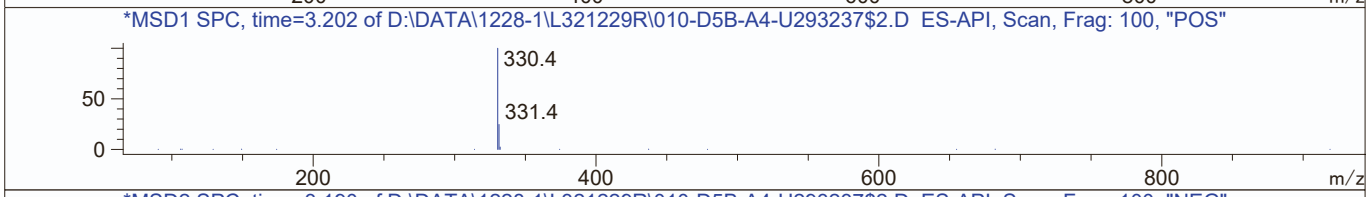




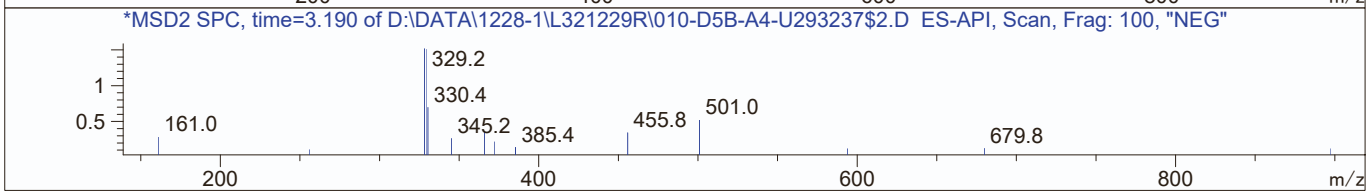
RT 2.908



RT 3.204

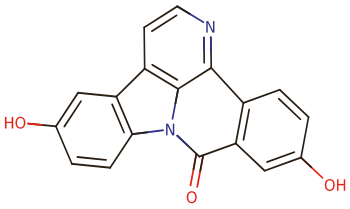


RT 3.194



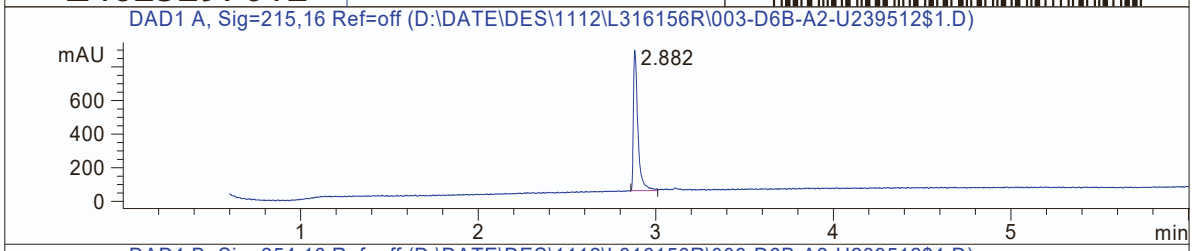
MaxPeak: 100.00%
Ret_Time: 2.882 min

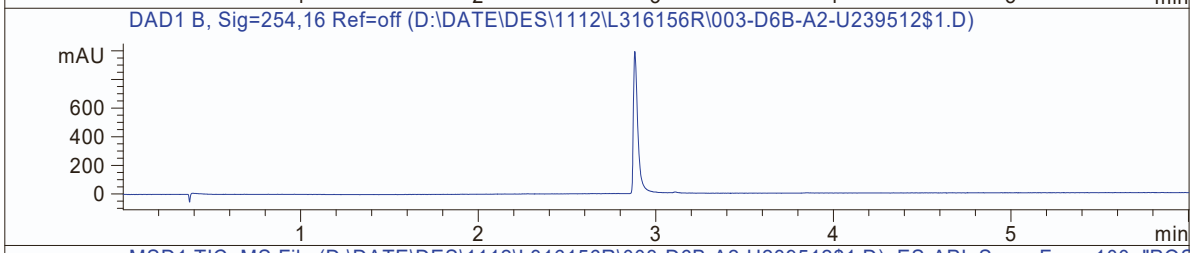
MW01-E10
Z4623297012

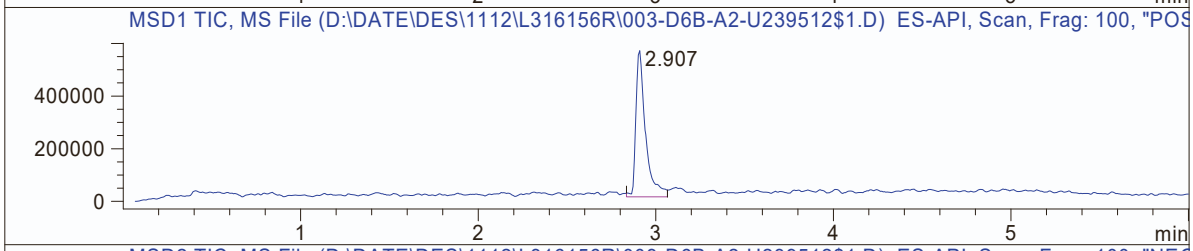


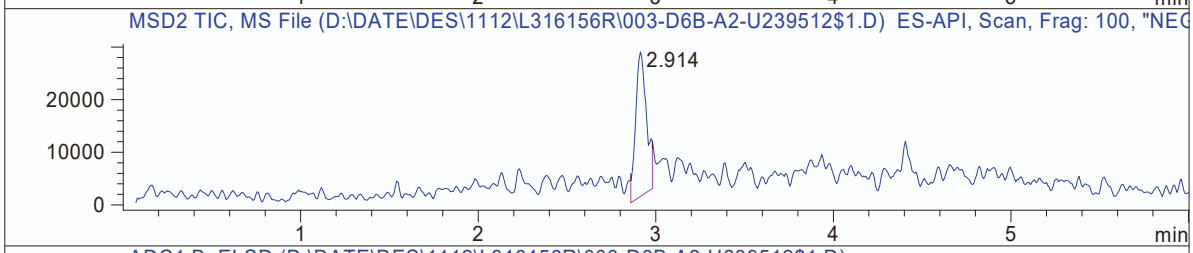
Mol Wt 302.28
Exact Mass 302.07

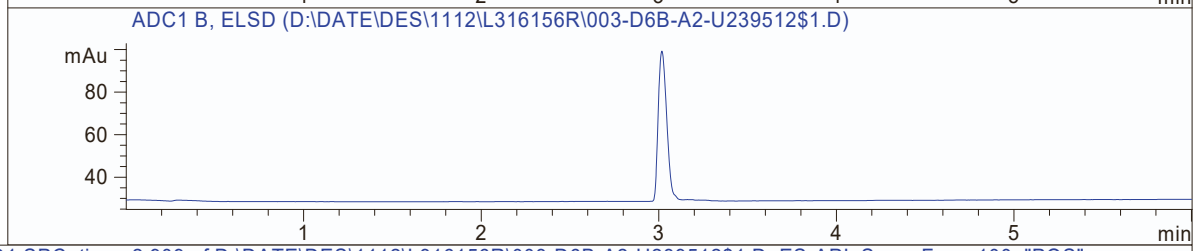
#	Time	Area%
1	2.882	100.00



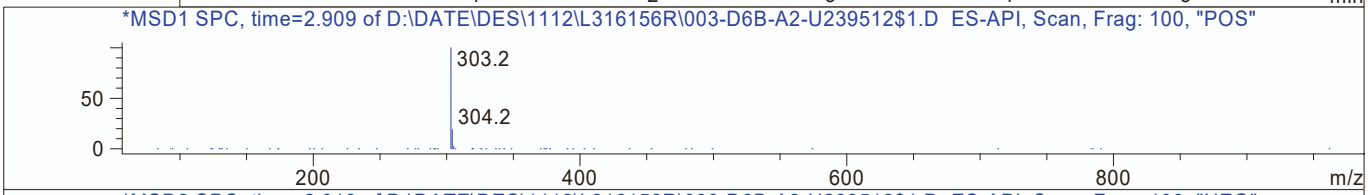




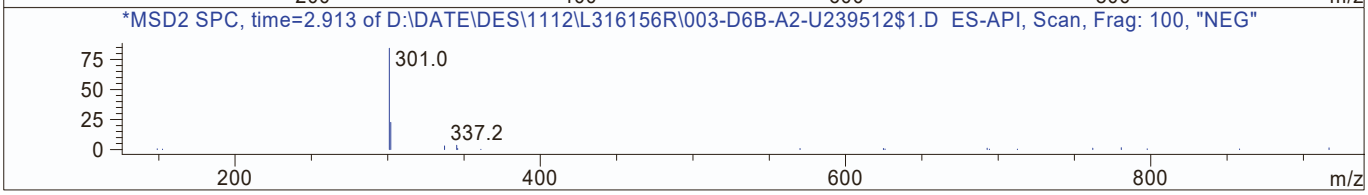




RT 2.907

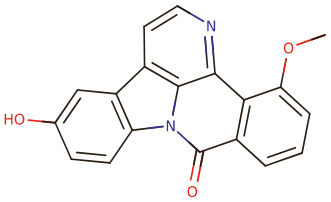


RT 2.914



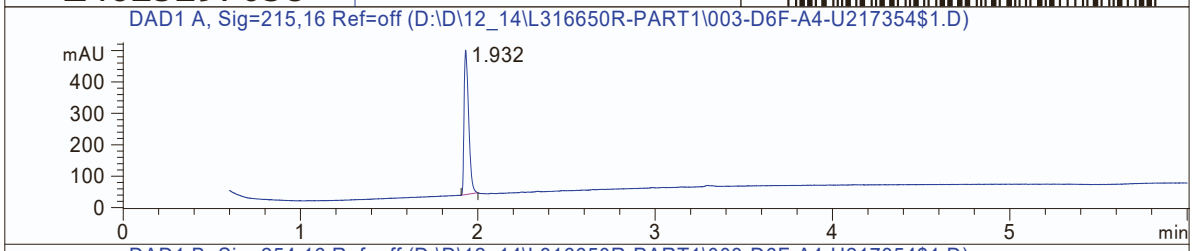
MaxPeak: 100.00%
Ret_Time: 1.932 min

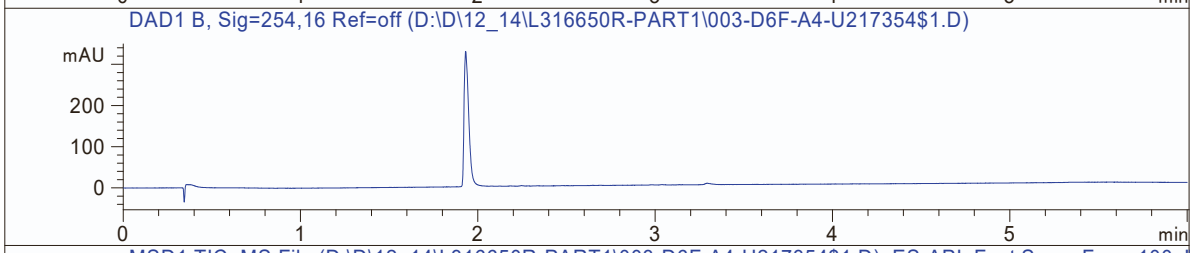
MW01-E11
Z4623297038

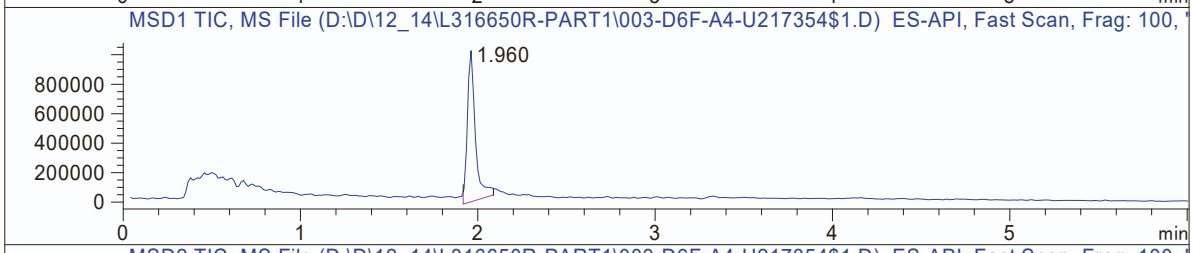


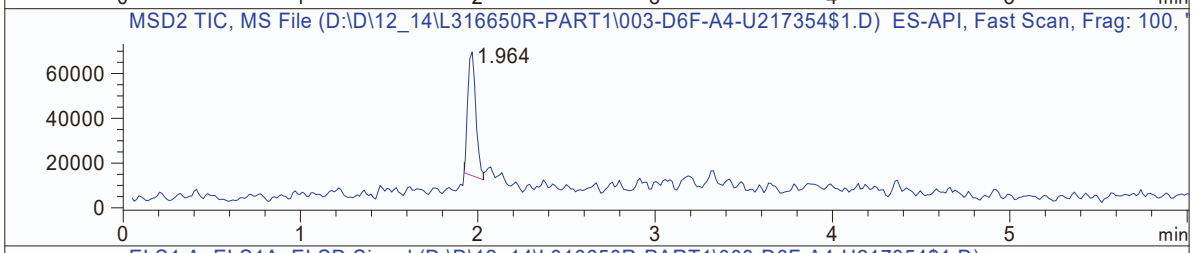
Mol Wt 316.31
Exact Mass 316.09

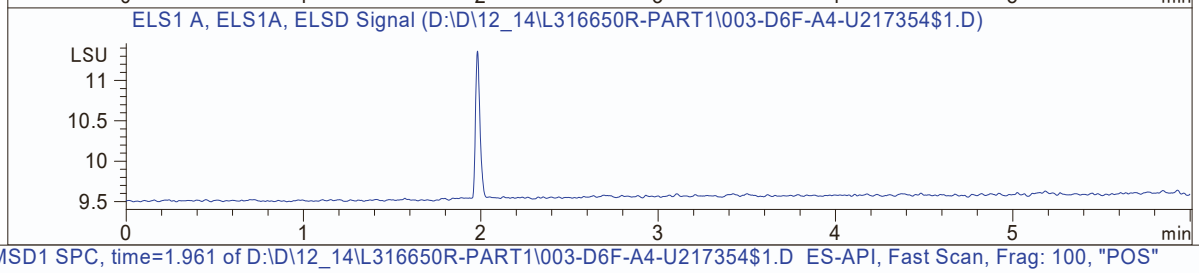
#	Time	Area%
1	1.932	100.00



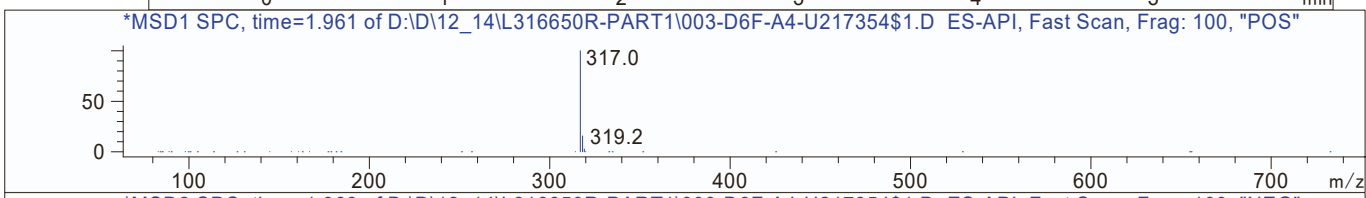




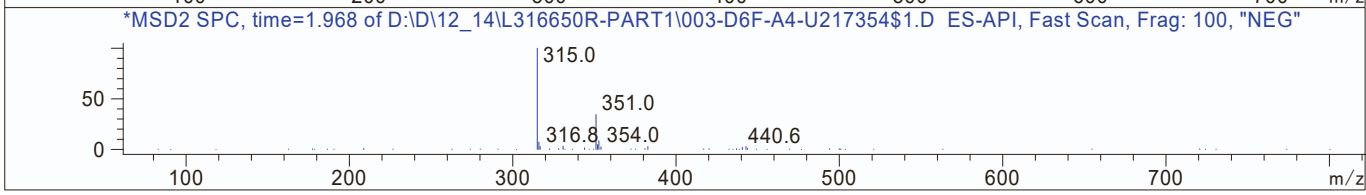




RT 1.960

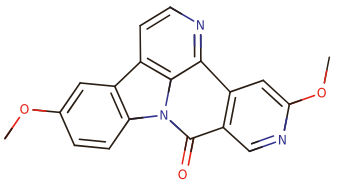


RT 1.964



Inj.Date 12/14/2020 N Acq. Method C:\Users\ -> ->

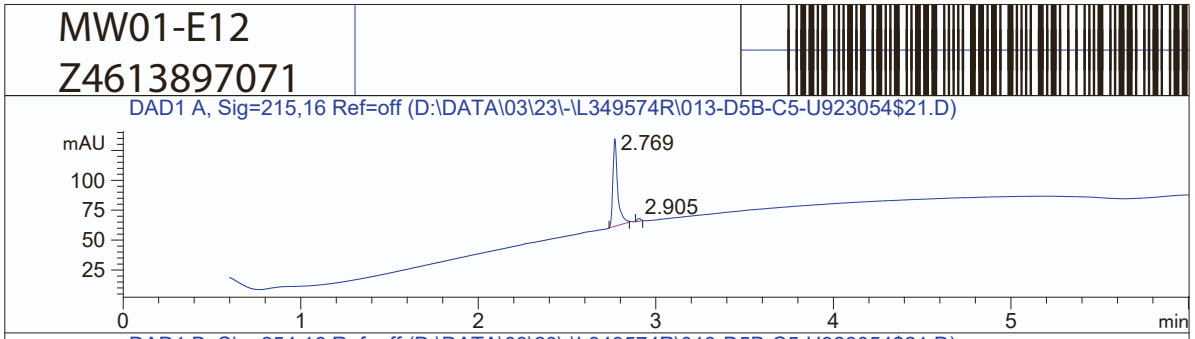
MaxPeak: 97.66%
Ret_Time: 2.769 min

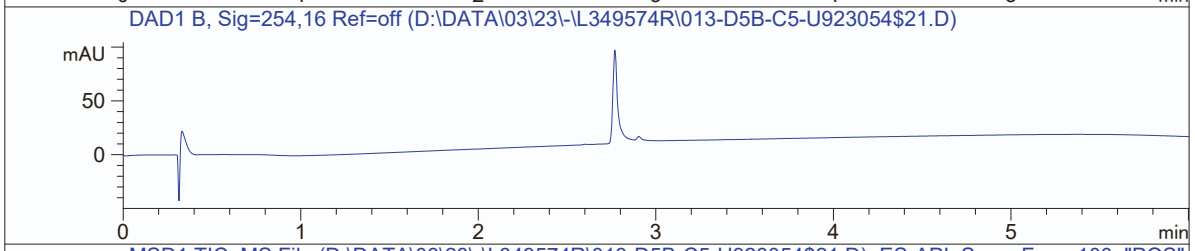


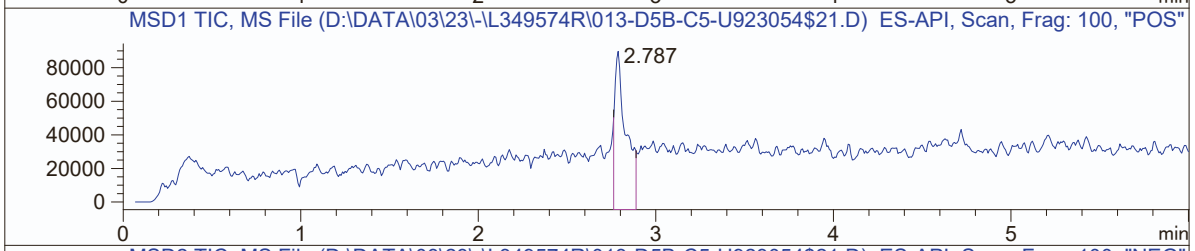
Mol Wt 331.32
Exact Mass 331.1

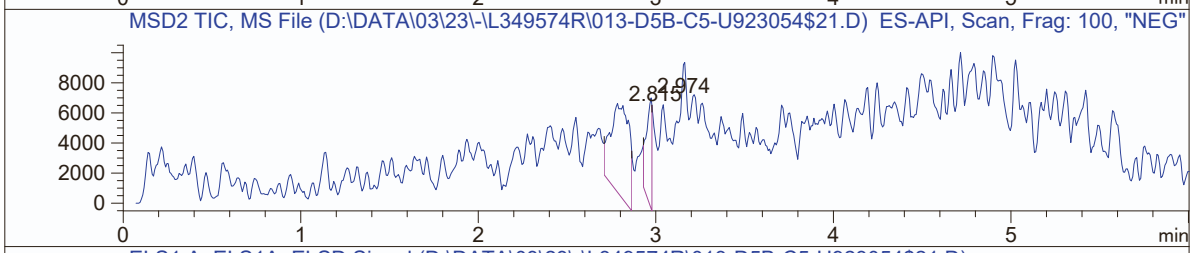
#	Time	Area%
1	2.769	97.66
2	2.905	2.34

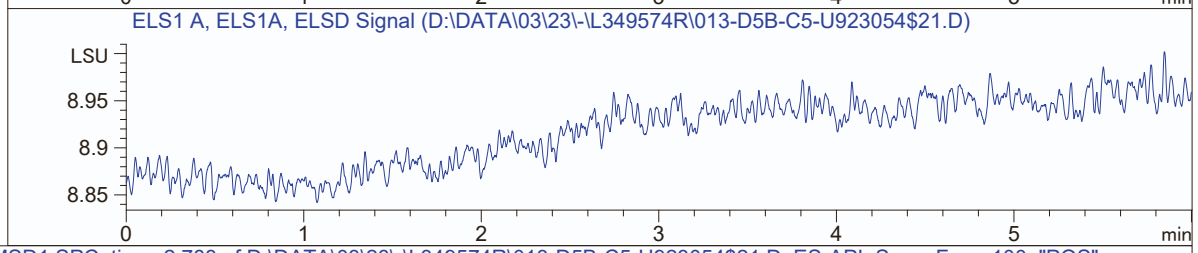
MW01-E12
Z4613897071



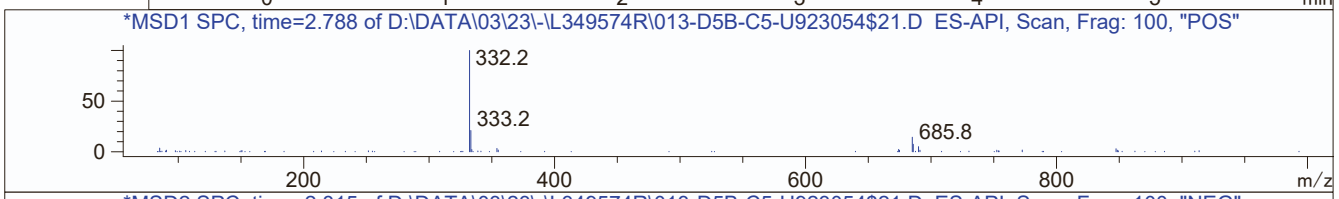




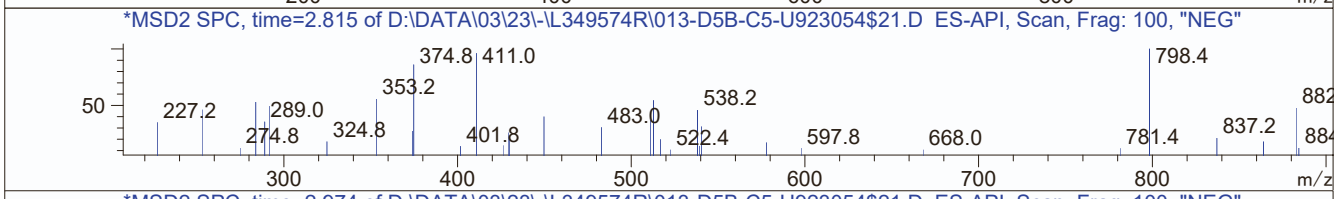




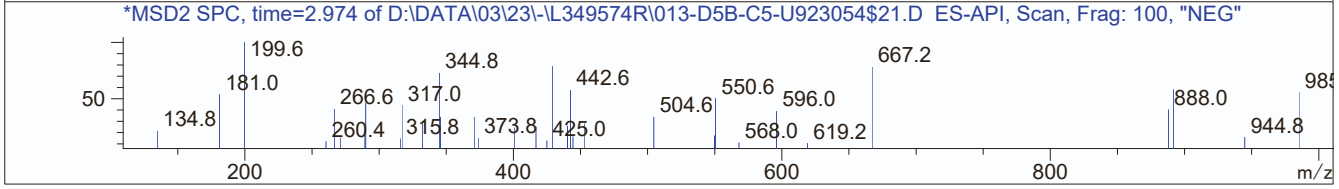
RT 2.787



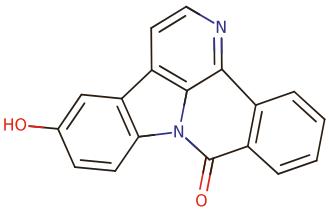
RT 2.815



RT 2.974



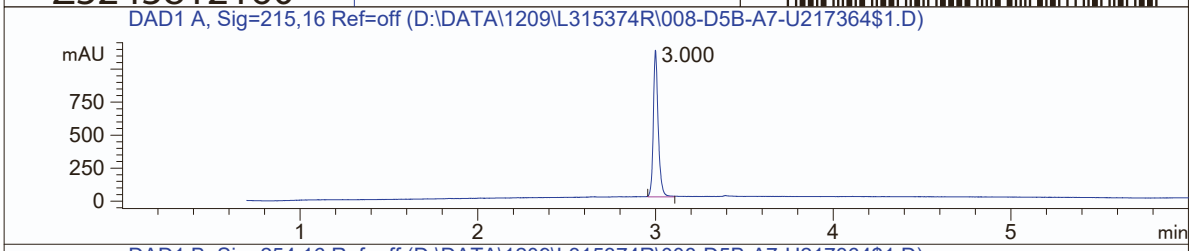
MaxPeak: 100.00%
Ret_Time: 3.000 min

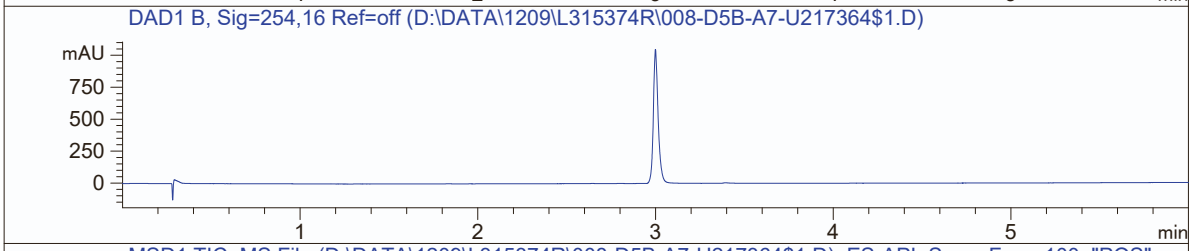


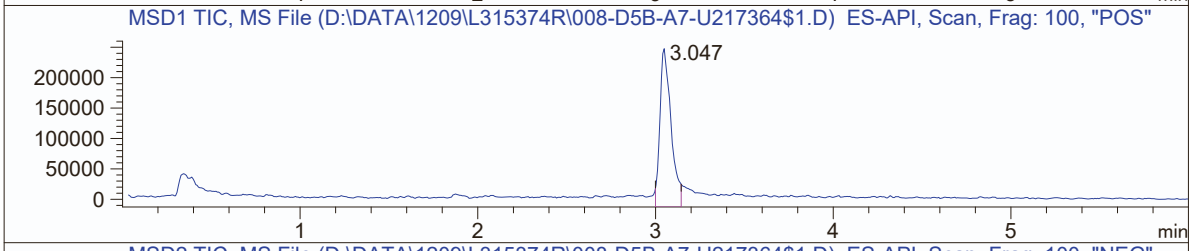
Mol Wt 286.28
Exact Mass 286.08

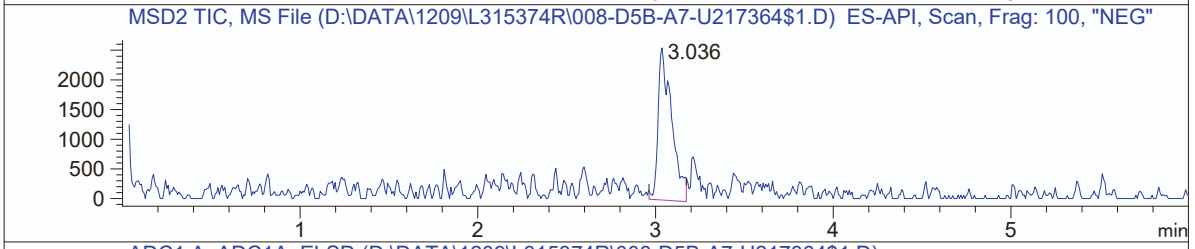
#	Time	Area%
1	3.000	100.00

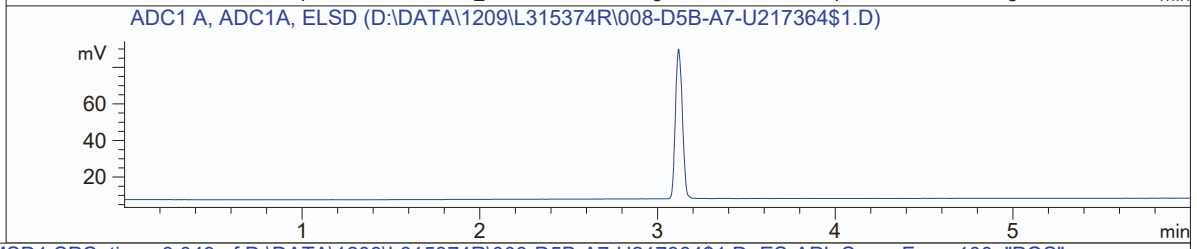
MW01-E13
Z3243812160



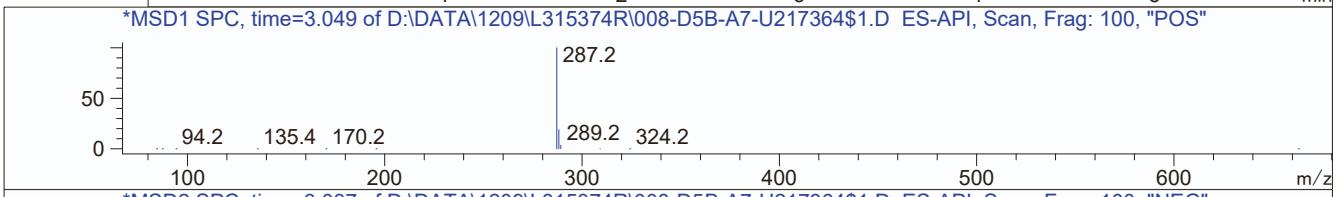




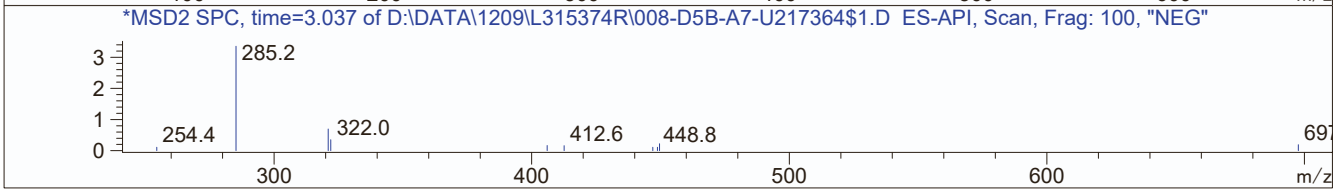




RT 3.047

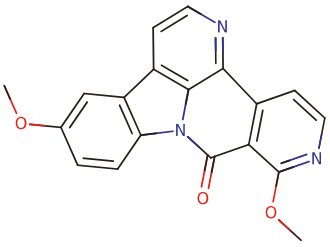


RT 3.036



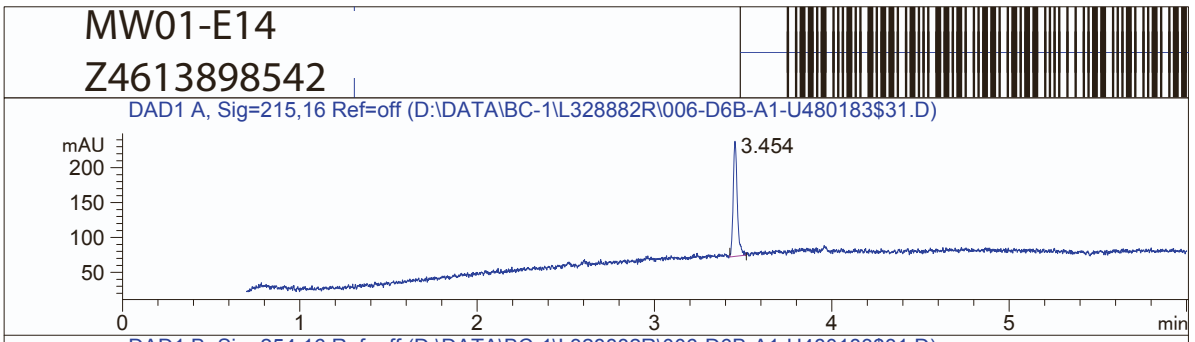
MaxPeak: 100.00%
Ret_Time: 3.454 min

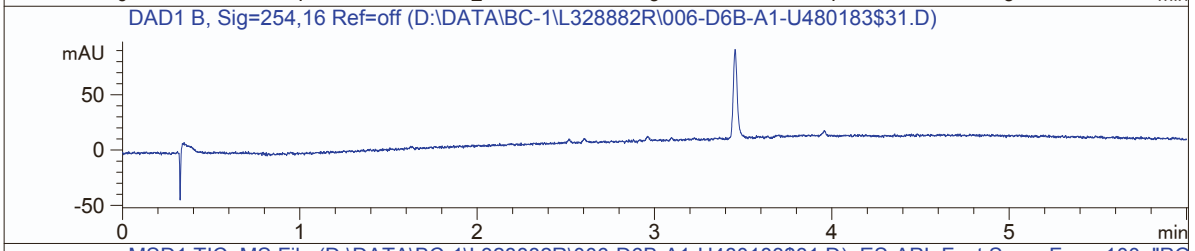
MW01-E14
Z4613898542

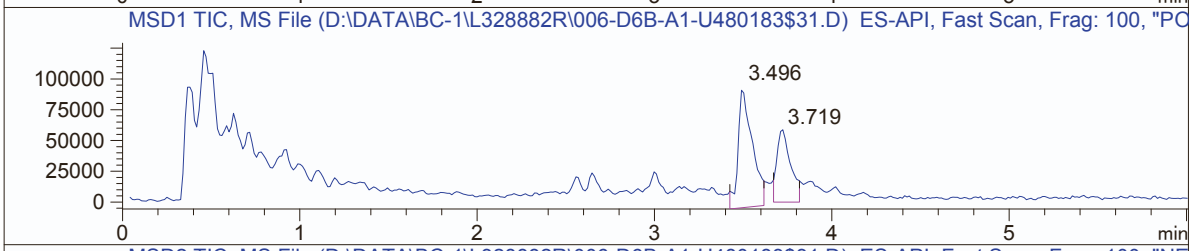


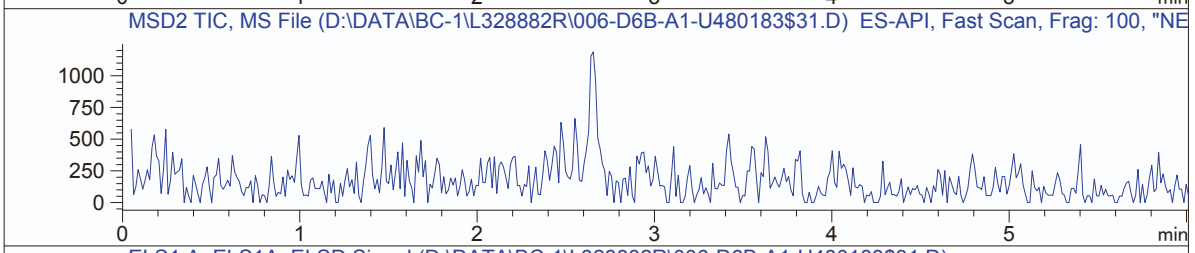
Mol Wt 331.32
Exact Mass 331.1

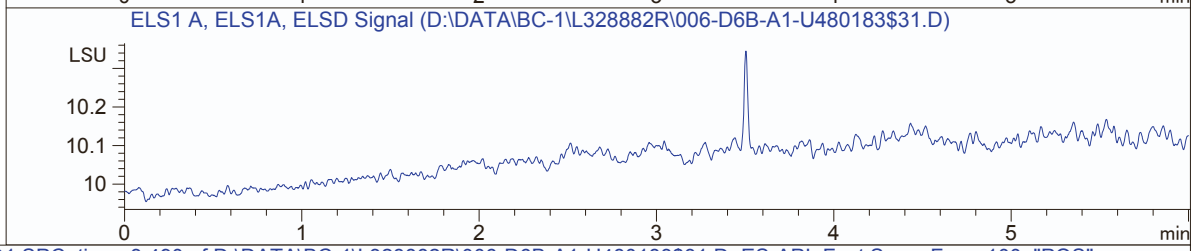
#	Time	Area%
1	3.454	100.00



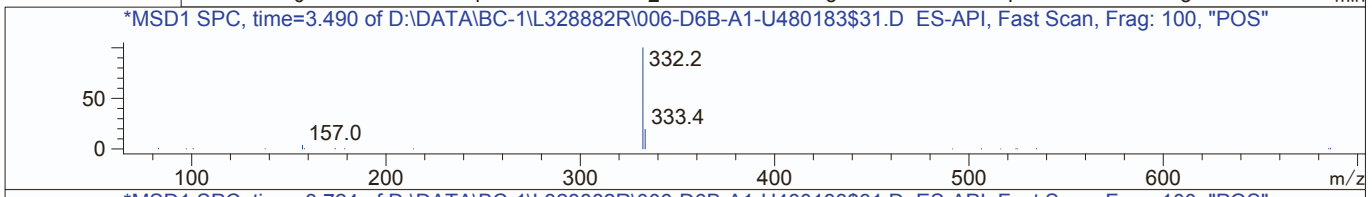




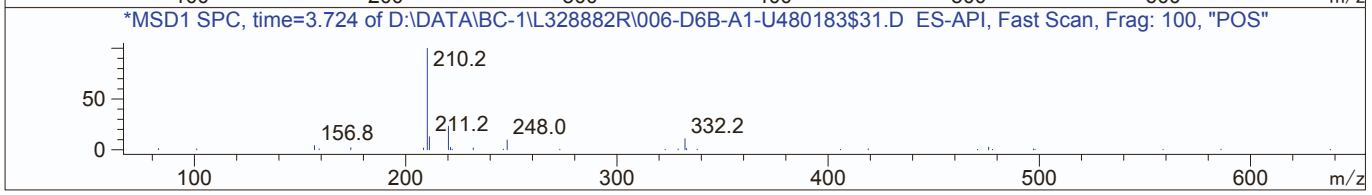




RT 3.496



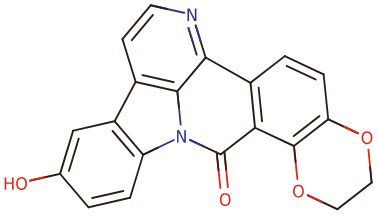
RT 3.719



Inj.Date 1/25/2021 M Acq. Method C:\Users\ -> ->

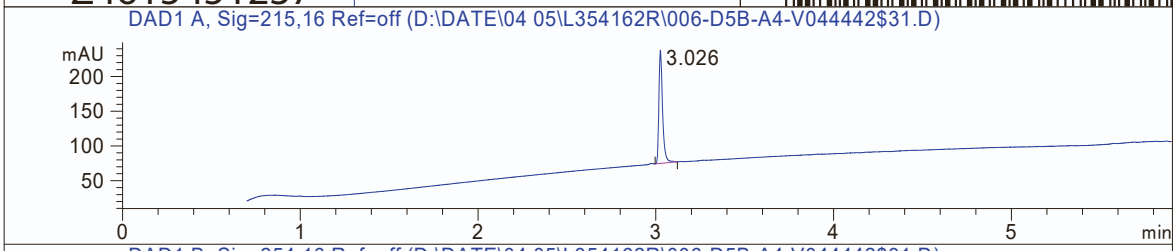
MaxPeak: 100.00%
Ret_Time: 3.026 min

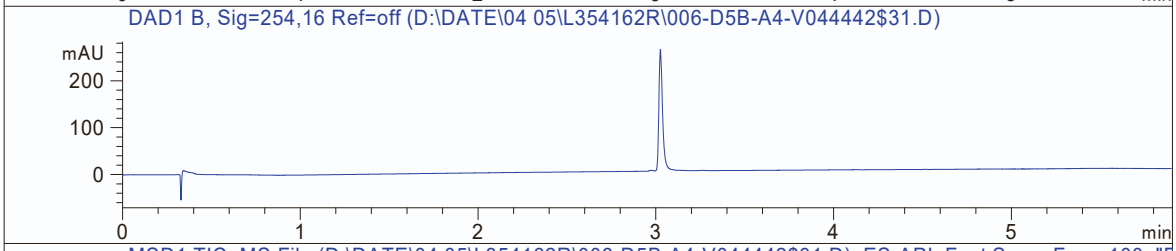
MW01-E15
Z4615451237

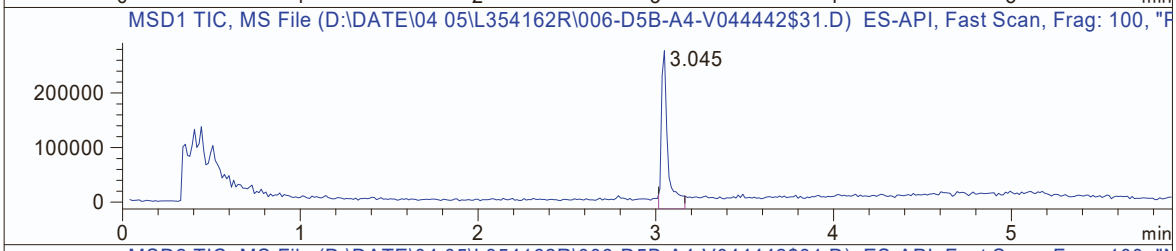


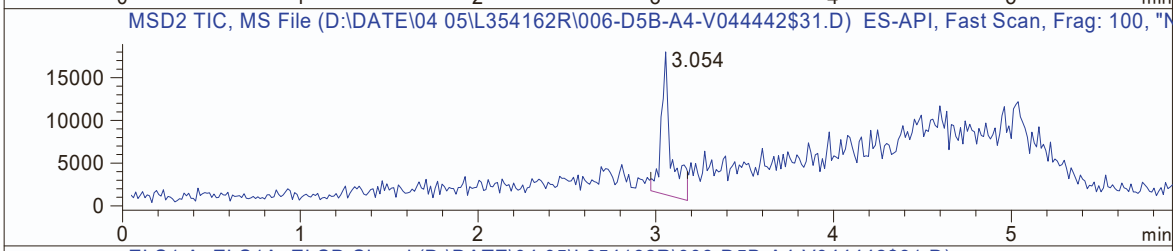
Mol Wt 344.32
Exact Mass 344.08

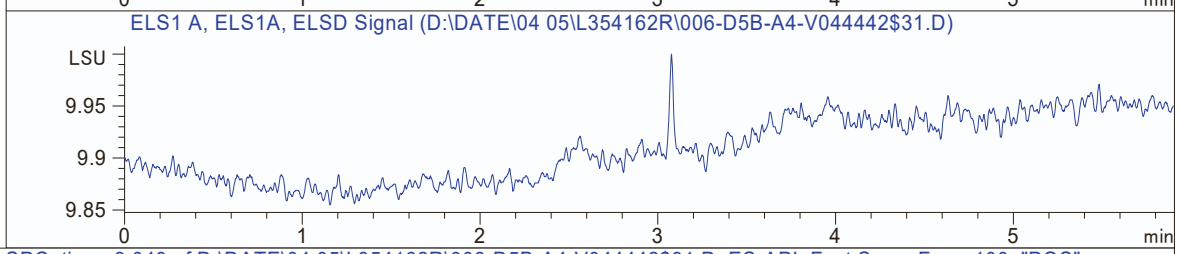
#	Time	Area%
1	3.026	100.00



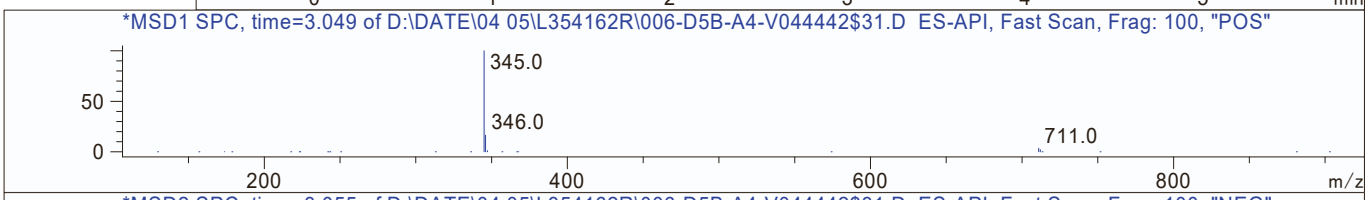




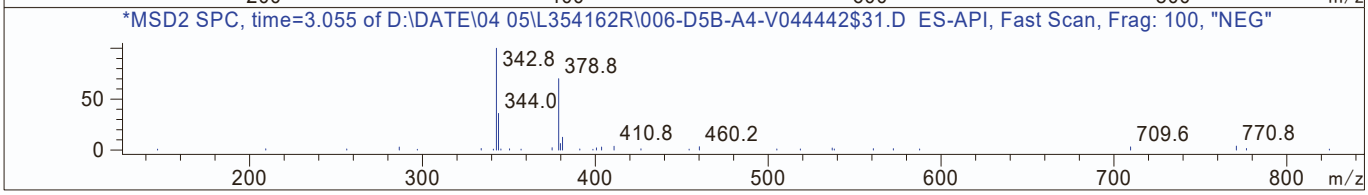




RT 3.045



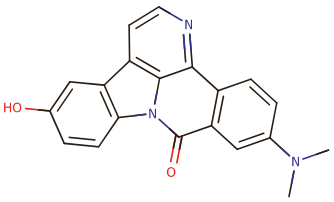
RT 3.054



Inj.Date 4/5/2021 E Acq. Method C:\Users\ -> ->

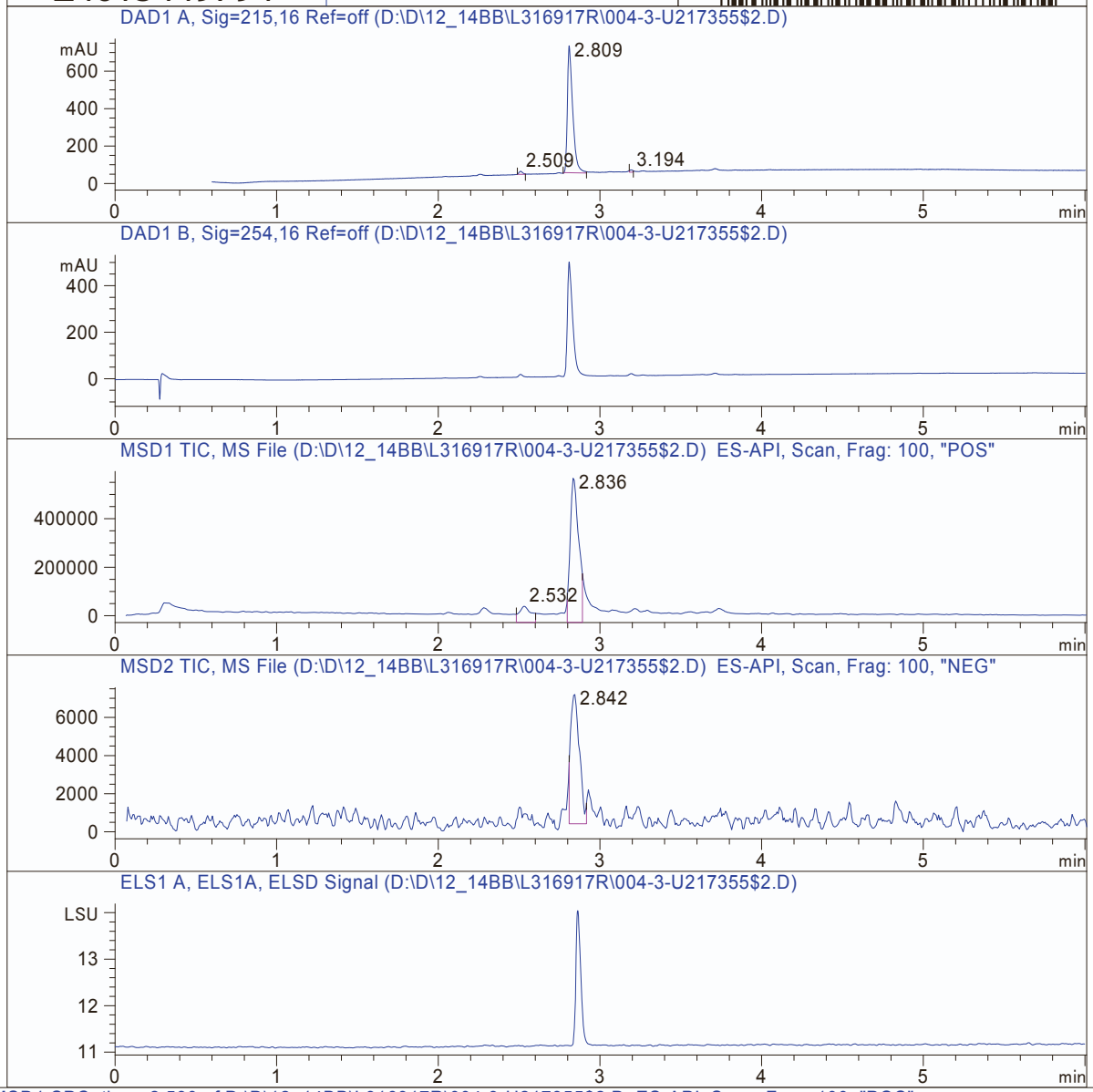
MaxPeak: 97.37%
Ret_Time: 2.809 min

MW01-E16
Z4613449794

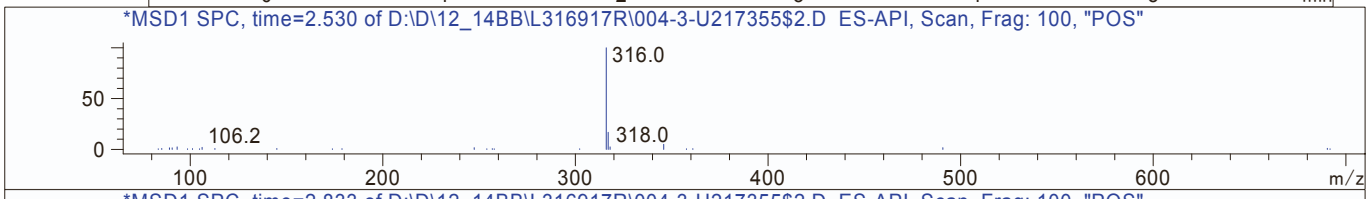


Mol Wt 329.35
Exact Mass 329.13

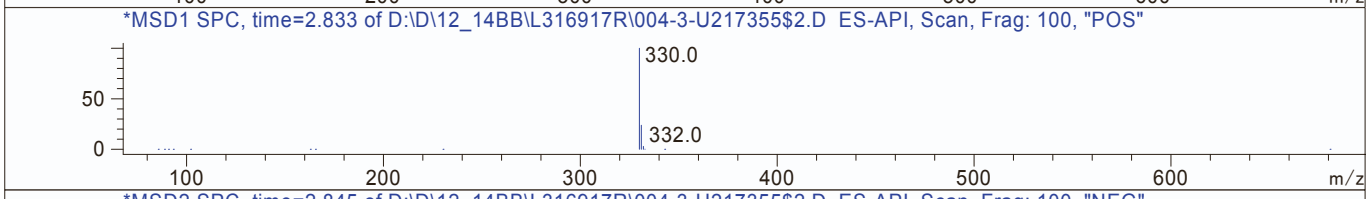
#	Time	Area%
1	2.509	1.66
2	2.809	97.37
3	3.194	0.97



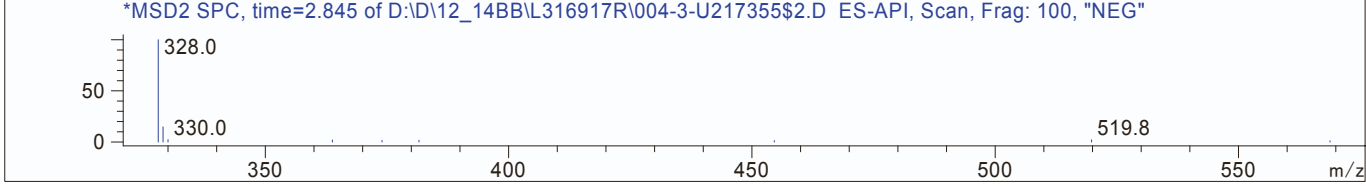
RT 2.532



RT 2.836



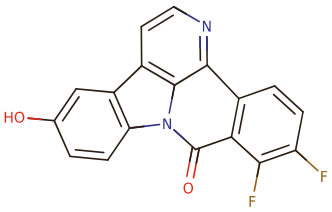
RT 2.842



Inj.Date 12/14/2020 N 13 Acq. Method C:\Chem32\-> ->

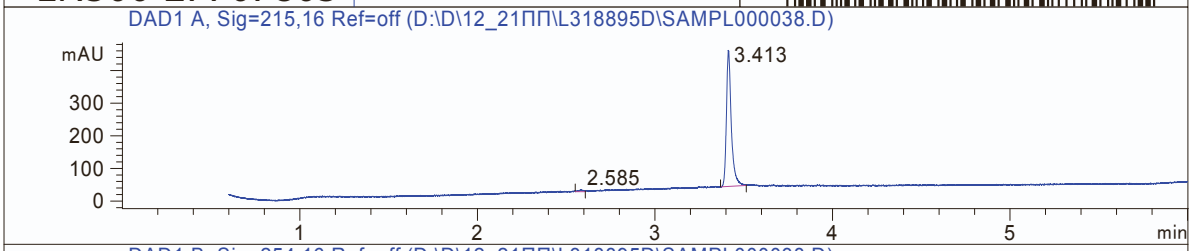
MW01-E17
EN300-27707803

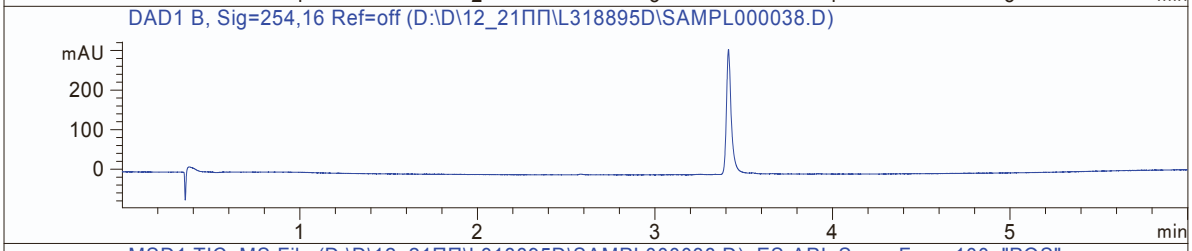
MaxPeak: 98.76%
Ret_Time: 3.413 min

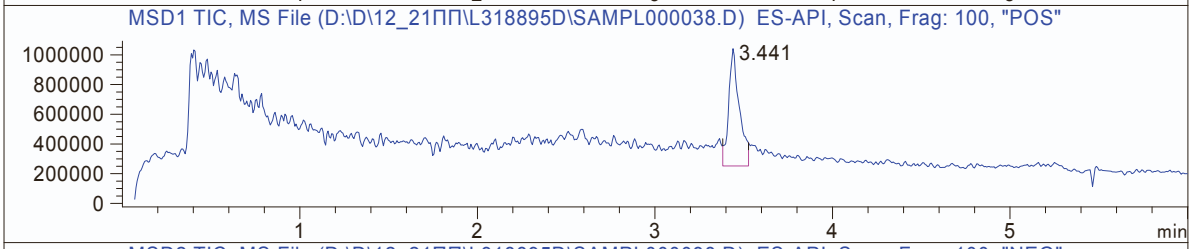


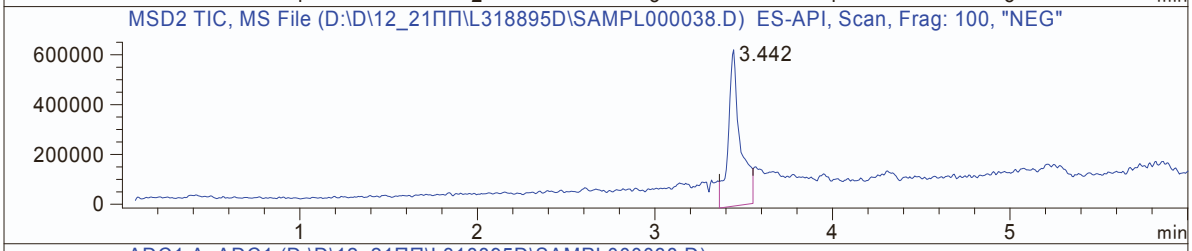
Mol Wt 322.26
Exact Mass 322.06

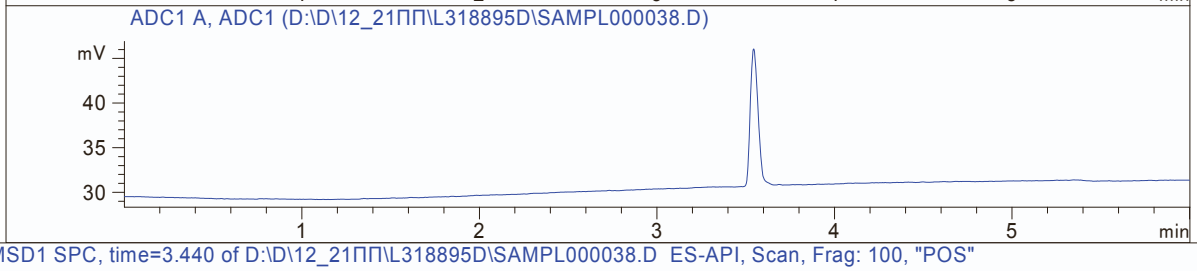
#	Time	Area%
1	2.585	1.24
2	3.413	98.76



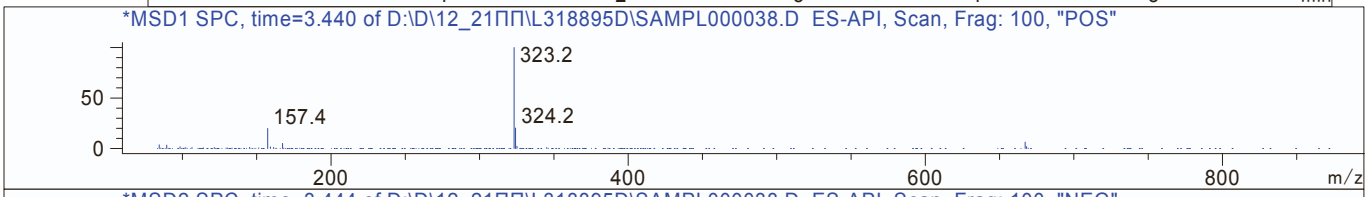




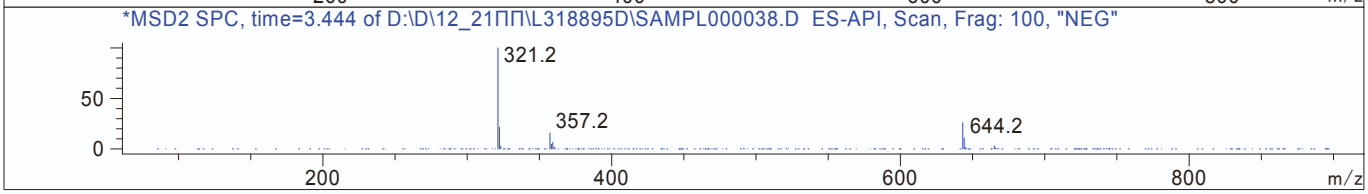




RT 3.441

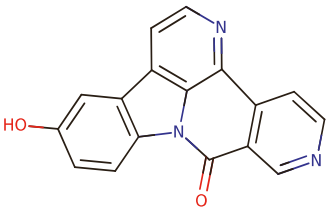


RT 3.442



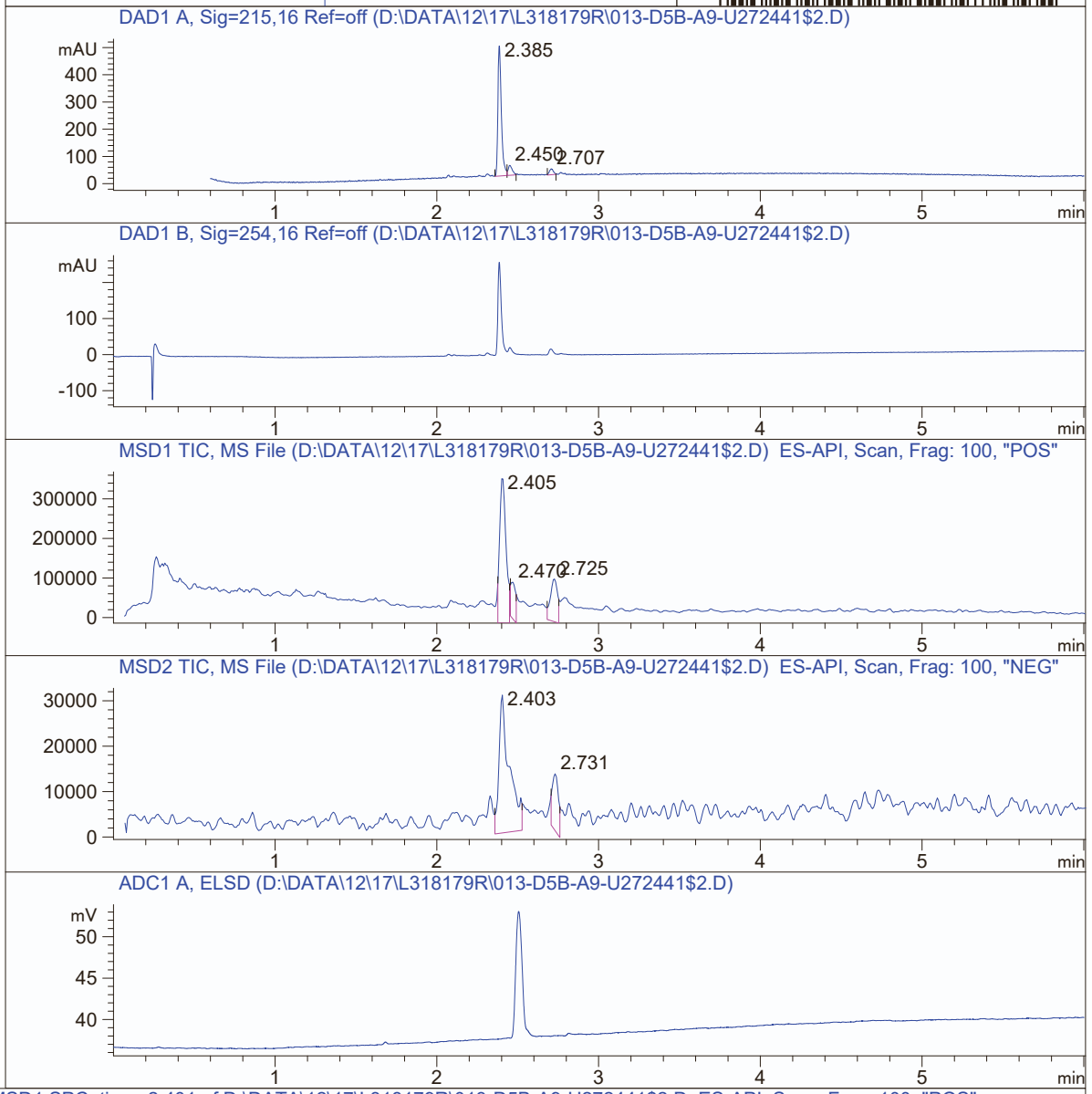
MW01-E18
EN300-27707927

MaxPeak: 87.58%
Ret_Time: 2.385 min

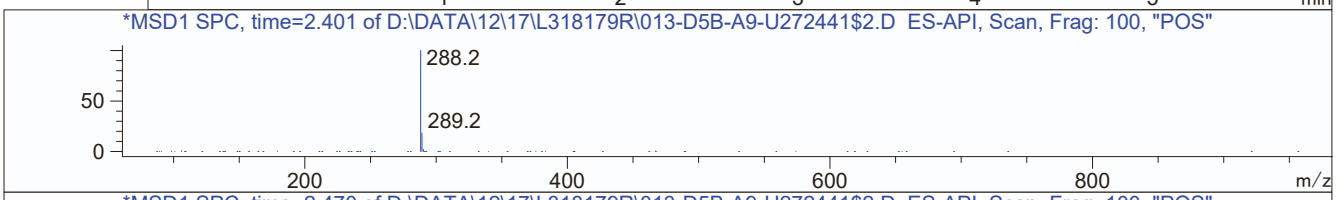


Mol Wt 287.27
Exact Mass 287.07

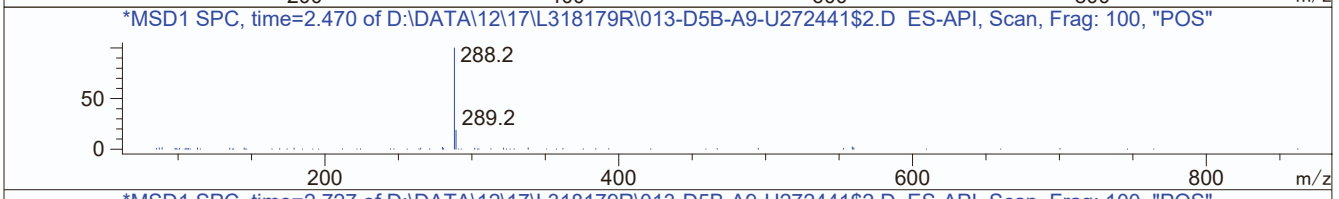
#	Time	Area%
1	2.385	87.58
2	2.450	8.01
3	2.707	4.41



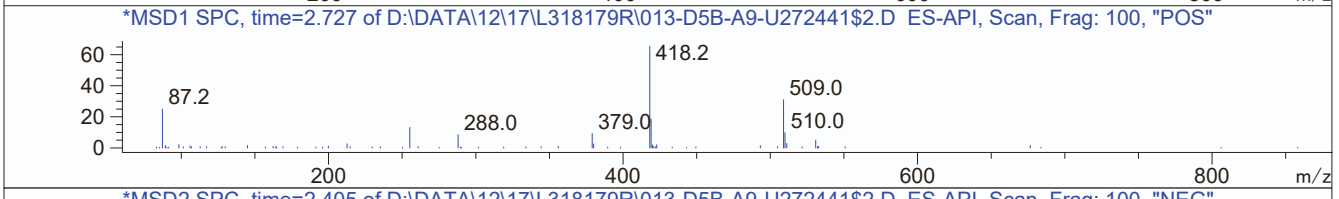
RT 2.405



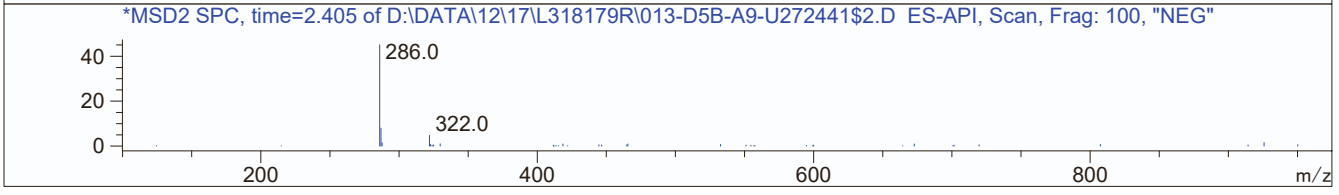
RT 2.470



RT 2.725

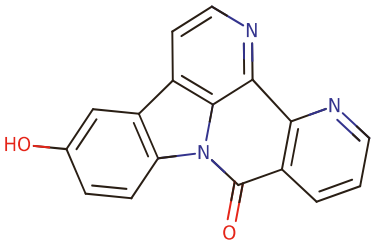


RT 2.403



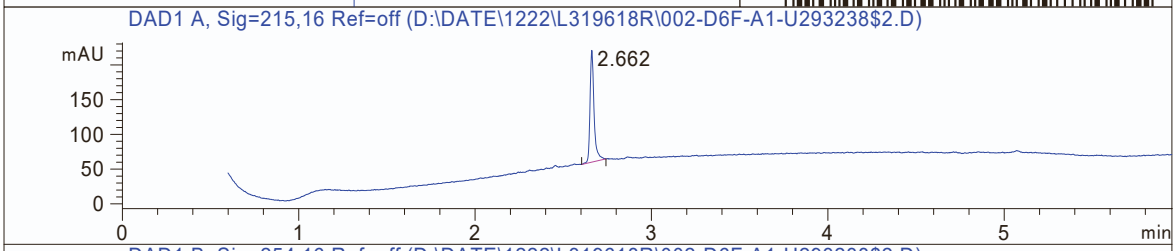
MaxPeak: 100.00%
Ret_Time: 2.662 min

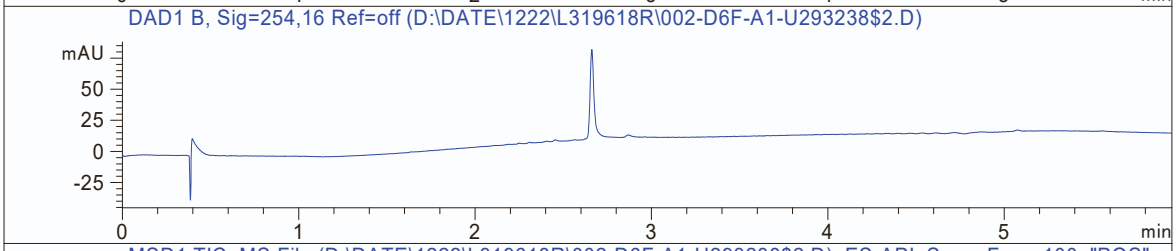
MW01-E19
EN300-27716224

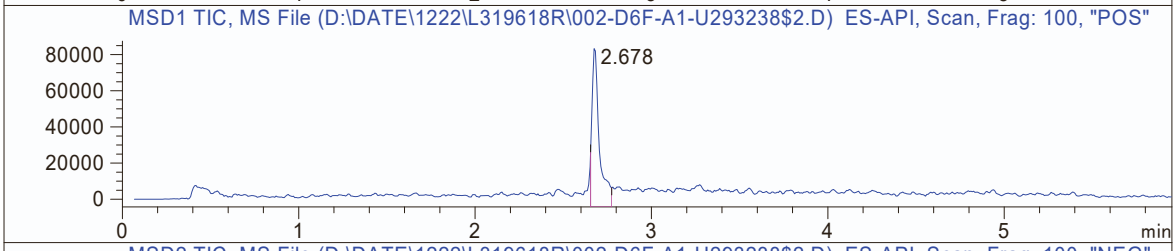


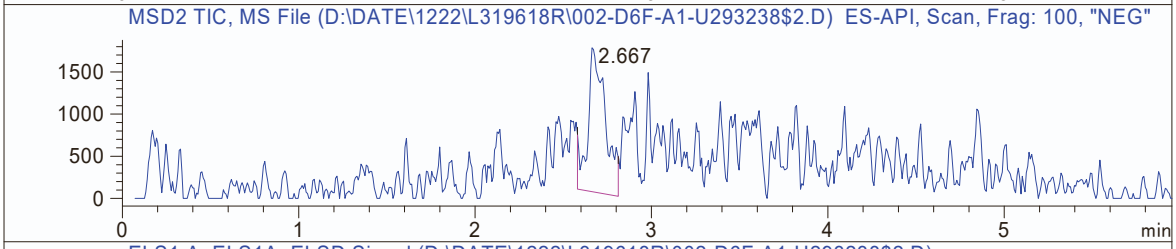
Mol Wt 287.27
Exact Mass 287.07

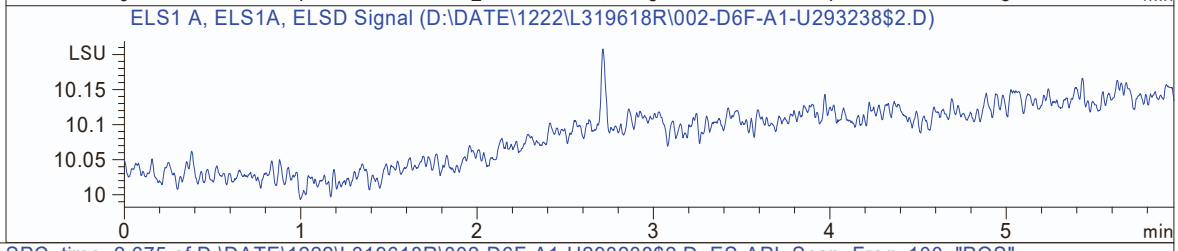
#	Time	Area%
1	2.662	100.00



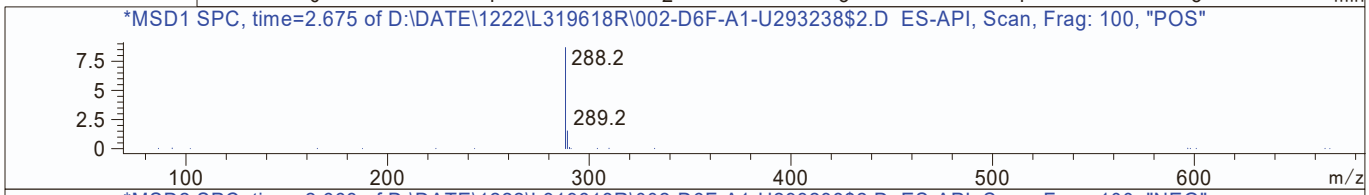




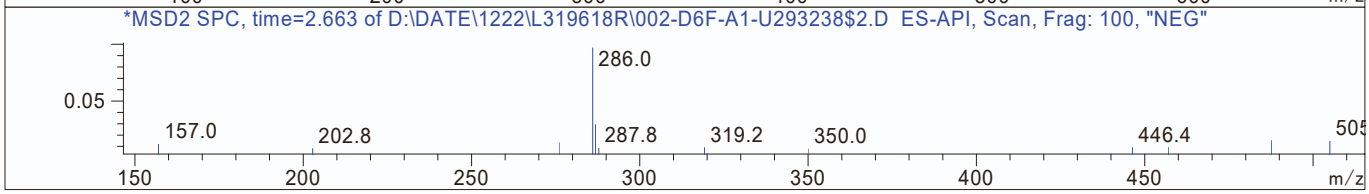




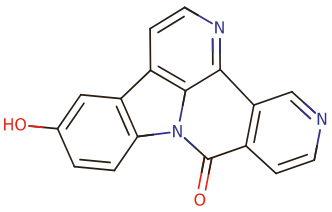
RT 2.678



RT 2.667



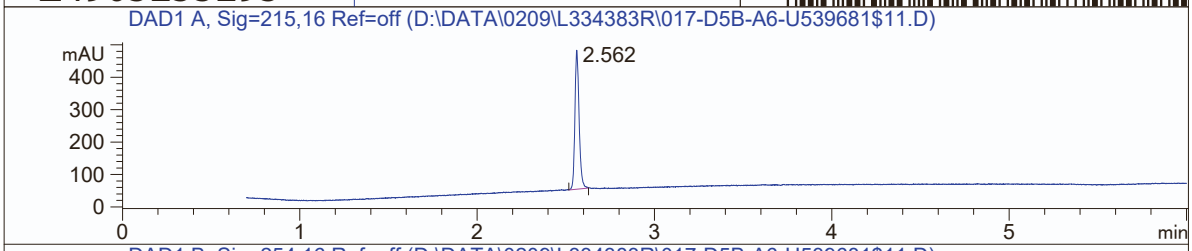
MaxPeak: 100.00%
Ret_Time: 2.562 min

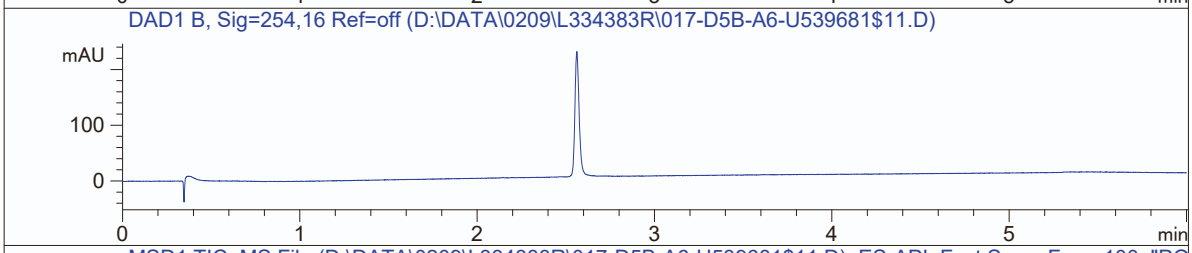


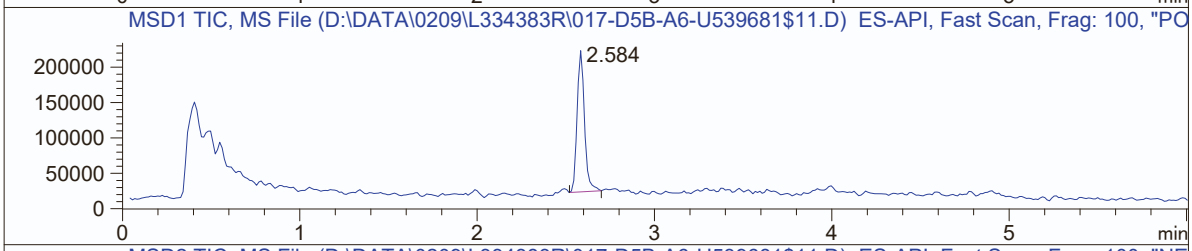
Mol Wt 287.27
Exact Mass 287.07

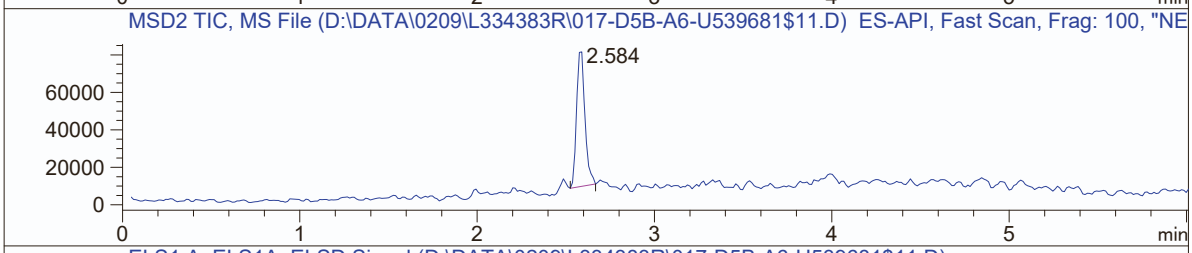
#	Time	Area%
1	2.562	100.00

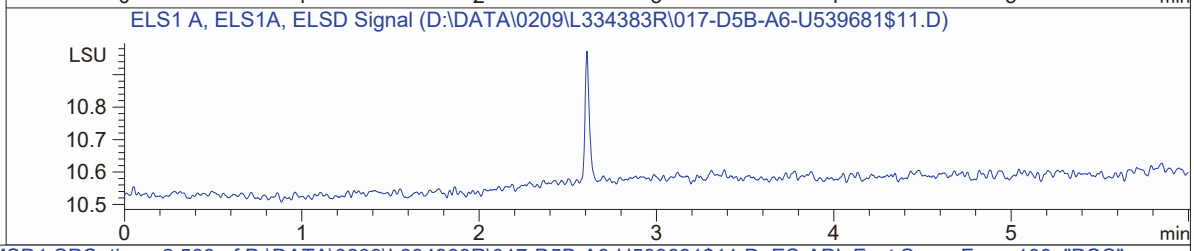
MW01-E20
Z4905255295



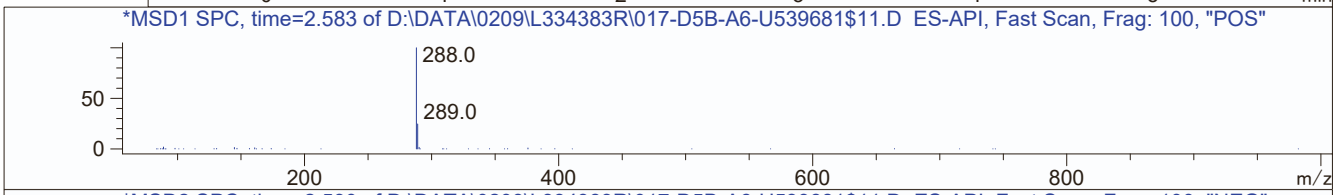




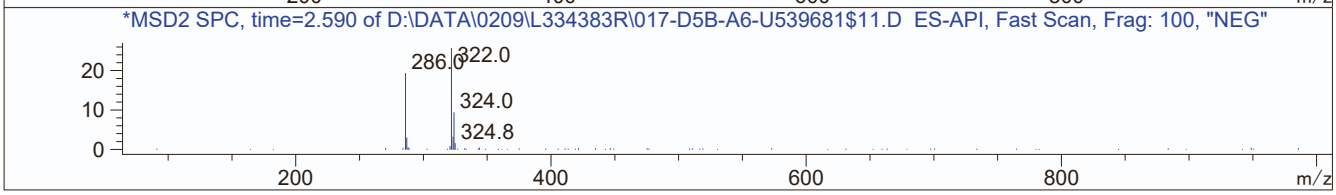




RT 2.584



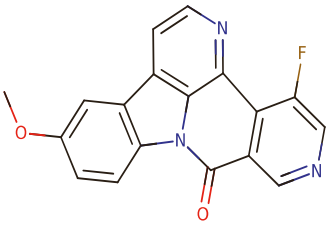
RT 2.584



Inj.Date 2/9/2021 OA Acq. Method C:\Users\ -> ->

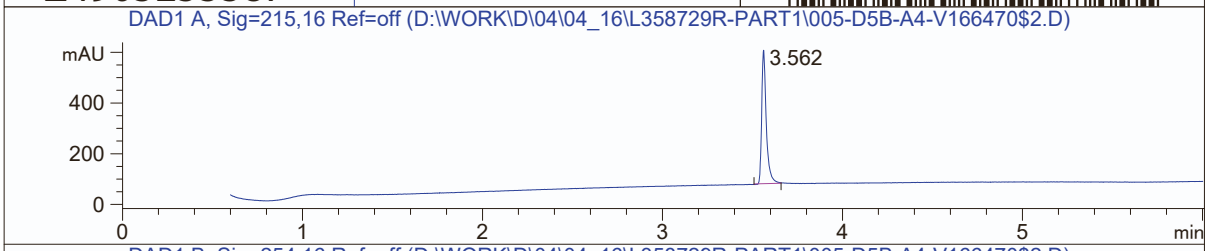
MaxPeak: 100.00%
Ret_Time: 3.562 min

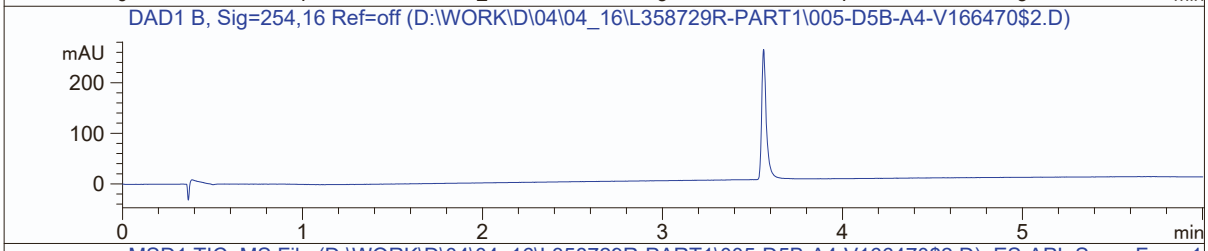
MW01-E21
Z4905255587

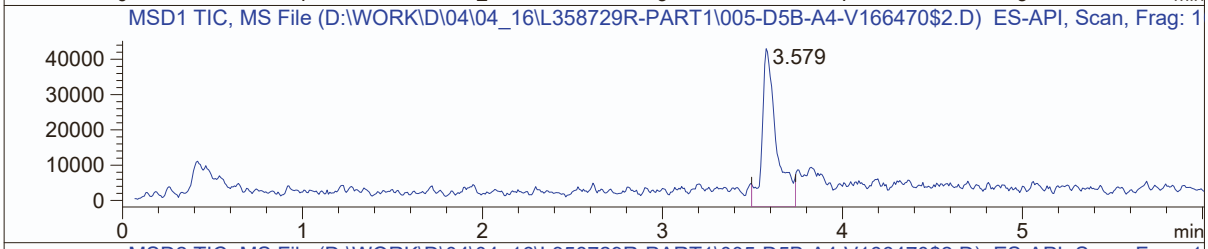


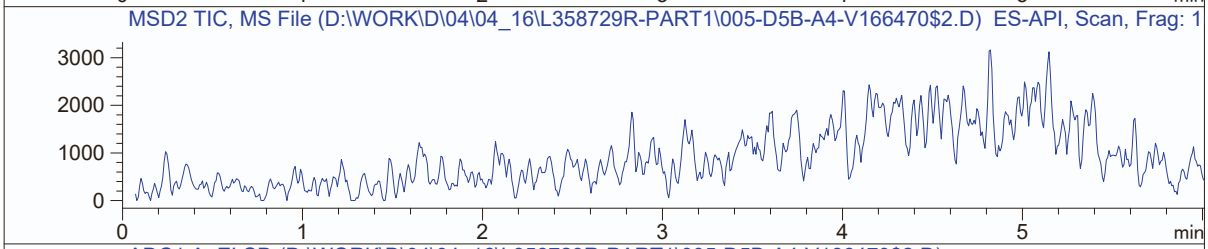
Mol Wt **319.29**
Exact Mass **319.08**

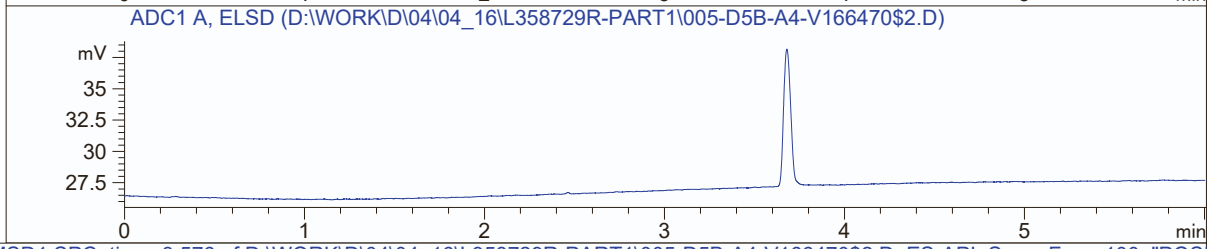
#	Time	Area%
1	3.562	100.00



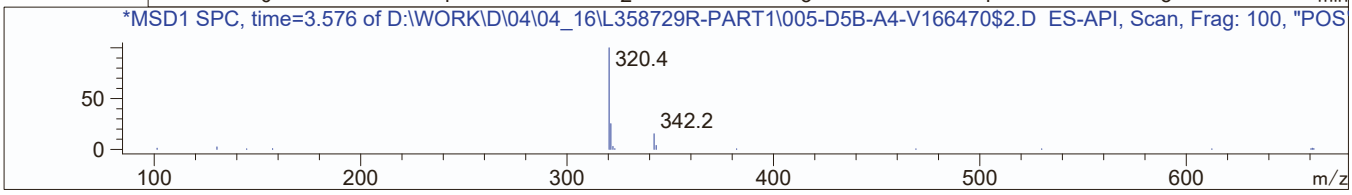




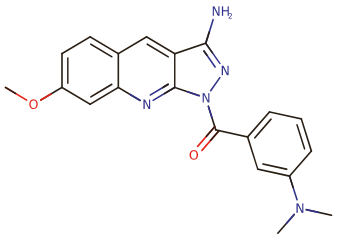




RT 3.579



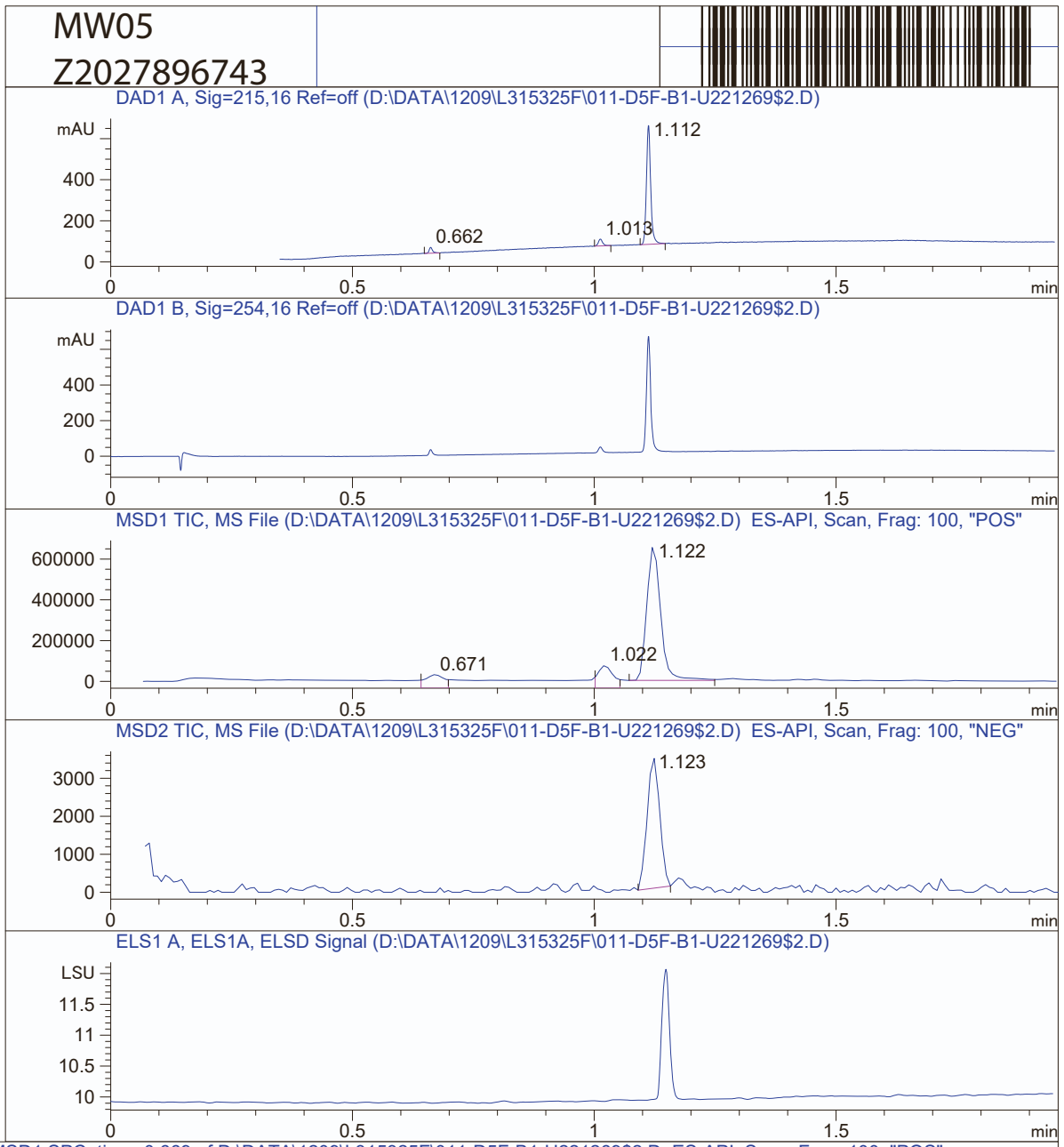
MaxPeak: 91.02%
Ret_Time: 1.112 min



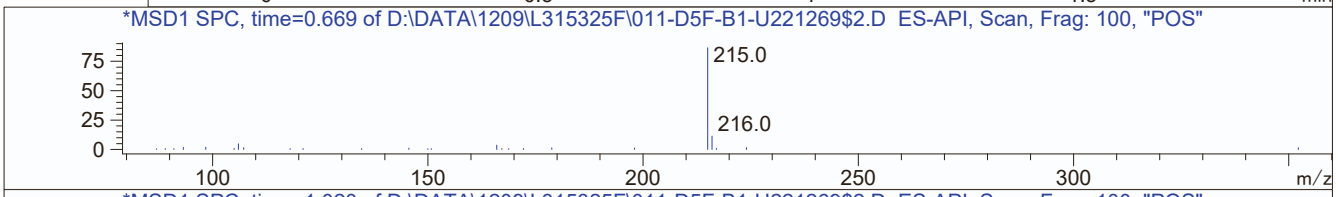
Mol Wt 361.4
Exact Mass 361.17

#	Time	Area%
1	0.662	3.70
2	1.013	5.29
3	1.112	91.02

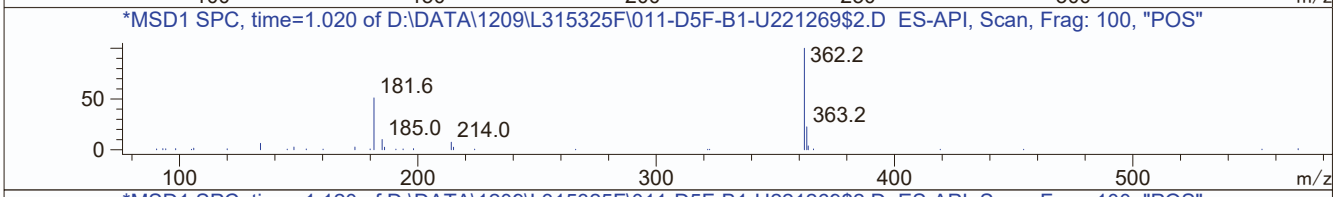
MW05
Z2027896743



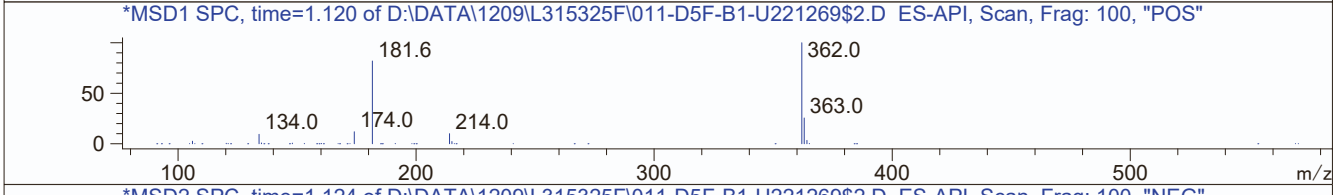
RT 0.671



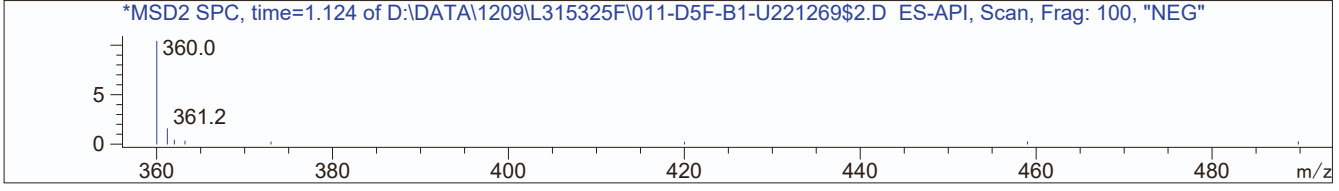
RT 1.022



RT 1.122

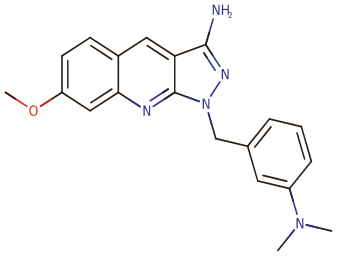


RT 1.123



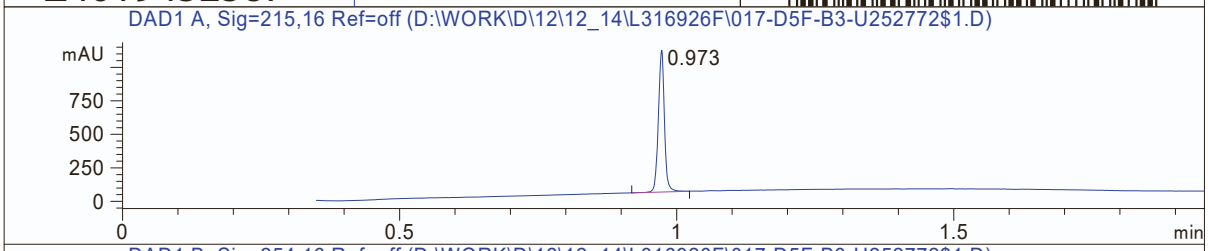
MaxPeak: 100.00%
Ret_Time: 0.973 min

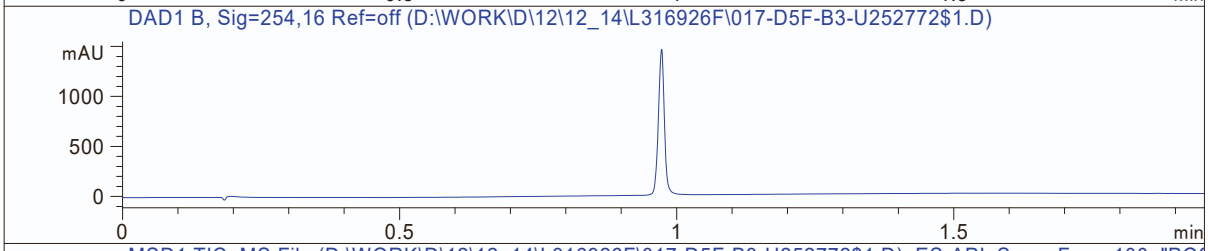
MW05-E1
Z4619432587

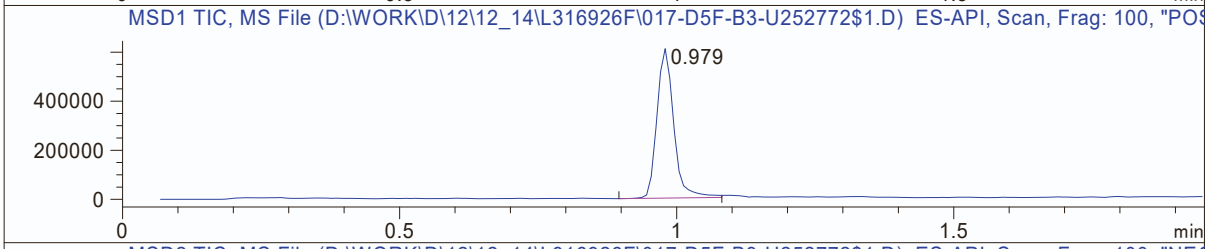


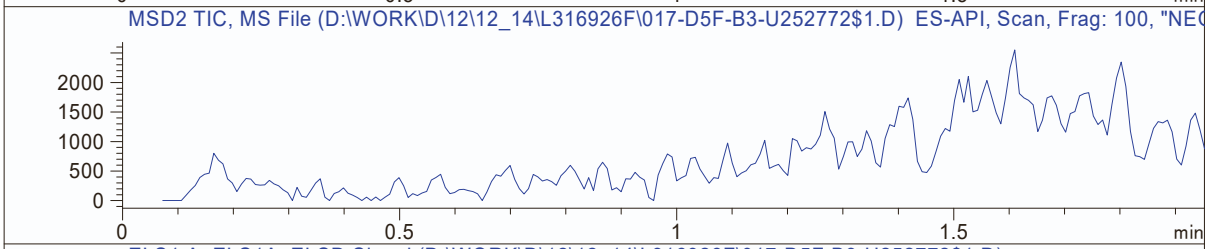
Mol Wt 347.41
Exact Mass 347.2

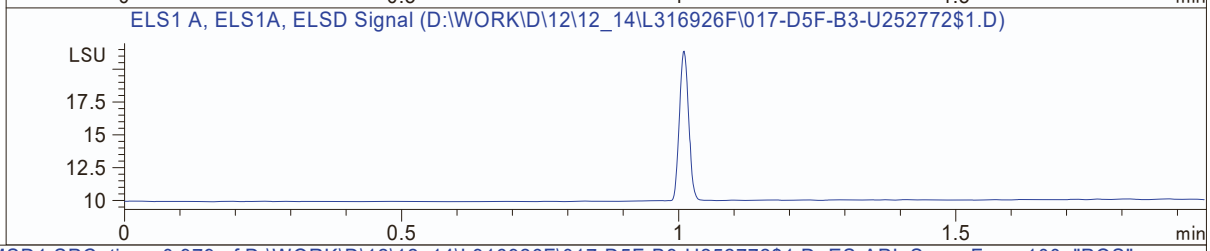
#	Time	Area%
1	0.973	100.00



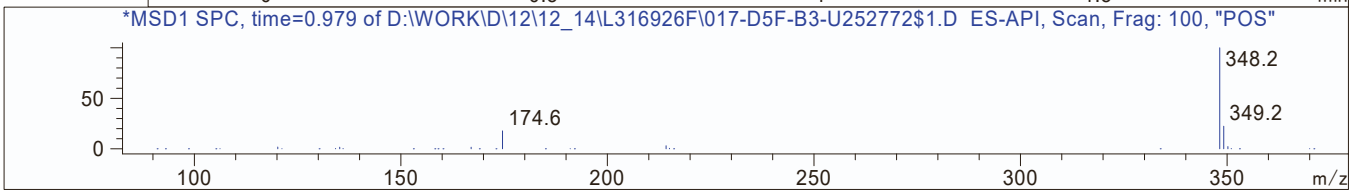




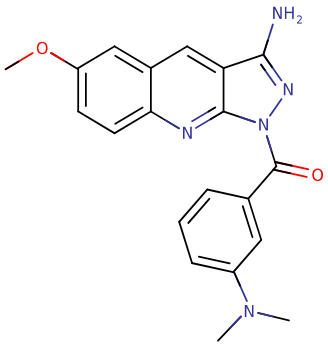




RT 0.979



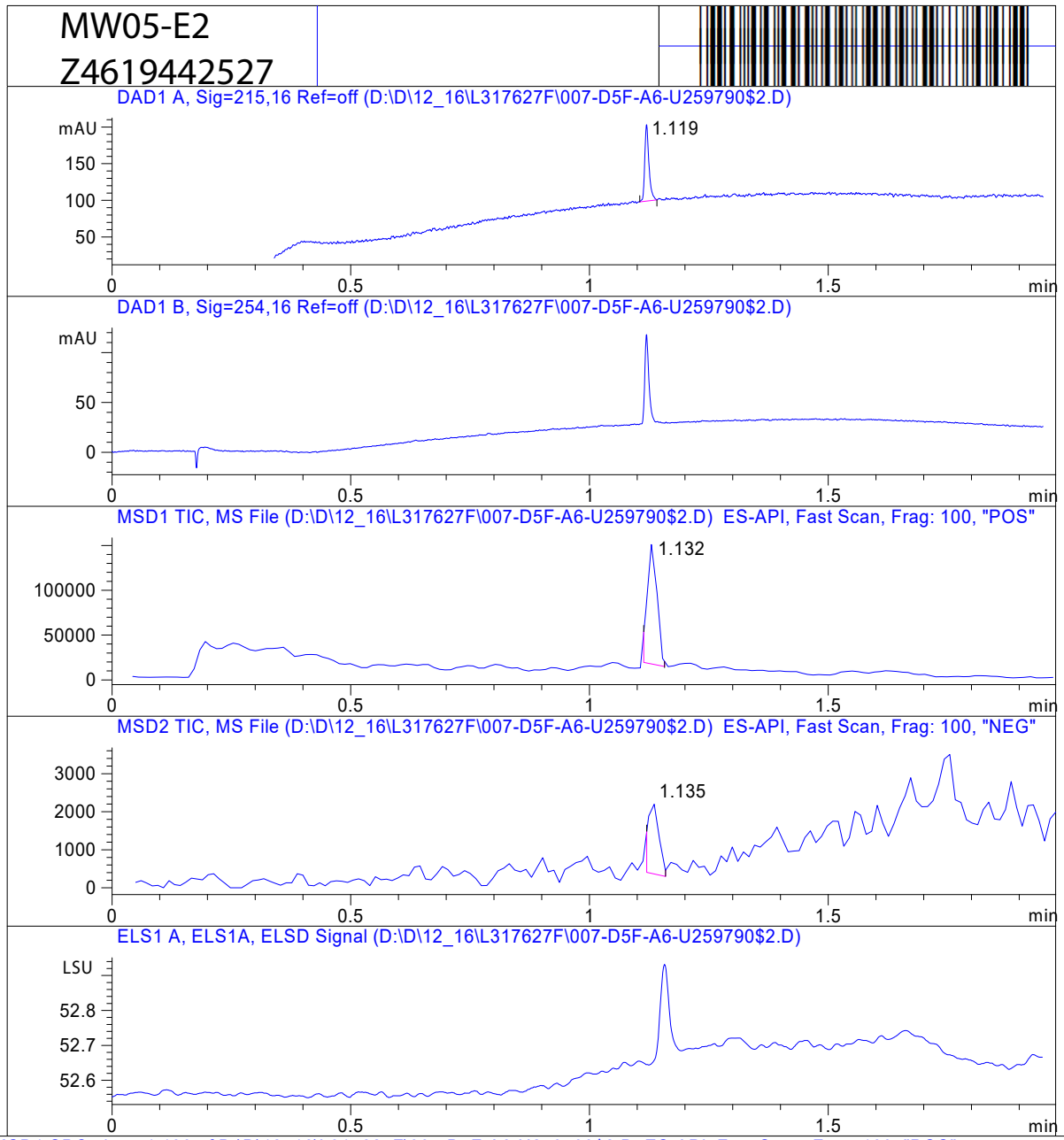
MaxPeak: 100.00%
Ret_Time: 1.119 min



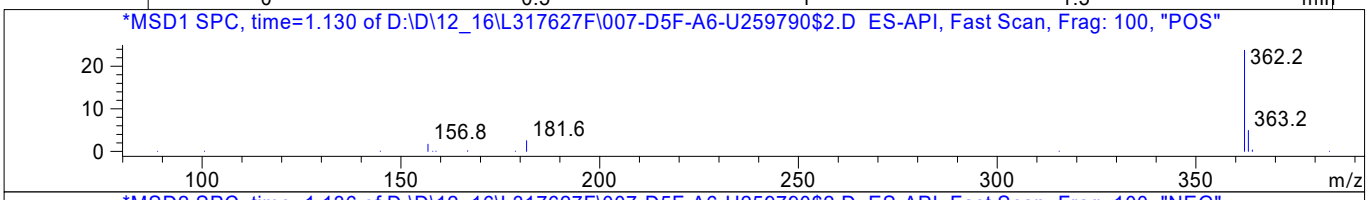
Mol Wt 361.4
Exact Mass 361.17

#	Time	Area%
1	1.119	100.00

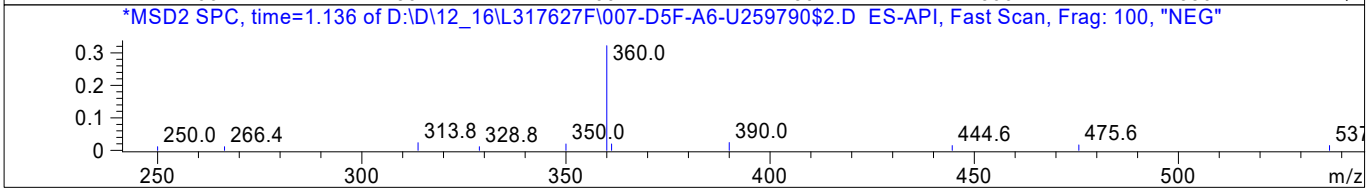
MW05-E2
Z4619442527



RT 1.132

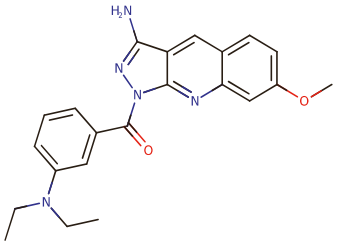


RT 1.135



Inj.Date 12/16/2020 N Acq. Method C:\Users\ -> ->

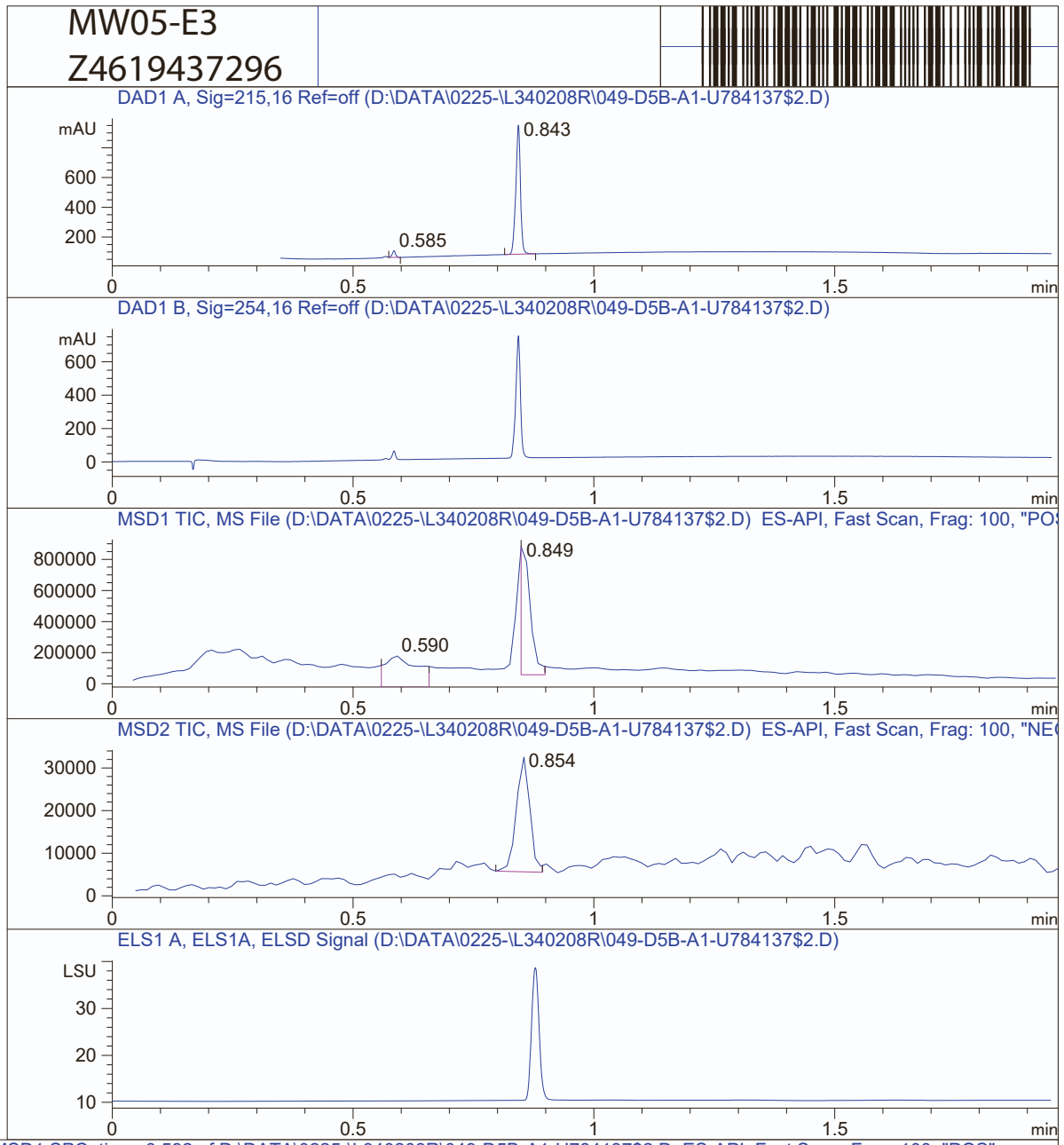
MaxPeak: 96.59%
Ret_Time: 0.843 min



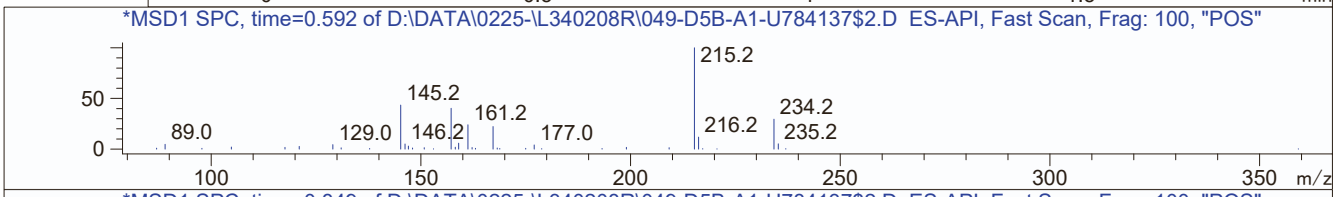
Mol Wt 389.45
Exact Mass 389.21

#	Time	Area%
1	0.585	3.41
2	0.843	96.59

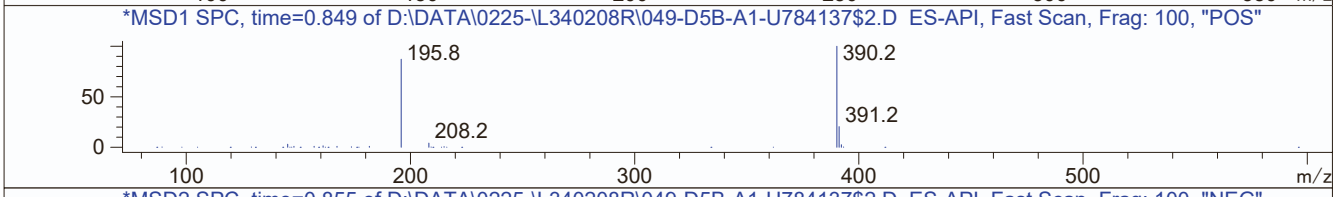
MW05-E3
Z4619437296



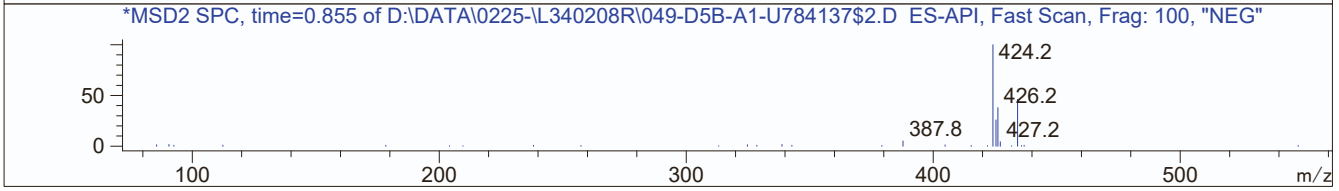
RT 0.590



RT 0.849

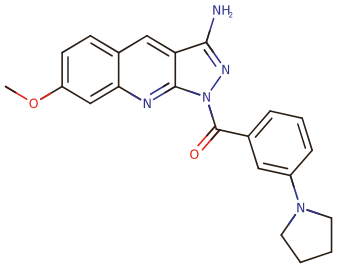


RT 0.854



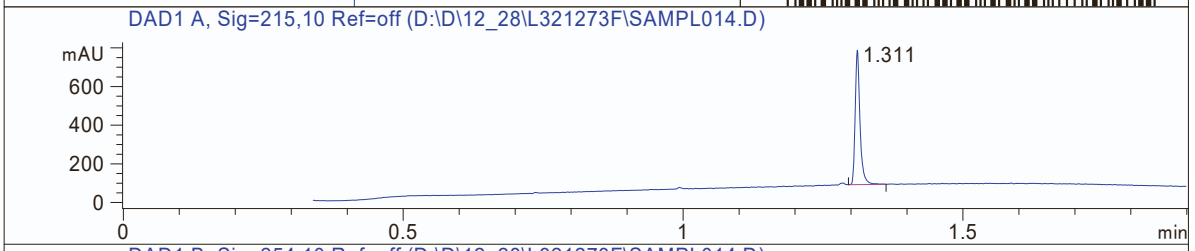
MaxPeak: 100.00%
Ret_Time: 1.311 min

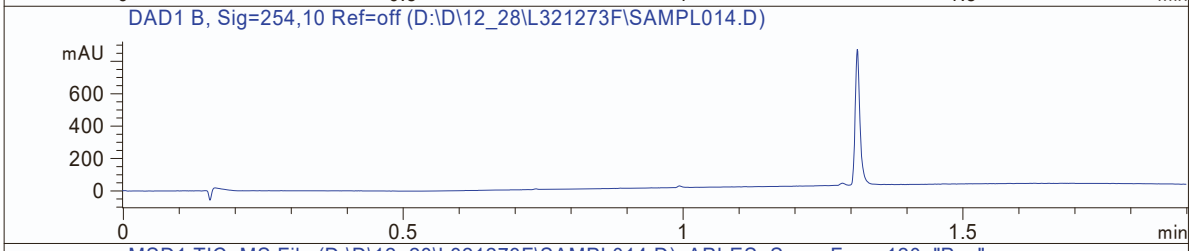
MW05-E4
Z4619437299

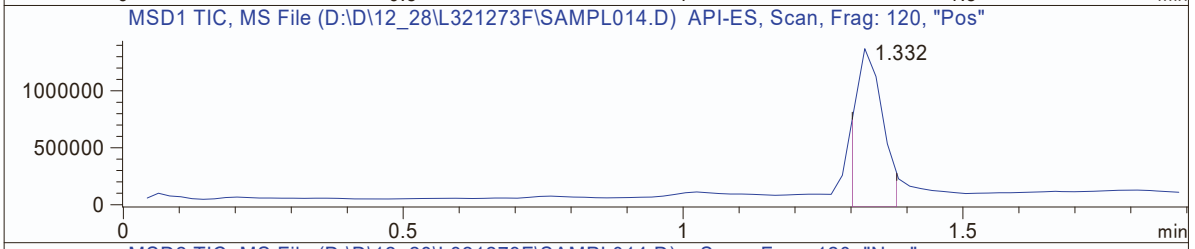


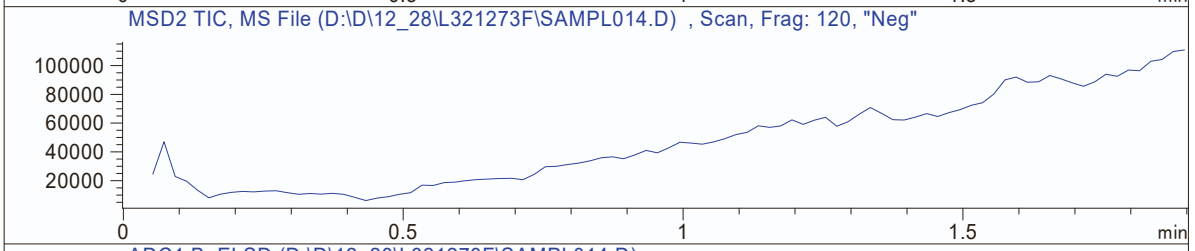
Mol Wt 387.43
Exact Mass 387.19

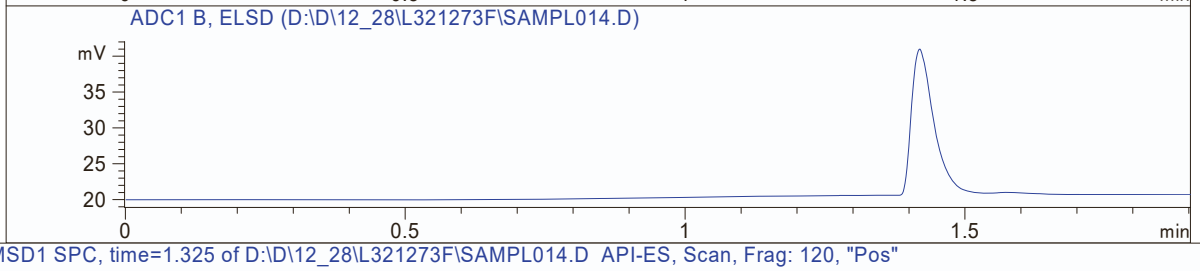
#	Time	Area%
1	1.311	100.00



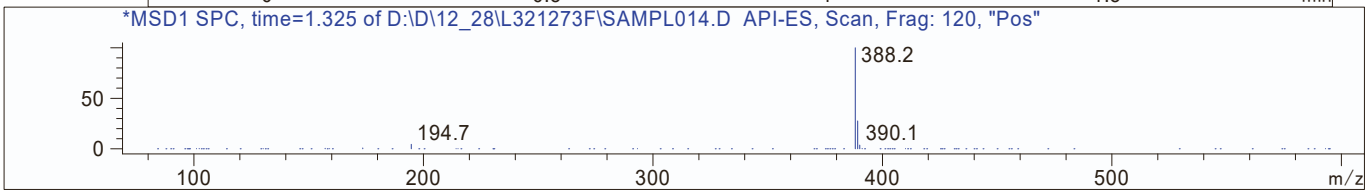








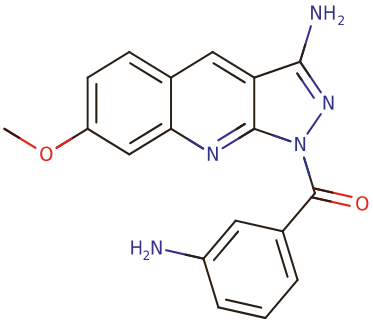
RT 1.332



Inj.Date 12/28/2020 N -SL- Acq. Method C:\HPCHEM\-> ->

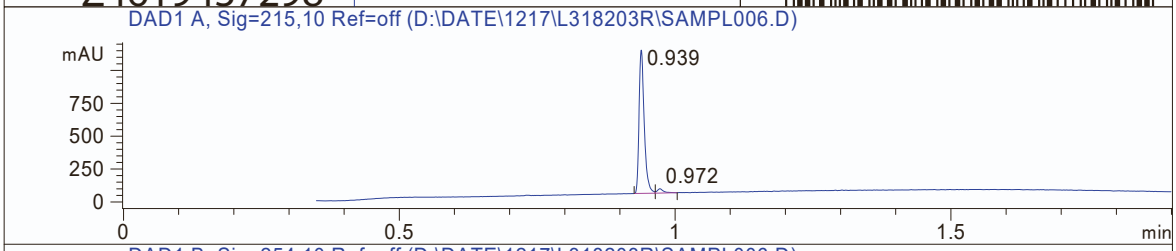
MaxPeak: 96.51%
Ret_Time: 0.939 min

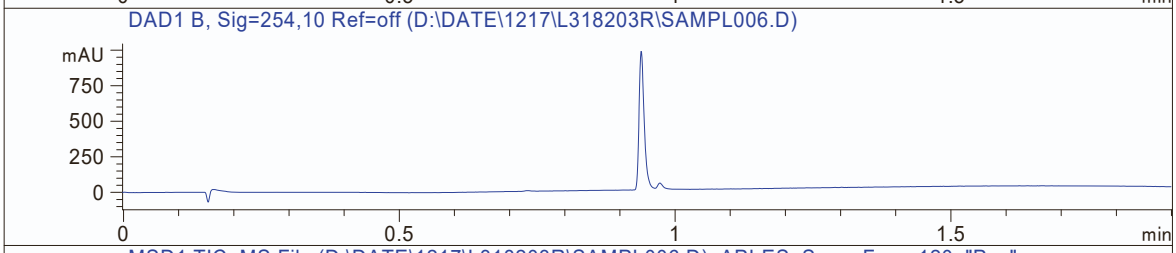
MW05-E5
Z4619437298

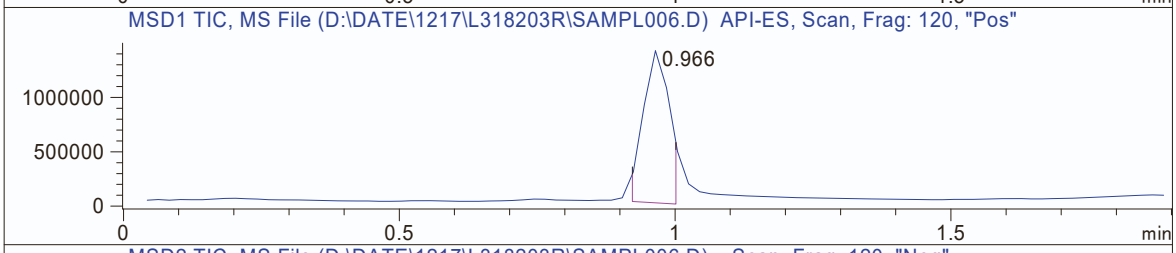


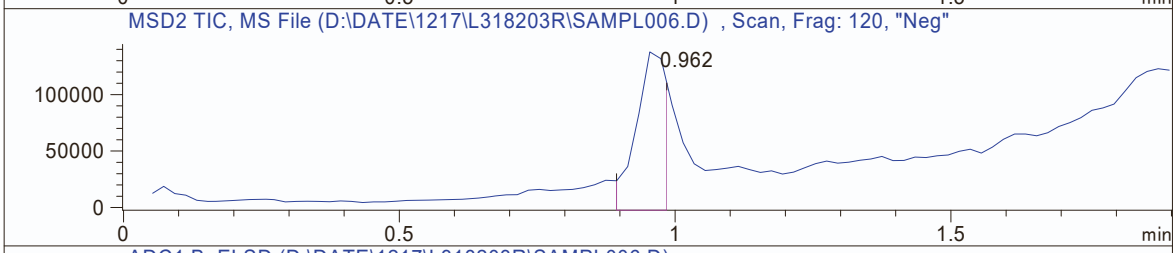
Mol Wt 333.34
Exact Mass 333.13

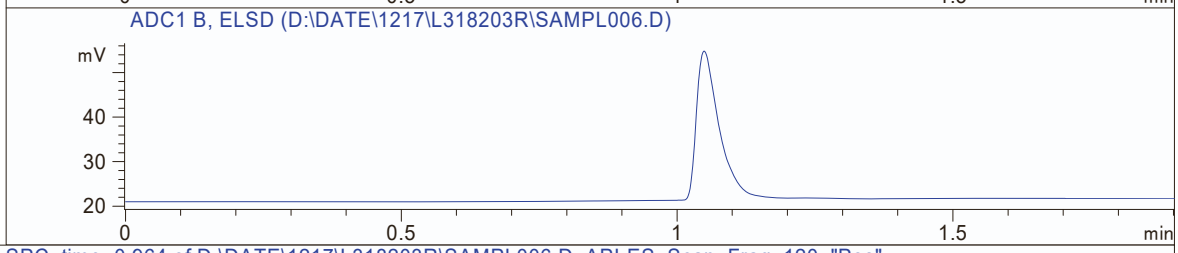
#	Time	Area%
1	0.939	96.51
2	0.972	3.49



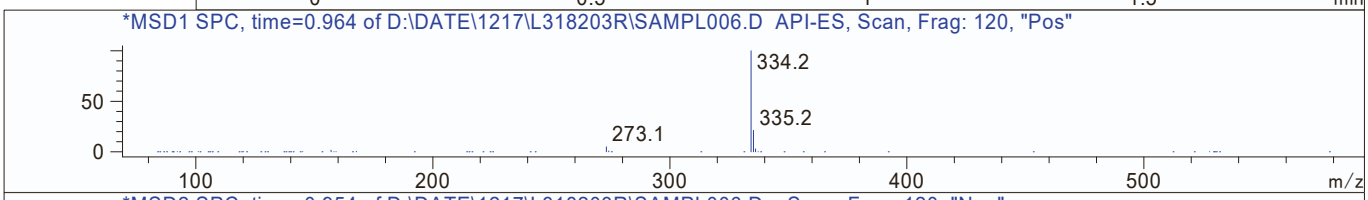




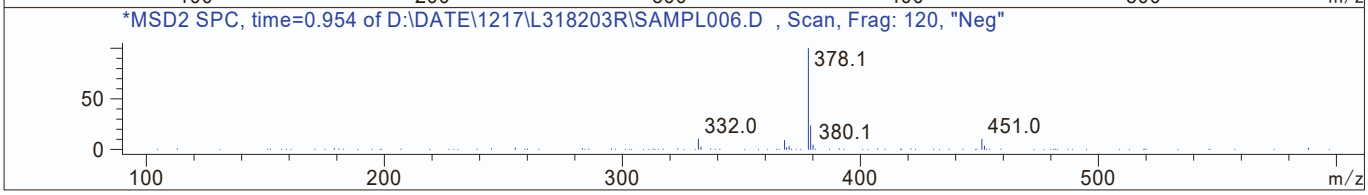




RT 0.966



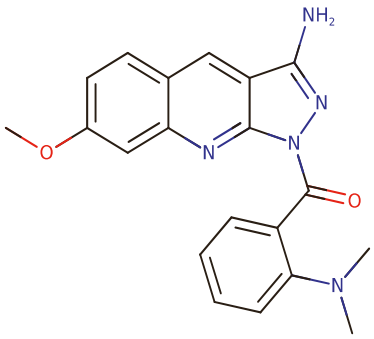
RT 0.962



Inj.Date 12/18/2020 E -SL- Acq. Method C:\HPCHEM\-> ->

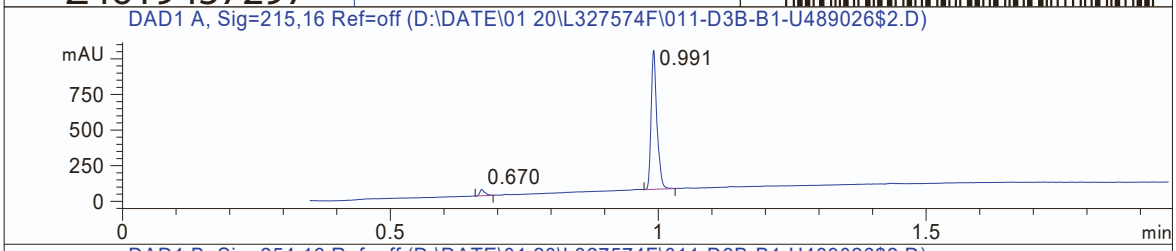
MaxPeak: 96.16%
Ret_Time: 0.991 min

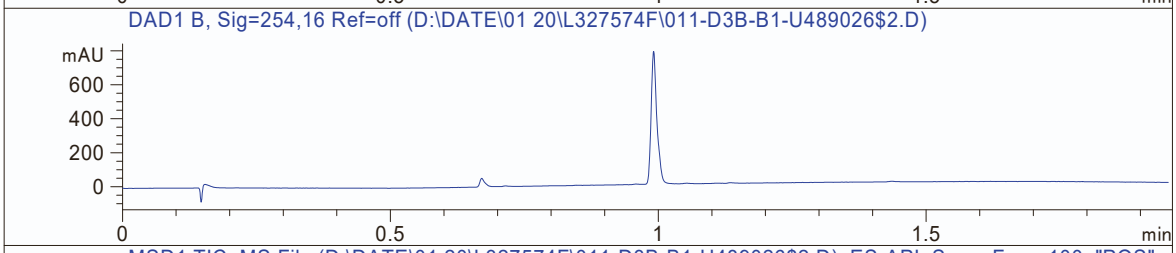
MW05-E6
Z4619437297

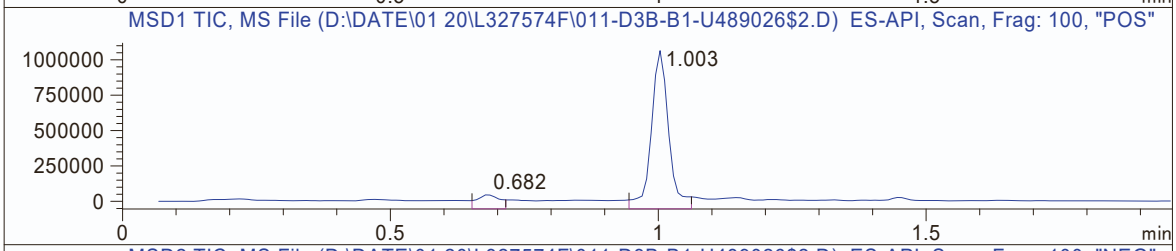


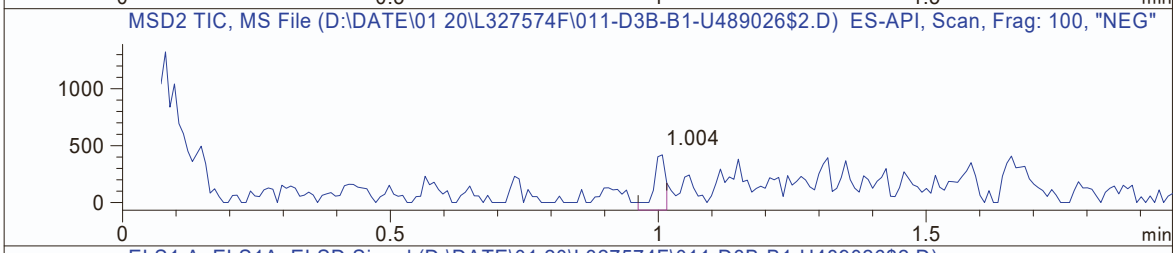
Mol Wt 361.4
Exact Mass 361.17

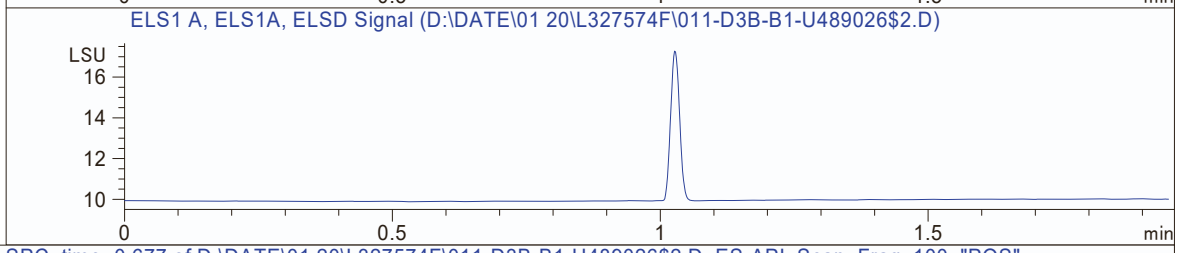
#	Time	Area%
1	0.670	3.84
2	0.991	96.16



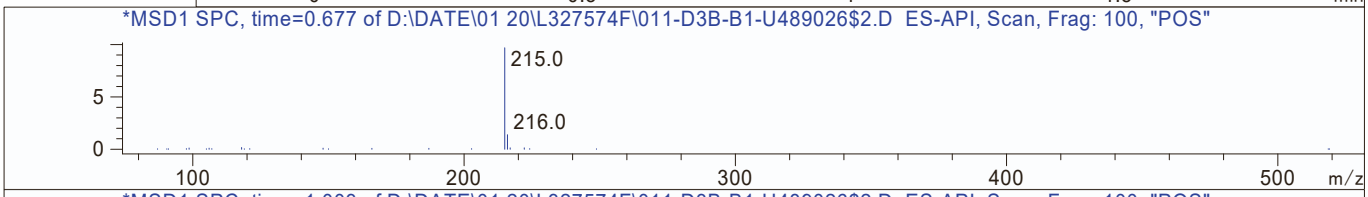




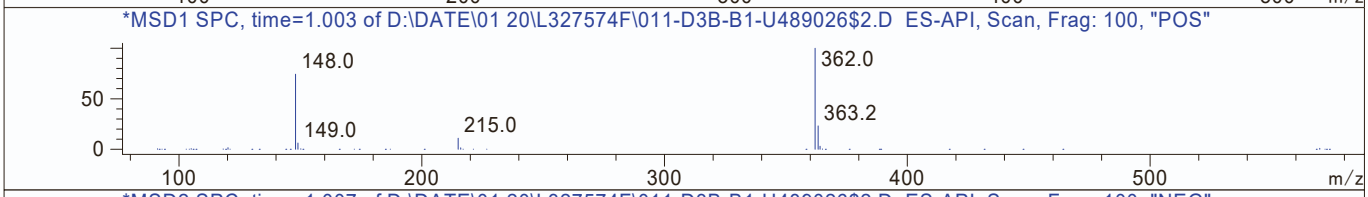




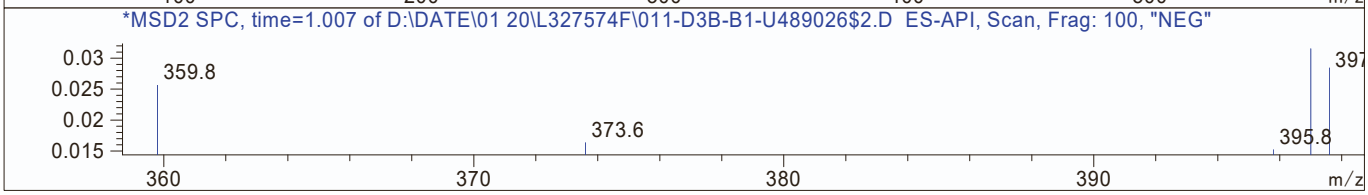
RT 0.682



RT 1.003

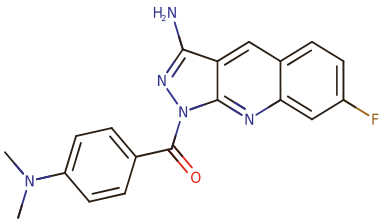


RT 1.004



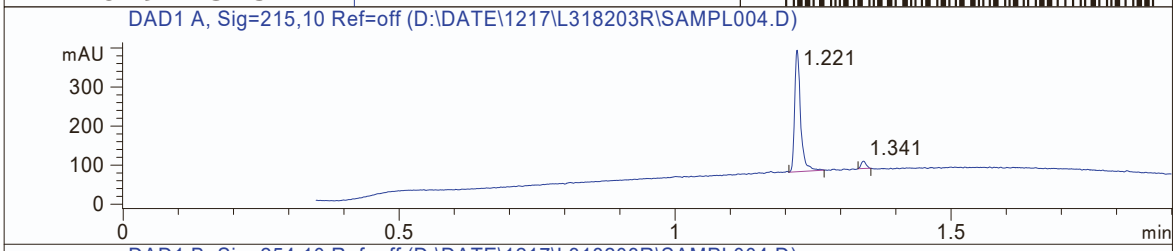
MaxPeak: 95.10%
Ret_Time: 1.221 min

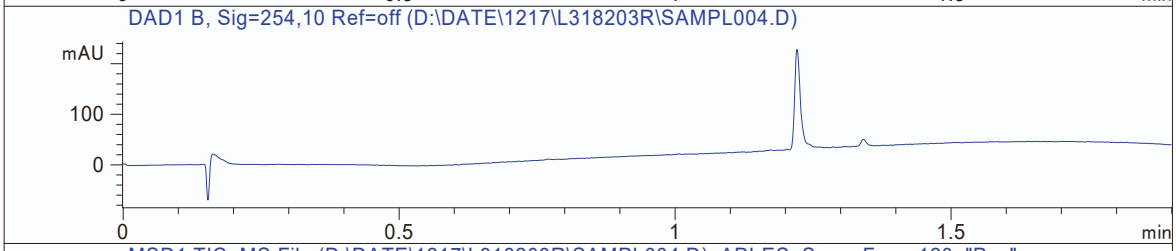
MW05-E7
Z4619443254

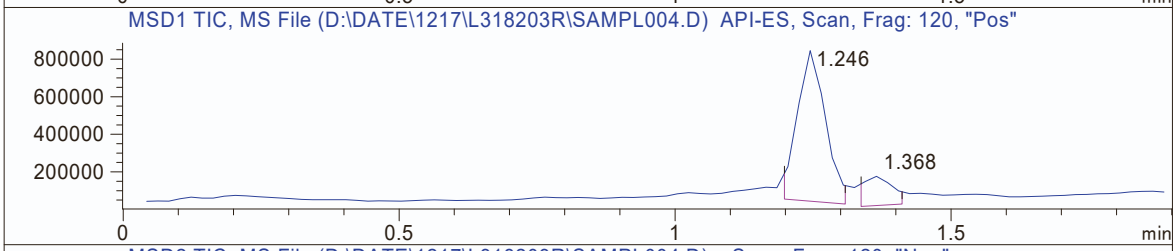


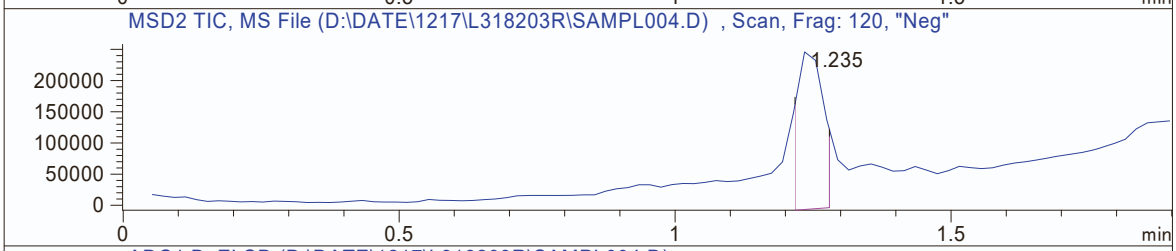
Mol Wt 349.36
Exact Mass 349.15

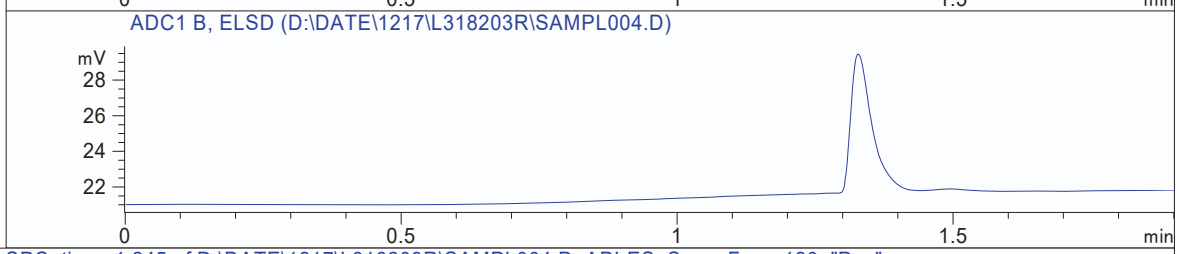
#	Time	Area%
1	1.221	95.10
2	1.341	4.90



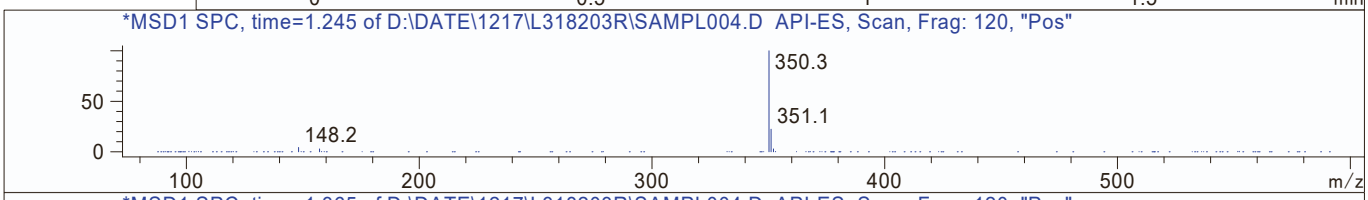




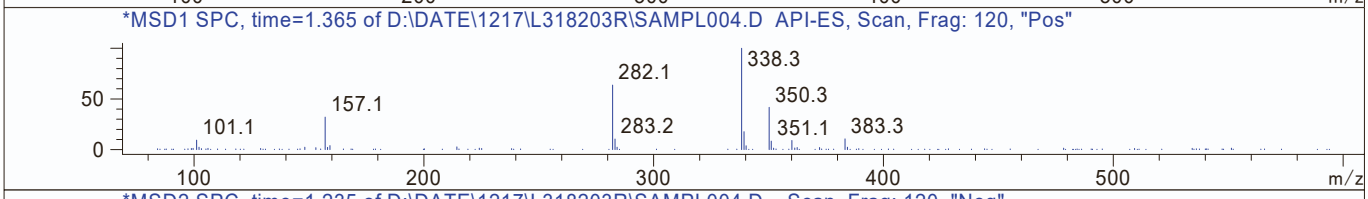




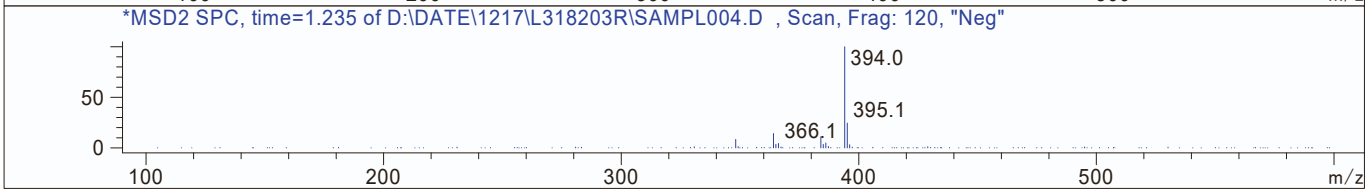
RT 1.246



RT 1.368

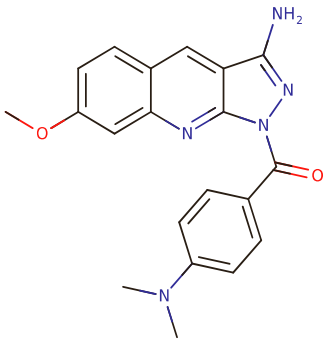


RT 1.235



Inj.Date 12/18/2020 E -SL- Acq. Method C:\HPCHEM\-> ->

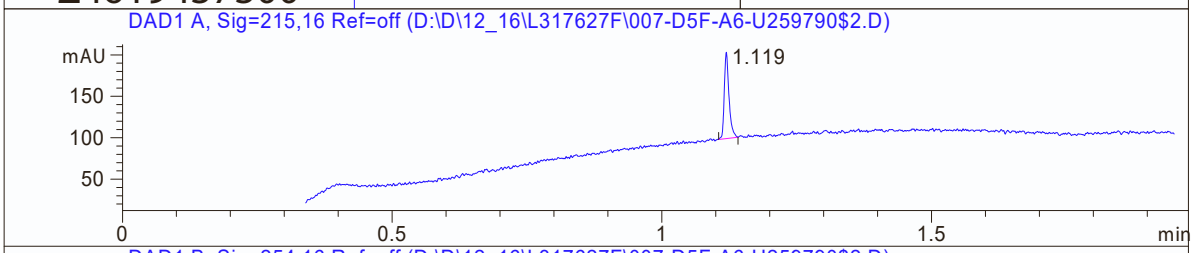
MaxPeak: 100.00%
Ret_Time: 1.119 min

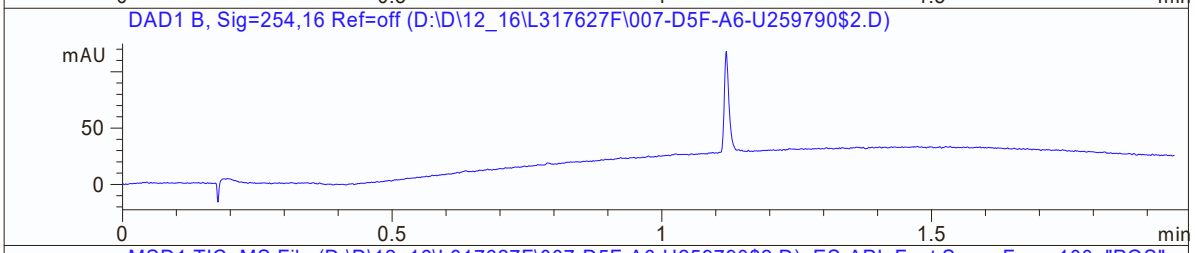


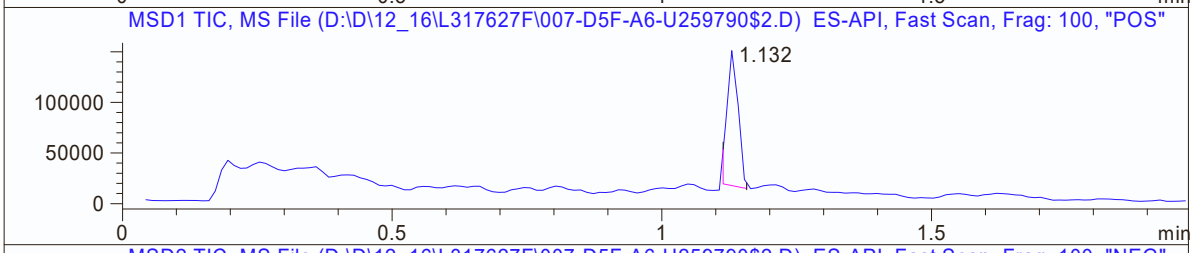
Mol Wt 361.4
Exact Mass 361.17

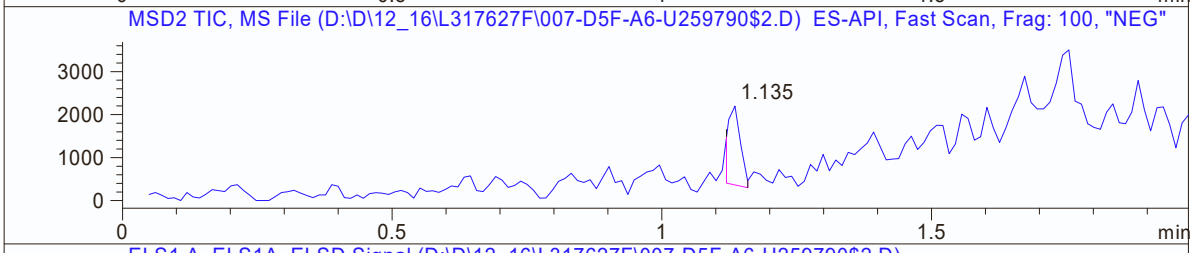
#	Time	Area%
1	1.119	100.00

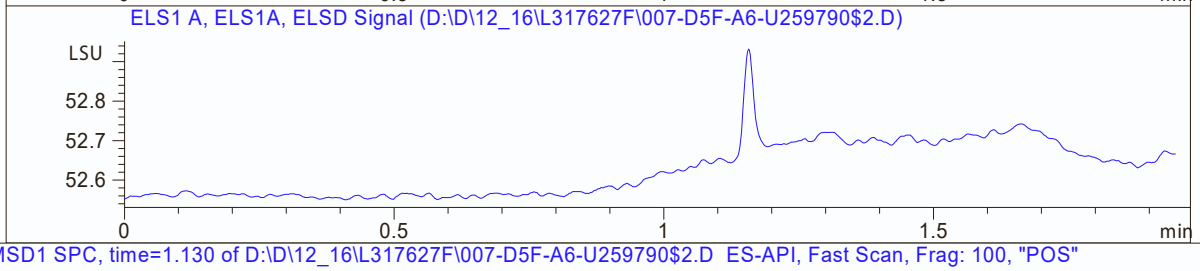
MW05-E8
Z4619437300



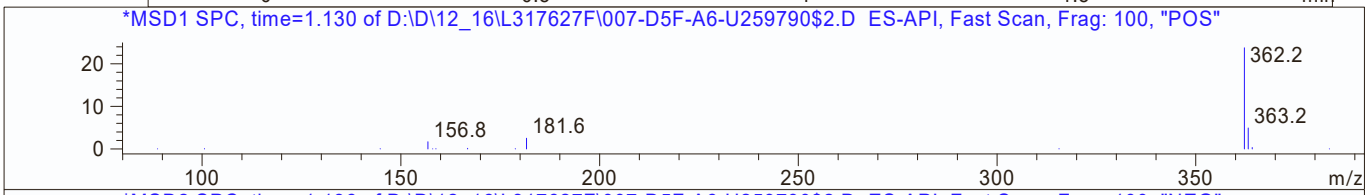




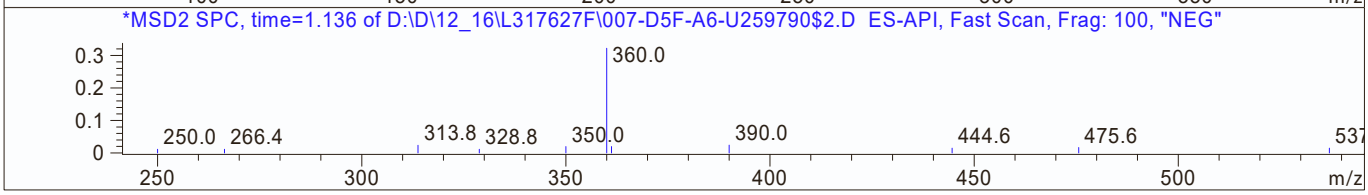




RT 1.132

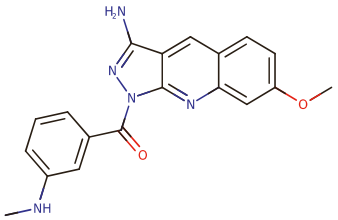


RT 1.135



Inj.Date 12/16/2020 N Acq. Method C:\Users\ -> ->

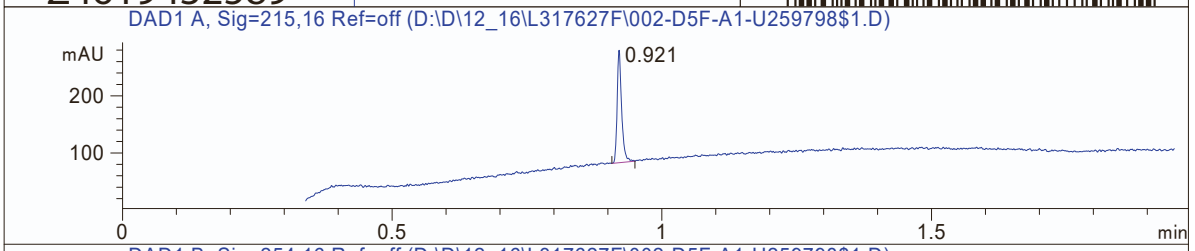
MaxPeak: 100.00%
Ret_Time: 0.921 min

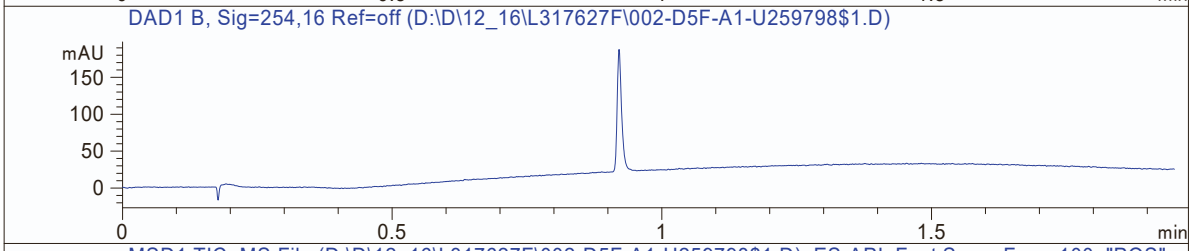


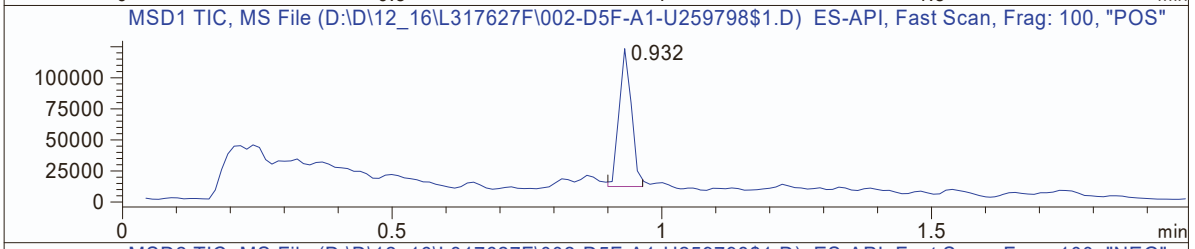
Mol Wt 347.37
Exact Mass 347.15

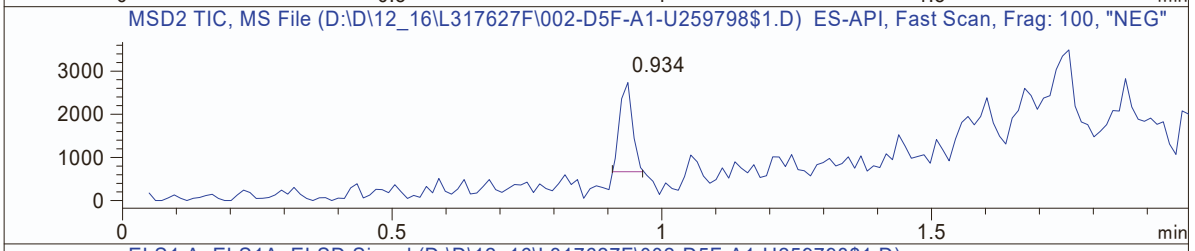
#	Time	Area%
1	0.921	100.00

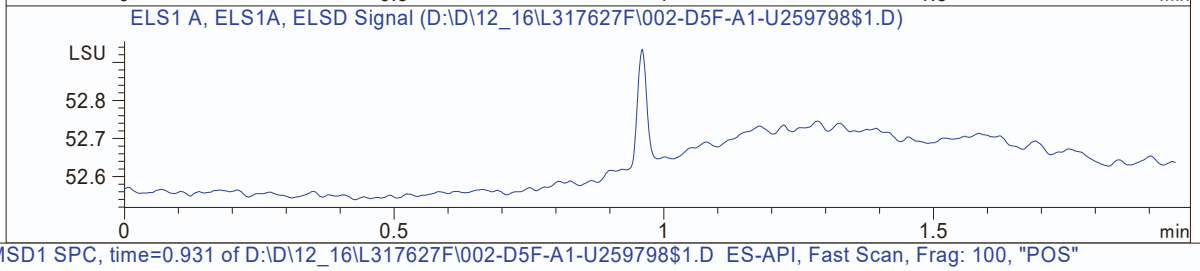
MW05-E9
Z4619432589



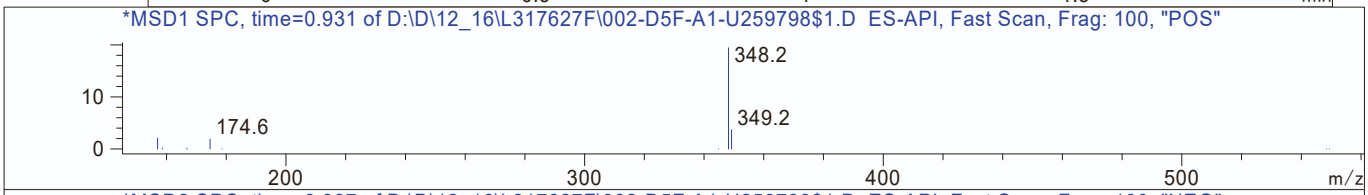




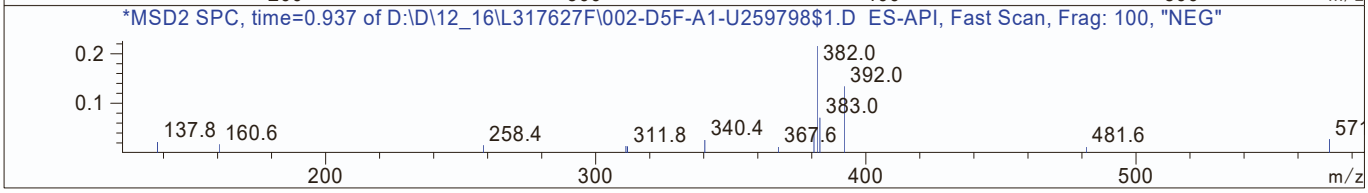




RT 0.932



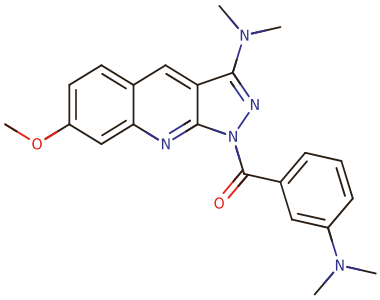
RT 0.934



Inj.Date 12/16/2020 N Acq. Method C:\Users\ -> ->

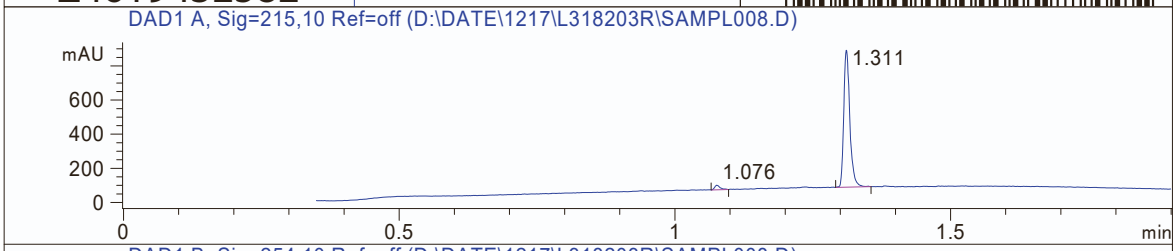
MaxPeak: 97.07%
Ret_Time: 1.311 min

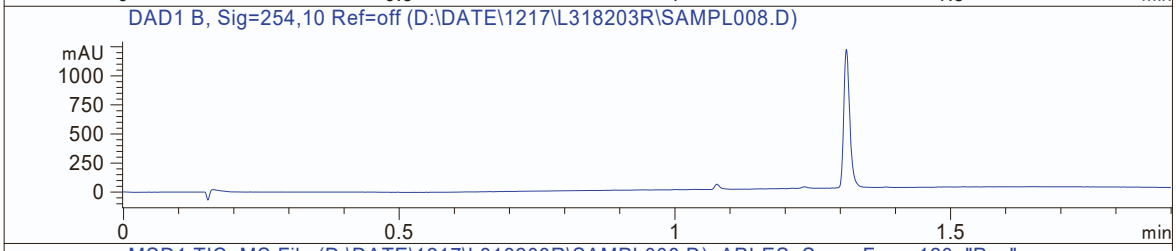
MW05-E10
Z4619432582

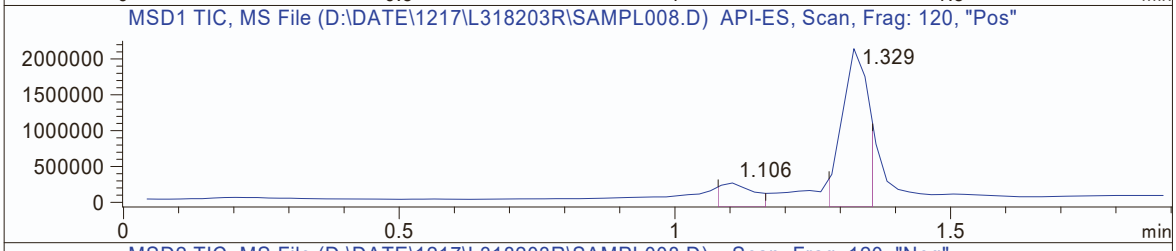


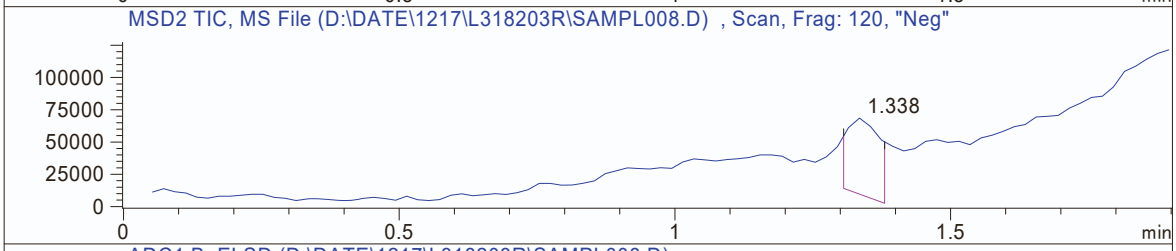
Mol Wt **389.45**
Exact Mass **389.21**

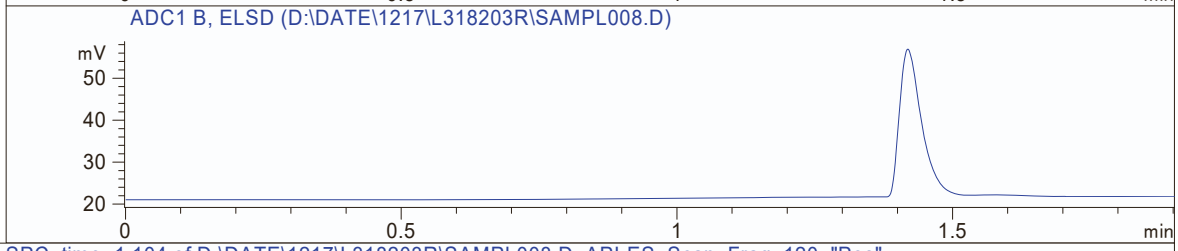
#	Time	Area%
1	1.076	2.93
2	1.311	97.07



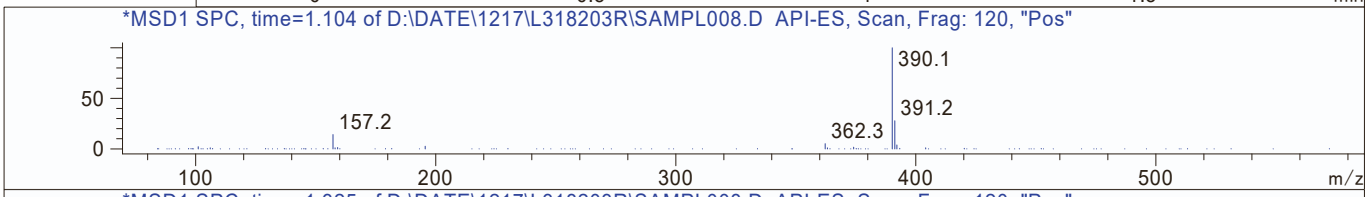




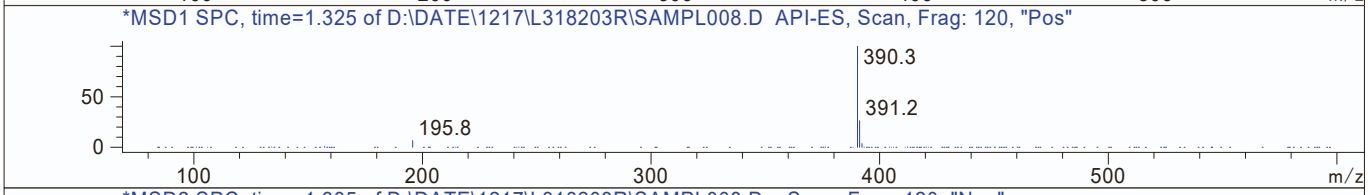




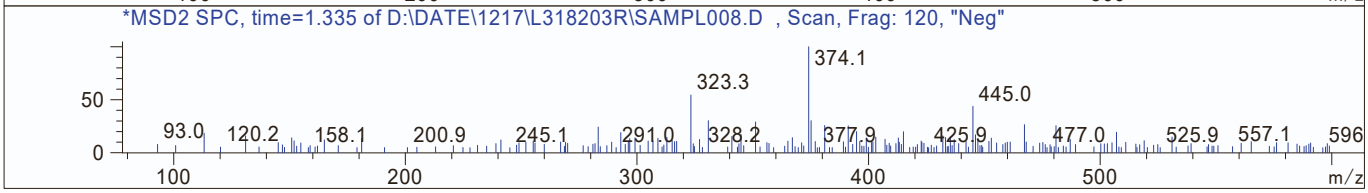
RT 1.106



RT 1.329



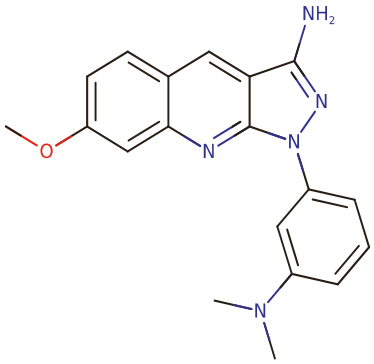
RT 1.338



Inj.Date 12/18/2020 E -SL- Acq. Method C:\HPCHEM\-> ->

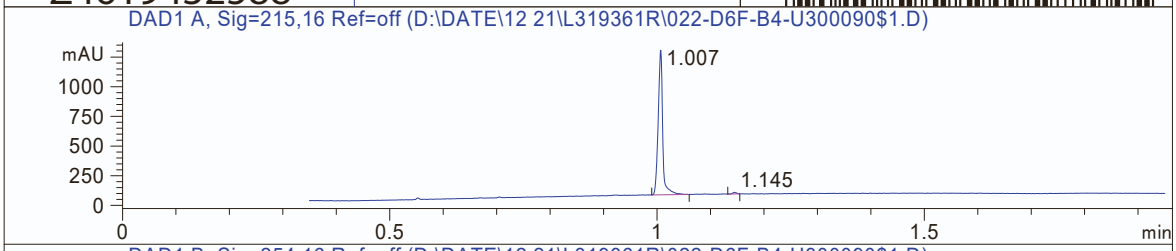
MaxPeak: 99.06%
Ret_Time: 1.007 min

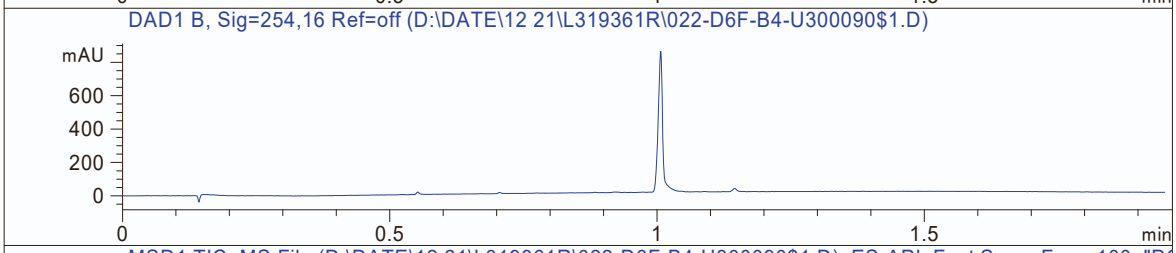
MW05-E11
Z4619432588

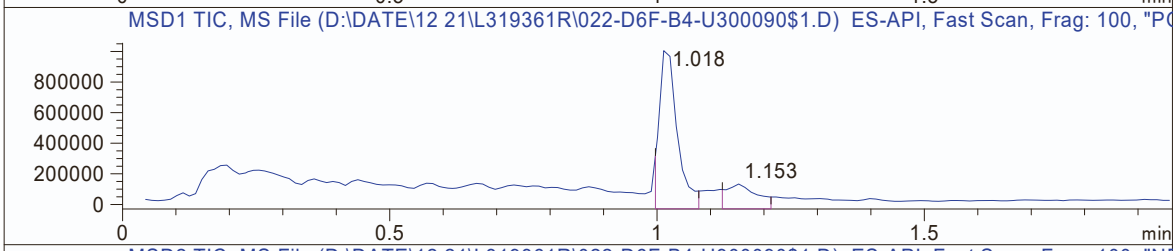


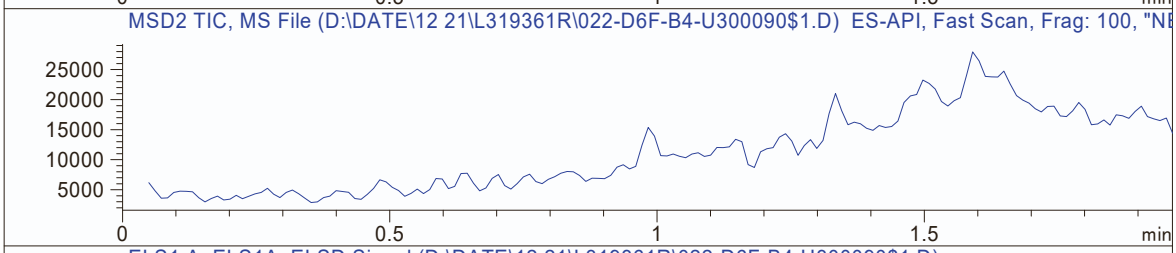
Mol Wt 333.39
Exact Mass 333.18

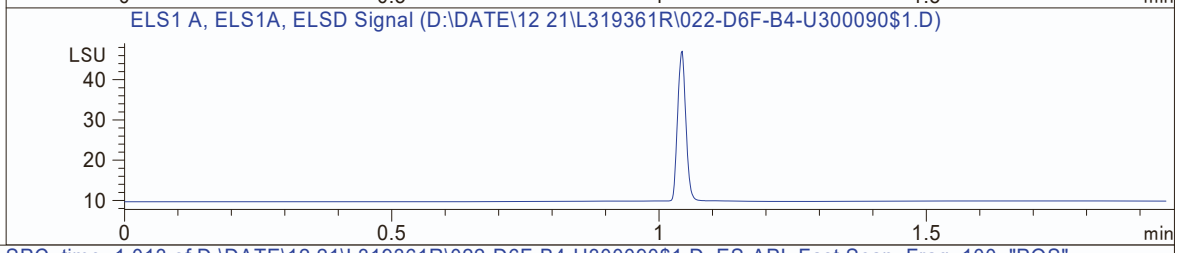
#	Time	Area%
1	1.007	99.06
2	1.145	0.94



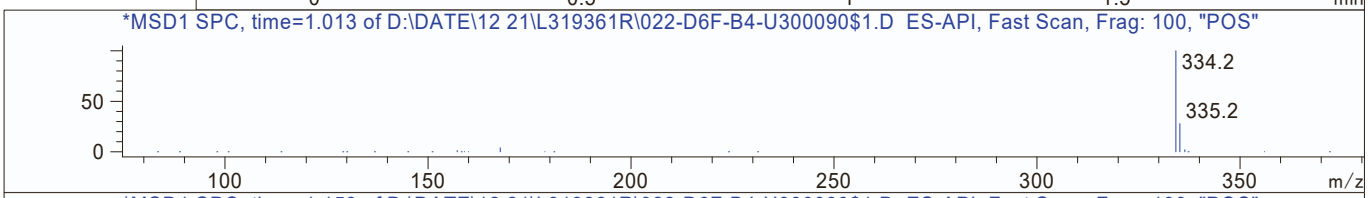




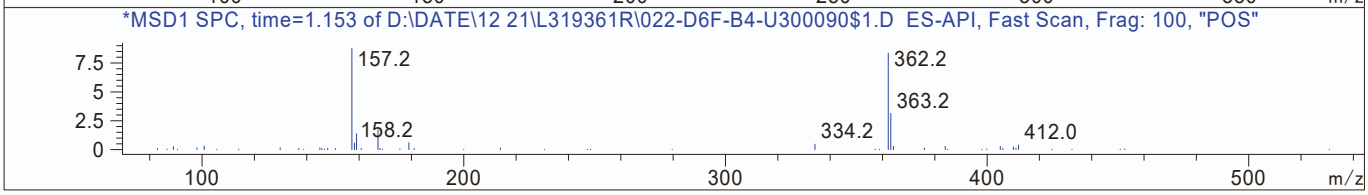




RT 1.018



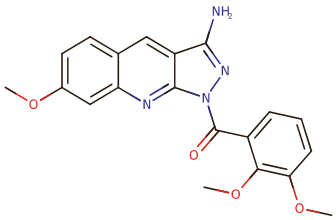
RT 1.153



Inj.Date 12/21/2020 E Acq. Method C:\Users\ -> ->

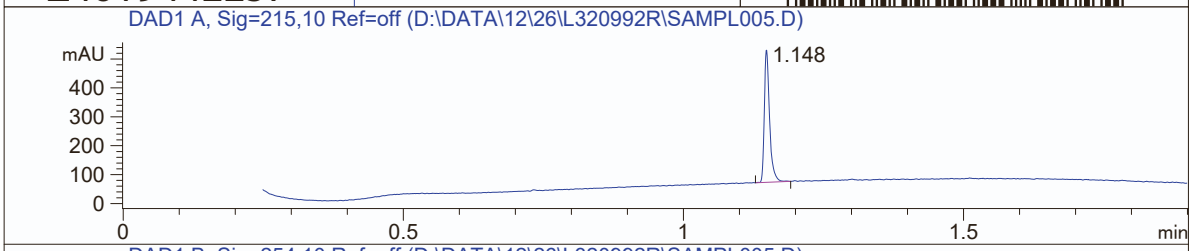
MaxPeak: 100.00%
Ret_Time: 1.148 min

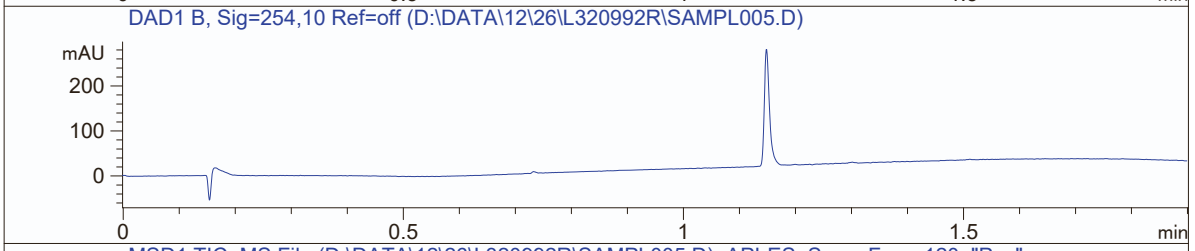
MW05-E13
Z4619442257

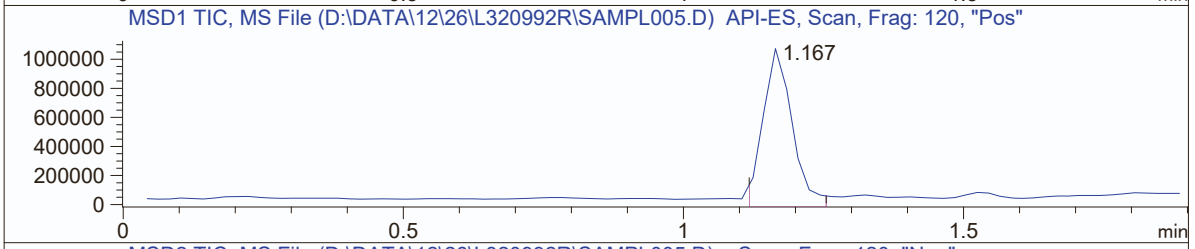


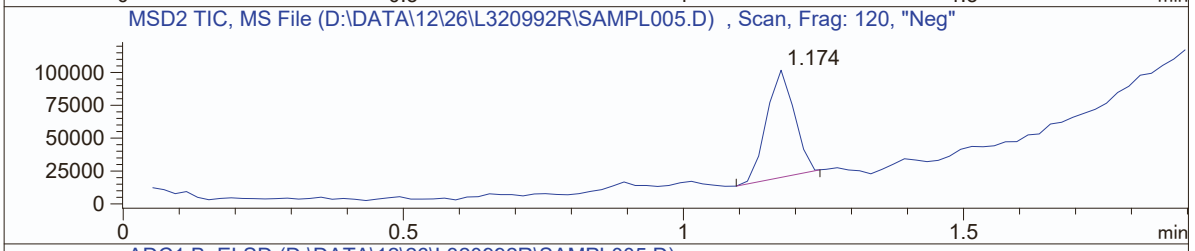
Mol Wt 378.38
Exact Mass 378.14

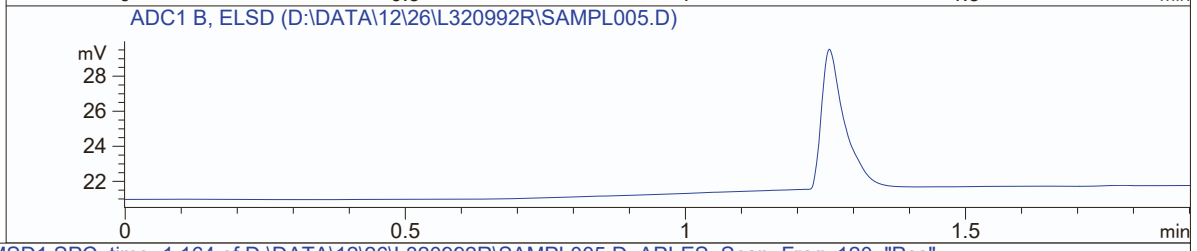
#	Time	Area%
1	1.148	100.00



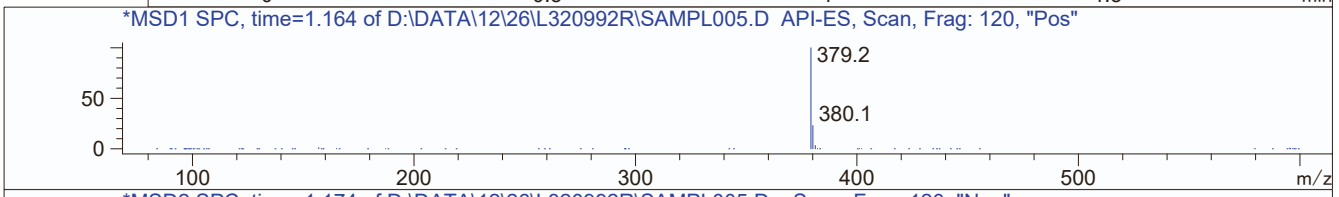




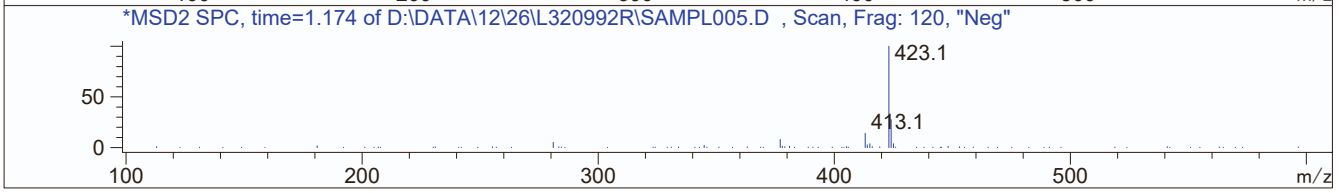




RT 1.167



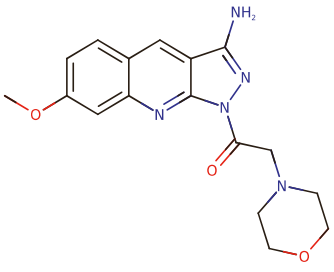
RT 1.174



Inj.Date 12/25/2020 LB -SL- Acq. Method C:\HPCHEM\-> ->

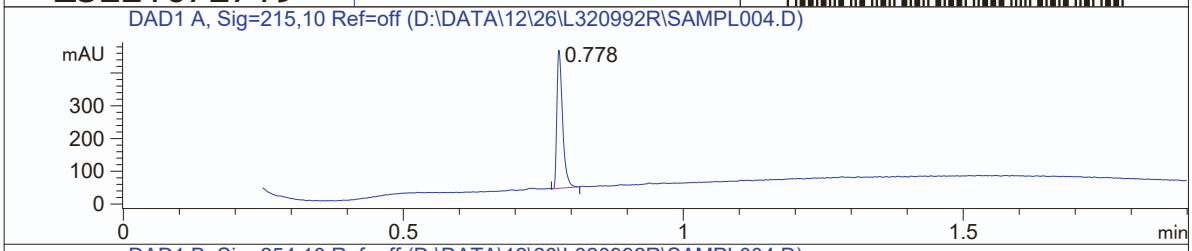
MaxPeak: 100.00%
Ret_Time: 0.778 min

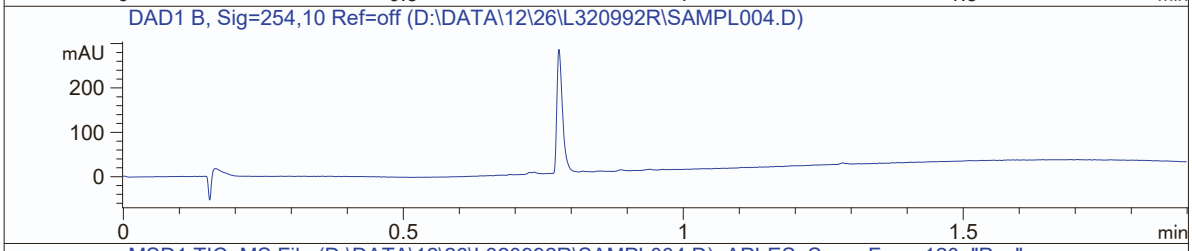
MW05-E14
Z3221672719

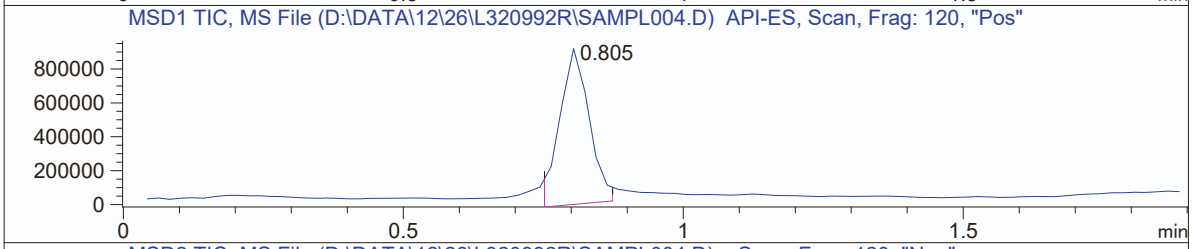


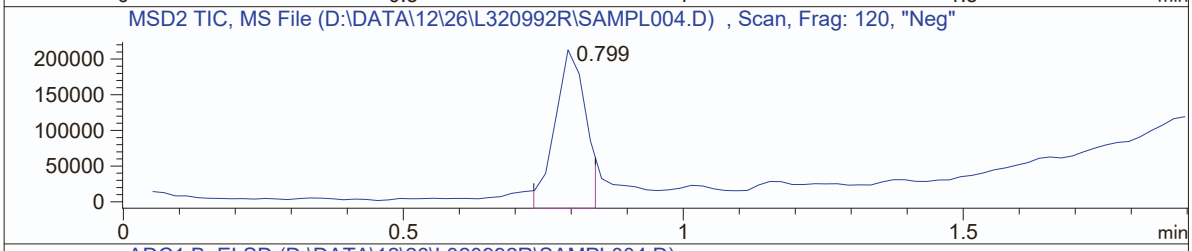
Mol Wt 341.36
Exact Mass 341.16

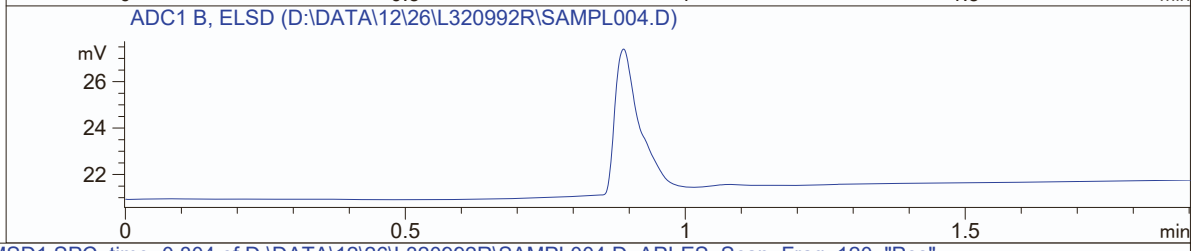
#	Time	Area%
1	0.778	100.00



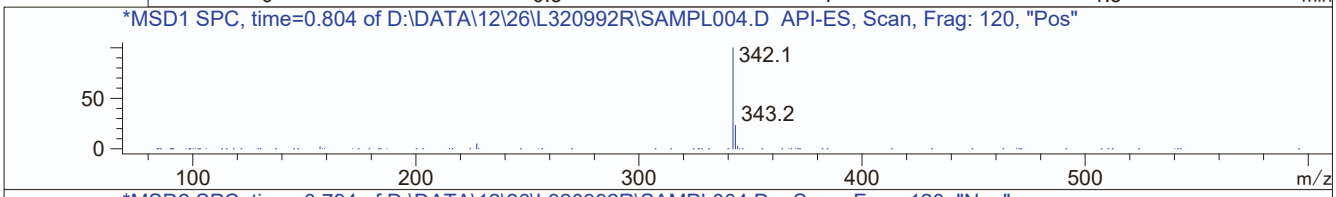




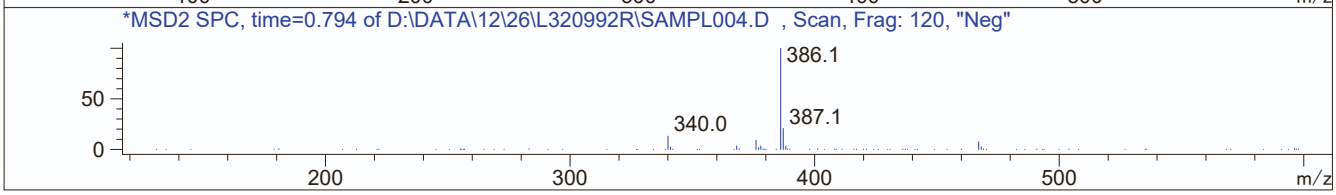




RT 0.805



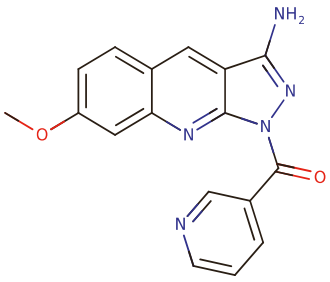
RT 0.799



Inj.Date 12/25/2020 LB -SL- Acq. Method C:\HPCHEM\-> ->

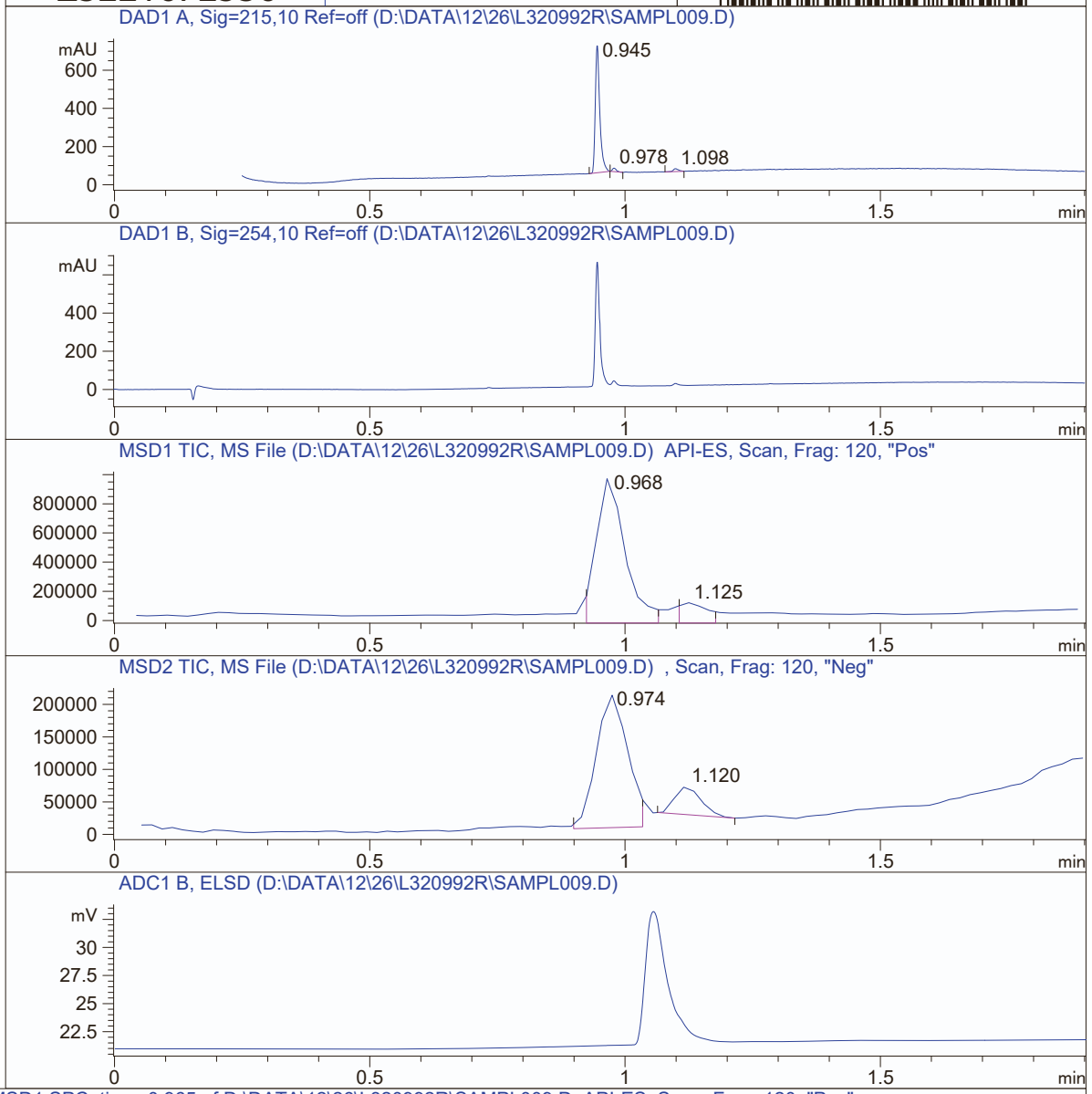
MaxPeak: 95.10%
Ret_Time: 0.945 min

MW05-E15
Z3221672536

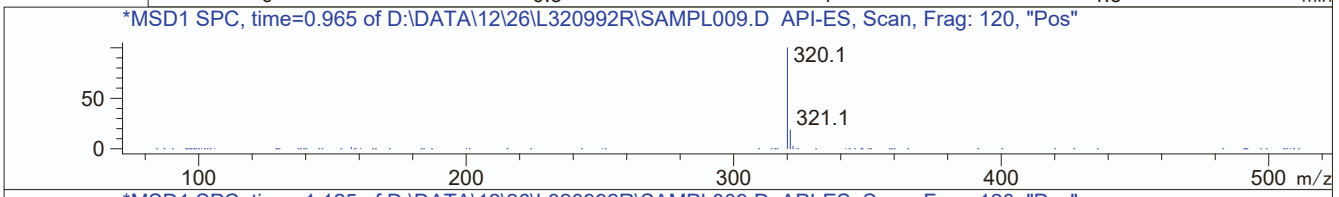


Mol Wt 319.32
Exact Mass 319.11

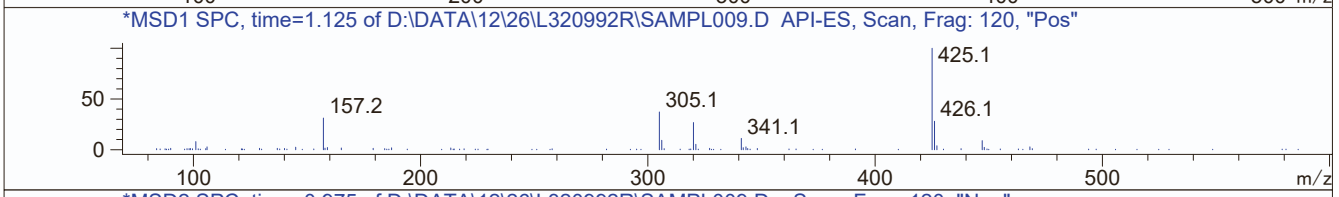
#	Time	Area%
1	0.945	95.10
2	0.978	2.28
3	1.098	2.62



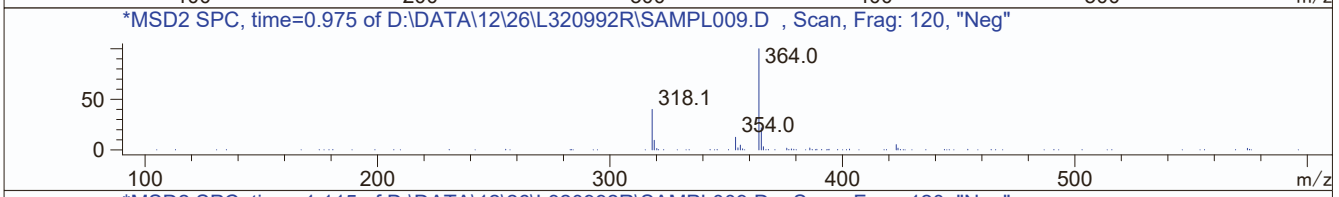
RT 0.968



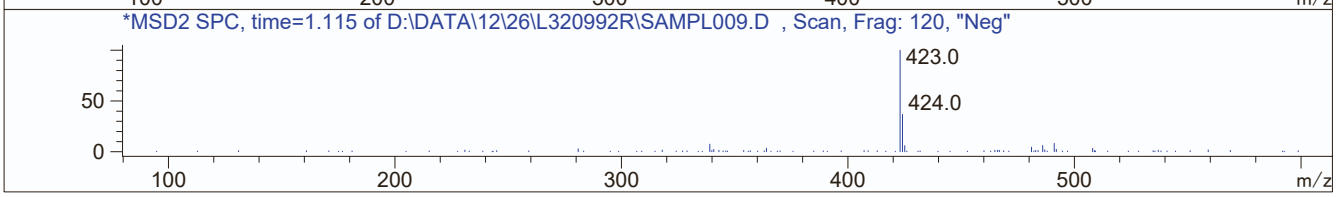
RT 1.125



RT 0.974



RT 1.120



Inj.Date 12/25/2020 LB -SL- Acq. Method C:\HPCHEM\-> ->

Compound	^{1}H NMR Description
MW01	^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 – 8.56 (m, 2H), 8.40 (dd, J = 8.9, 2.2 Hz, 1H), 8.29 (dd, J = 6.3, 2.2 Hz, 1H), 7.64 (dd, J = 9.0, 2.3 Hz, 1H), 7.45 (dt, J = 9.1, 2.4 Hz, 1H), 4.10 (d, J = 2.2 Hz, 3H), 4.05 (d, J = 2.4 Hz, 4H)
MW01-E10	^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 9.93 (s, 1H), 8.75 (d, J=5.5 Hz, 1H), 8.59 (d, J=5.5 Hz, 1H), 8.45 (d, J=8.4 Hz, 1H), 8.15 (d, J=8.5 Hz, 1H), 7.83 (d, J=1.6 Hz, 1H), 7.65 (d, J=1.6 Hz, 1H), 7.42 (dd, J=8.4, 1.6 Hz, 1H), 7.20 (dd, J=8.4, 1.6 Hz, 1H)
MW01-E18	^{1}H NMR(500 MHz, DMSO-d_6) δ 10.01 (s, 1H), 9.59 (s, 1H), 9.08 (dd, J=8.3, 1.7 Hz, 1H), 8.85 (d, J=5.6, 1H), 8.53 (dd, J=5.5, 1.7 Hz, 1H), 8.38 (m, 2H), 7.72 (s, 1H), 7.22 (dd, J=8.5, 1.8 Hz, 1H)
MW01-E6	^{1}H NMR(500 MHz, DMSO-d_6) δ 8.92 (d, J=8.6 Hz, 1H), 8.65 (d, J=5.6 Hz, 1H), 8.45 (d, J=5.6 Hz, 1H), 8.35 (d, J=8.5 Hz, 1H), 8.00 (d, J=1.6 Hz, 1H), 7.33 (dd, J=8.5, 1.6 Hz, 1H), 7.20 (d, J=8.6 Hz, 1H), 4.15 (s, 3H), 3.91 (s, 3H)
MW01-E14	^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (d, J = 4.9 Hz, 1H), 8.64 (d, J = 5.1 Hz, 1H), 8.51 (d, J = 8.8 Hz, 1H), 8.38 (d, J = 5.0 Hz, 1H), 8.21 (d, J = 5.3 Hz, 1H), 8.02 (d, J = 1.6 Hz, 1H), 7.46 – 7.38 (m, 1H), 4.09 (s, 3H), 3.93 (s, 3H)
MW05	^{1}H NMR(300 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.03 (d,J = 9.08Hz, 1H), 7.39 (d,J = 2.67Hz, 1H), 7.33 – 7.23 (m, 2H), 7.17 – 7.06 (m, 2H), 6.91 (dd,J = 2.90, 8.32Hz, 1H), 6.69 (s, 2H), 3.97 (s, 3H), 2.93 (s, 6H).
MW05-E3	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.09 (d, J=8.2 Hz, 1H), 7.37 (m, 1H), 7.25 (m, 2H), 7.10-6.95 (m, 2H), 6.84 (m, 1H), 6.65 (s, 2H), 4.00 (s, 3H), 3.32 (m, 4H), 1.10 (m, 6H)
MW05-E9	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.05 (d, J=8.2 Hz, 1H), 7.42 (d, J=1.9 Hz, 1H), 7.33-7.12 (m, 2H), 7.00 (m, 2H), 6.70 (m, 3H), 5.87 (m, 1H), 4.00 (s, 3H), 2.70 (m, 3H)
MW05-E2	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (s, 1H), 7.95 (d, J=8.2 Hz, 1H), 7.60-7.45 (m, 2H), 7.30 (m, 1H), 7.10 (m, 2H), 6.90 (d, J=8.2 Hz 1H), 6.75 (s, 2H), 4.00 (s, 3H), 2.95 (s, 6H)
MW05-E10	^{1}H NMR(500 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.92 (d, J=8.4 Hz, 1H), 7.30 (s, 1H), 7.15 (m, 1H), 6.95 (m, 1H), 6.72 (s, 1H), 6.65 (m, 1H), 6.53 (m, 1H), 4.00 (s, 6H), 3.50 (s, 3H), 2.65 (s, 6H)