Supporting Information

for

Design, Synthesis and Preclinical Evaluation of 3-Methyl-6-(5-thiophenyl)-1,3-dihydro-imidazo[4,5-*b*]pyridin-2-ones as Selective GluN2B Negative Allosteric Modulators for the Treatment of Mood Disorders

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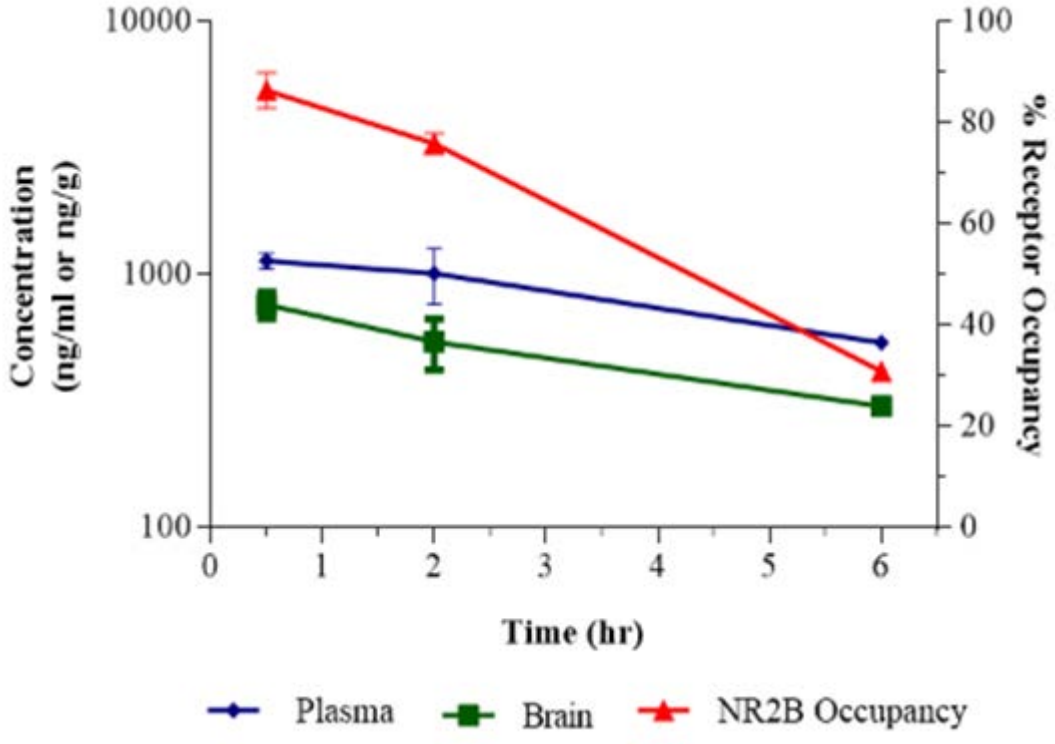
SI Table 1. Mean Plasma and Brain Levels of 12 over time at 3 mg/kg PO in the Rat; vehicle=20% HP-β-CD.

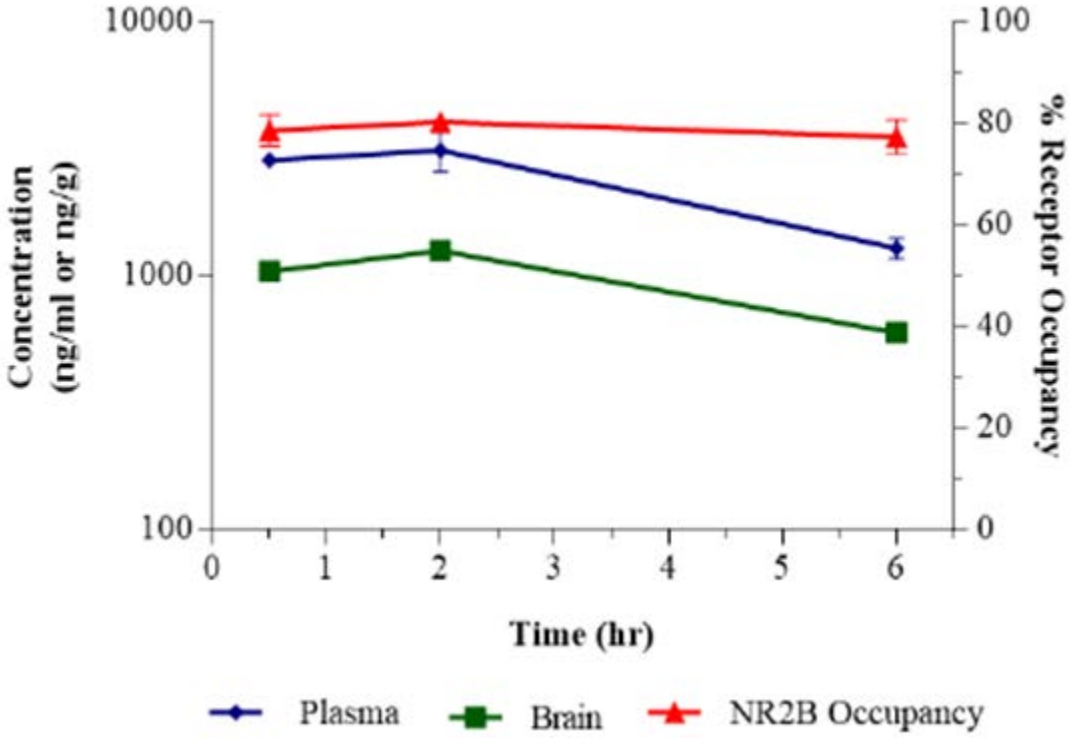
Time (h)	Mean Plasma (ng/ml)	Mean Brain (ng/g)	Mean Brain/Plasma Ratio
0.5	1140.1	765.0	0.68
2.0	1019.3	546.8	0.54
6.0	542.9	304.0	0.56



SI Figure 1. Concentration vs Occupancy of 12 at 3 mg/kg PO in the Rat, n=2 ± SEM.

SI Table 2. Mean Plasma and Brain Levels of 13 over time at 3 mg/kg PO in the Rat; vehicle=20% HP-β-CD.

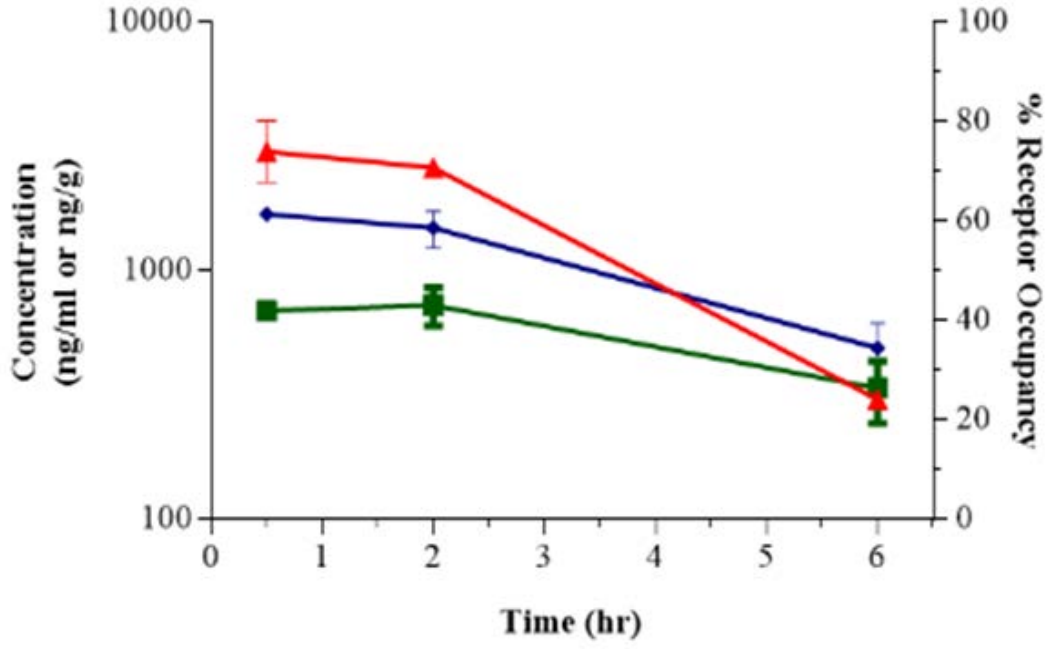
Time (h)	Mean Plasma (ng/ml)	Mean Brain (ng/g)	Mean Brain/Plasma Ratio
0.5	2860.9	1042.4	0.37
2.0	3119.3	1257.4	0.42
6.0	1286.8	597.8	0.47



SI Figure 2. Concentration vs Occupancy of 13 at 3 mg/kg PO in the Rat, n=2 ± SEM.

SI Table 3. Mean Plasma and Brain Levels for 14 over time at 3 mg/kg PO in the Rat; vehicle=20% HP-β-CD.

Time (h)	Mean Plasma (ng/ml)	Mean Brain (ng/g)	Mean Brain/Plasma Ratio
0.5	1696.5	694.4	0.41
2.0	1490.8	728.2	0.49
6.0	491.1	338.8	0.69



SI Figure 3. Concentration vs Occupancy of 14 at 3 mg/kg PO in the Rat, n=2 ± SEM.

SI Table 4. Rat ex vivo GluN2B receptor occupancy of 12 in rat hippocampus and plasma and brain exposures at 60 min after p.o. administration, n=3 per dose ± SD.

Dose (mg/kg)	% NR2B receptor occupancy [a]	Brain conc. (ng/g)	Plasma conc. (ng/mL)	Brain/plasma ratio
0.01	0 ± 0	5 ± 0	3 ± 0	2.01 ± 0.17
0.03	0 ± 0	10 ± 1	9 ± 1	1.18 ± 0.10
0.1	7 ± 5	41 ± 7	27 ± 3	1.59 ± 0.43
0.3	11 ± 6	94 ± 5	99 ± 6	0.96 ± 0.06
1	55 ± 3	414 ± 28	454 ± 13	0.91 ± 0.04
3	78 ± 4	1,311 ± 132	1,516 ± 34	0.86 ± 0.08
10	92 ± 2	6,432 ± 1,024	6,024 ± 753	1.07 ± 0.13
30[b]	92 ± 2	5,372 ± 547	5,554 ± 305	0.97 ± 0.09

[a] Ex vivo GluN2B labelling was expressed as the percentage of GluN2B labelling in corresponding brain areas of vehicle-treated animals. [b] The compound was dosed as a suspension at 30 mg/mg.

SI Table 5. Biodistribution of 12 in rat brain regions at different time points after a single i.v. injection of 30 μg/kg.

Time(min)	**Cerebellum**	**Cortex**	**Striatum**	**Hippocampus**
5	25.6 ± 3.1	20.1 ± 1.5	23.8±2.7	17.7 ± 0.8

10	25.3 ± 2.1	28.1 ± 6.2	26.6 ± 7.0	29.2 ± 2.7
30	19.7 ± 3.0	21.9 ± 4.7	21.6 ± 4.6	30.7 ± 5.3
60	15.2 ± 3.5	17.8 ± 2.2	17.0 ± 2.9	27.5 ± 5.8
120	8.8 ± 1.4	9.8 ± 2.1	11.9 ± 1.1	12.3 ± 0.2

Biological assay result details:

SI Table 6. Metabolic stability[1] of 12 in liver microsomes of various species.

Species	$T_{1/2}$ in microsomes (min)	CL_{int} in microsomes (μL/min/mg)
Human	105.5; 165.6[a]	6.6; 4.2[a]
Monkey	26.4	26.3
Dog	47.3	14.7
Rat	68.0	10.2
Mouse	41.9	16.6

NT, not tested; $T_{1/2}$, half life; CL_{int}, intrinsic clearance.
[a] From two independent experiments.

Plasma Protein Binding. Plasma protein binding of **12** was determined at 0.5, 5, and 25 μM using the equilibrium dialysis method.[2]

SI Table 7. Protein binding of 12 in human and rat plasma.

Species	Concentration (μM)	% Free
Human	0.5	4.6
	5	5.0

	25	5.6
Rat	0.5	5.3
	5	5.2
	25	6.5

SI Table 8: Compound 12 inhibition of major CYP isoforms in human liver microsomes.

Isoform	CYP Probe Substrate	12 IC_{50} (μM)	Positive Control IC_{50} (μM)
1A2	Phenacetin	n/a	α-Naphthoflavone 0.018
2A6	Coumarin	n/a	Tranylcypromine 0.79
2B6	Bupropion	n/a	Ticlopidine 0.083
2C8	Amodiaquine	n/a	Quercetin 3.0
2C9	Tolbutamide	n/a	Sulfaphenazole 0.37
2C9	Diclofenac	n/a	Sulfaphenazole 0.25
2C19	S-Mephenytoin	n/a	N-3-BenzylPB 0.11
2D6	Dextromethorphan	15	Quinidine 0.038

2E1	Chlorzoxazone	n/a	4-Methylpyrazole 0.57
3A4	Testosterone	n/a	Ketoconazole 0.016
3A4	Midazolam	n/a	Ketoconazole 0.014
3A4	Nifedipine	n/a	Ketoconazole 0.010

n/a, no concentration-dependent inhibition observed.

Solubility of 12 in enabling excipients. Formulation studies were conducted using the following excipient combinations to achieve final concentrations of 10 mg/mL. Each of the conditions were prepared and stirred overnight. None of the attempts resulted in a solution.

SI Table 9. Solubility Results for 12 in various excipient combinations.

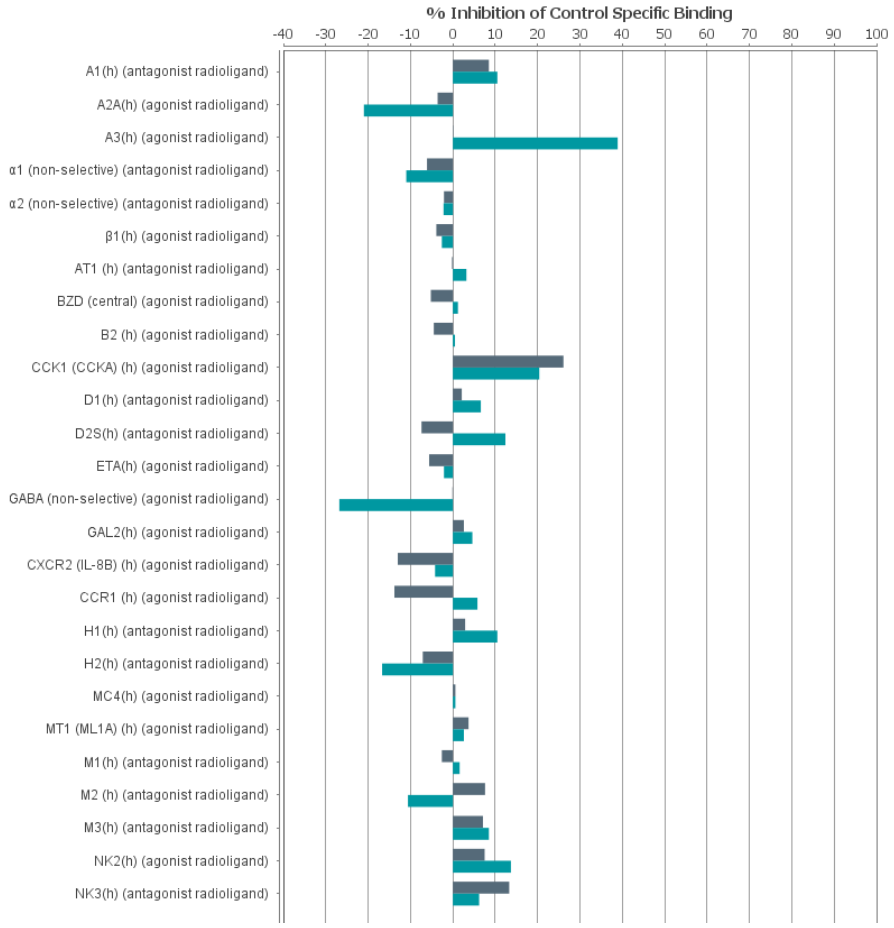
	Excipient 1	Excipient 2	Excipi-ent 3	Excipient 1 (uL)	Excipient 2 (uL)	Excipient 3 (uL)	Max. vol. mice/ rat ml/kg	Max. vol. dog (ml/kg)	weighed (mg)	Soluble?
1	Propylene Glycol			893			5	2.5	8.93	no
2	Labrafac CC			798			7	2	7.98	no
3	Labrasol			994			5	3	9.94	no
4	PEG 400 (25%)	Propylene Glycol (75%)		237	709		6.7	2.5	9.46	no

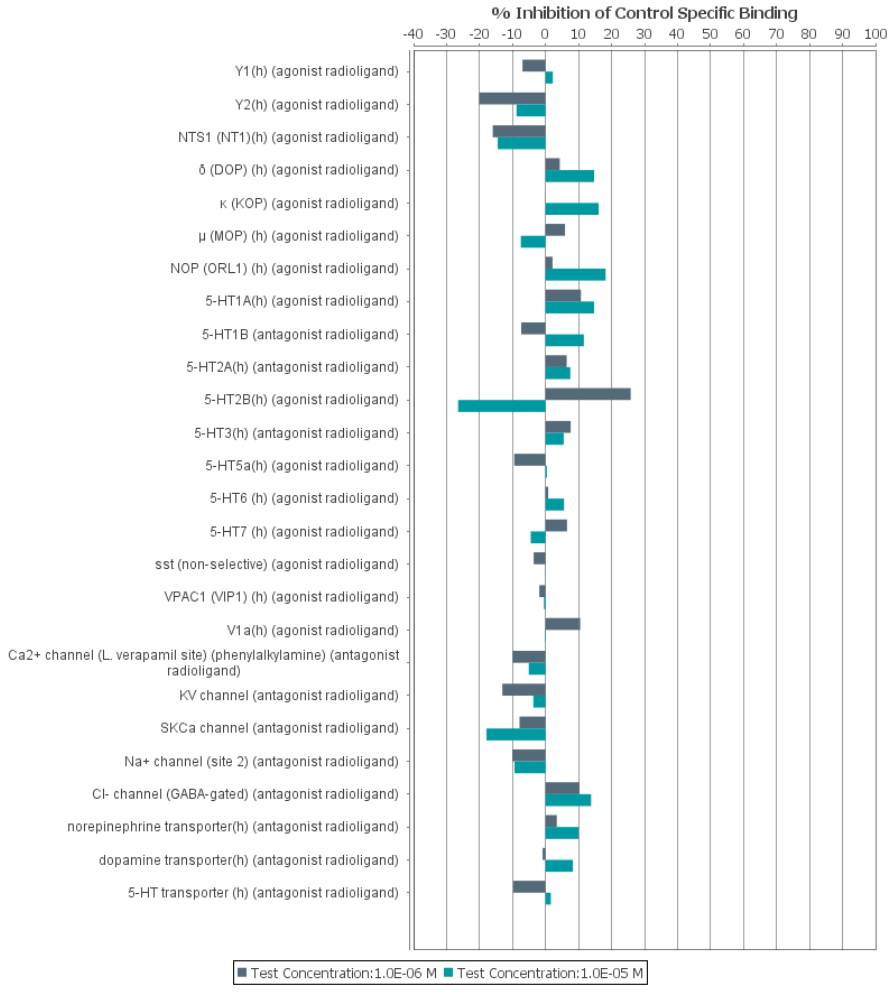
5	Labrasol (10%)	PEG 400 (90%)		109	976		10	3	10.85	no
6	Labrasol (10%)	Propylene Glycol (90%)		99	893		5	2.5	9.92	no
7	Labrasol (25%)	PEG 400 (75%)		287	860		10	3	11.47	no
8	Labrasol (25%)	Propylene Glycol (75%)		224	673		5	3	8.97	no
9	Labrasol (25%)	PEG 400 (50%)	Propylene Glycol (25%)	233	464	233	10	3	9.3	no
10	Safflower Oil (90%)	Transcutol HP (10%)		1026	114		10	2	11.4	no
11	Ethanol (10%)	Safflower Oil (90%)		99	896		5	2	9.95	no
12	Safflower Oil (80%)	Transcutol HP (20%)		901	225		5	2	11.26	no
13	Safflower Oil			988			10	2	9.88	no
14	Sesame Oil (90%)	Transcutol HP (10%)		1000	113		10	1	11.12	no
15	Ethanol (10%)	Sesame Oil (90%)		113	1019		5	1	11.32	no
16	Sesame Oil (80%)	Transcutol HP (20%)		718	180		5	1.2	8.98	no

References:

1) Letavic et al. *ACS Med. Chem. Lett.*, **2013**, *4*, 419-422.
2) Swanson,et al. *J. Med. Chem.* **2016**, *59*, 8535–8548

APPENDIX A: SELECTIVITY OF 12 IN A PANEL OF ION CHANNELS, GPCRS AND RECPTORS





APPENDIX B: LCMS PURITY DETERMINATION FOR 11-14

Compound 11

Openlynx Report - CLF

Page 1

Vnumber:44598148#137654#C4 | Sample ID:5900150934 | Description:9101045882;C4

Vial:4:4,C | Date:24-Jul-2015 | Time:22:10:59

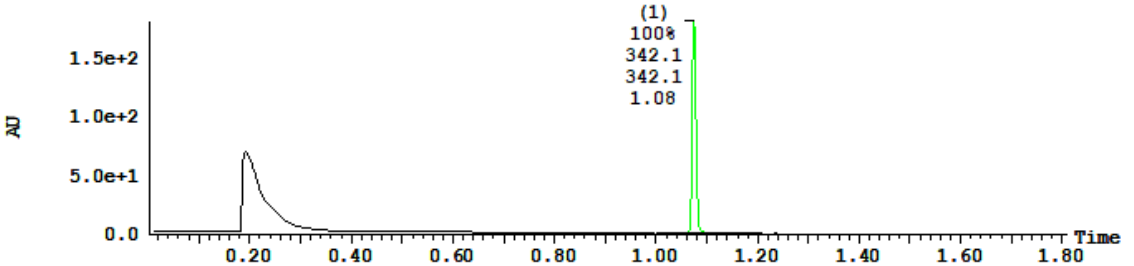
Instrument:ACQ-SQD#K06SQD006W | Method:M:\UPLC_Nugenesis_P30_N30@B5501B5501.olp

Printed: Tue Sep 22 09:41:13 2015

3: UV Detector: TIC

1.794e+2

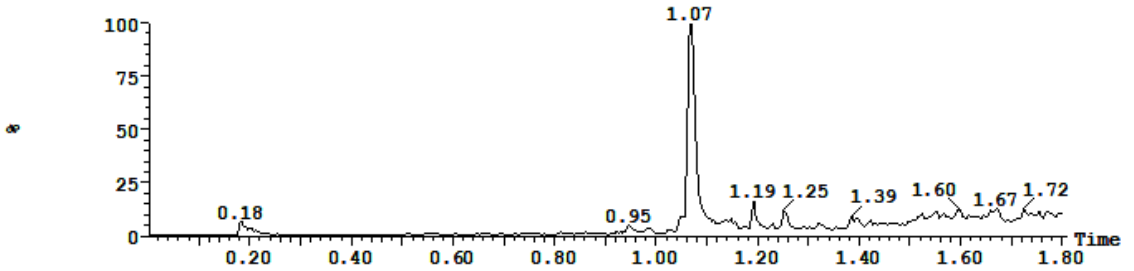
Range: 1.817e+2



Peak Number	Time	State	Area %Total	Mass Found
1	1.08	OK	100.00	342.12, 342.12

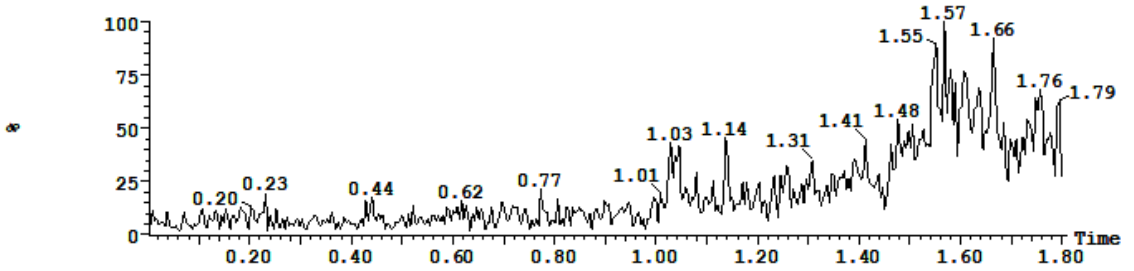
1: MS ES+ :TIC

1.5e+008



2: MS ES- :TIC

1.4e+006

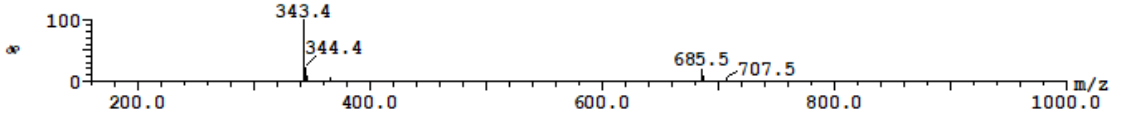


Peak ID	Time	Mass Found	BPM	State
1	1.08	365.12,343.12	343	OK

1:(Time: 1.08) Combine (254:261-(235:247+278:290))

1:MS ES+

4.2e+007



Compound 12

Openlynx Report - CLF — Page 1

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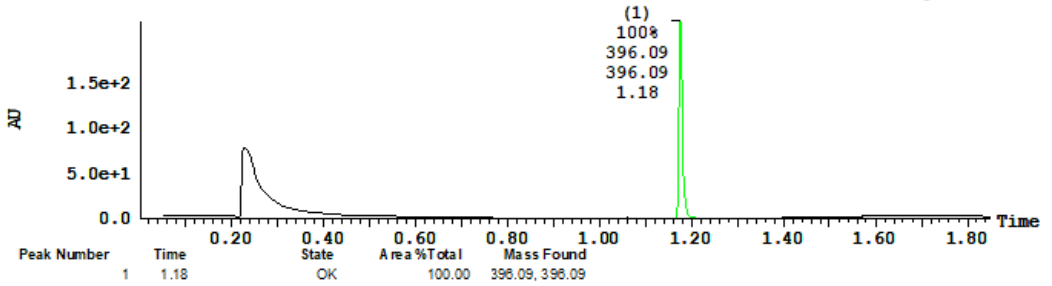
Vial:2:5,B | Date:22-Jun-2016 | Time:13:26:01
Instrument:ACQ-SQD#NotSet | Method:K:\LJ_CL_UPLC_Nugenesis_P30_N30@L2101L2101.olp | FreeText1

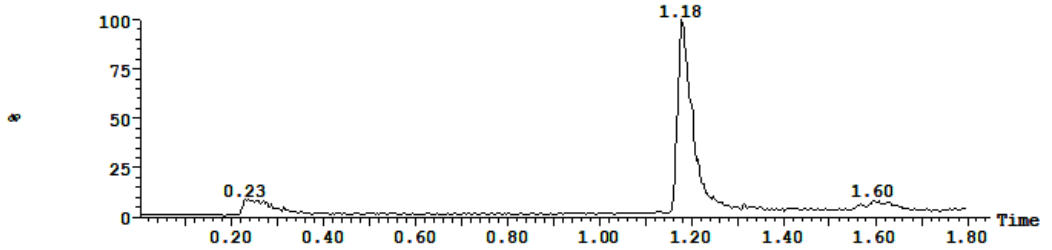
Printed: Fri Aug 05 16:26:22 2016

3: UV Detector: TIC — 2.157e+2, Range: 2.179e+2

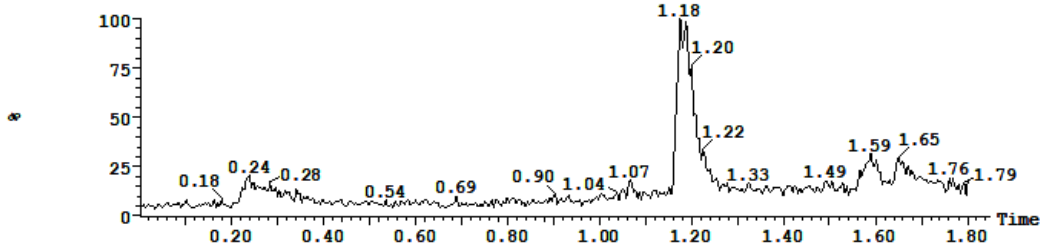


Peak Number	Time	State	Area %Total	Mass Found
1	1.18	OK	100.00	396.09, 396.09

1: MS ES+ :TIC — 1.5e+008



2: MS ES- :TIC — 1.1e+007



Peak ID	Time	Mass Found	BPM	State
1	1.18	419.09,397.09	397	OK

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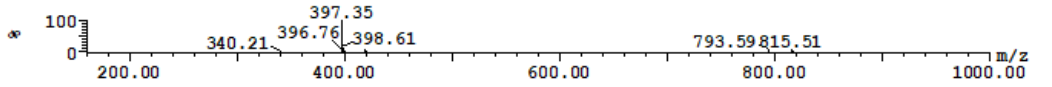

Peak ID	Time	Mass Found	BPM	State
1	1.18	455.09,395.09	455	OK

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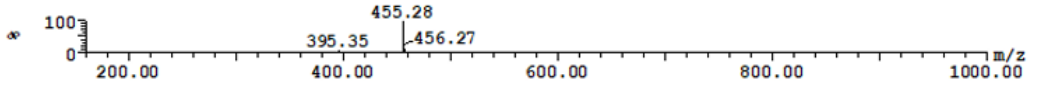

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1	1.08	401.12,	294	OK

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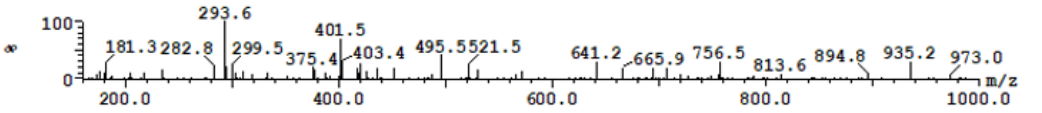
2:MS ES-
1.8e+004



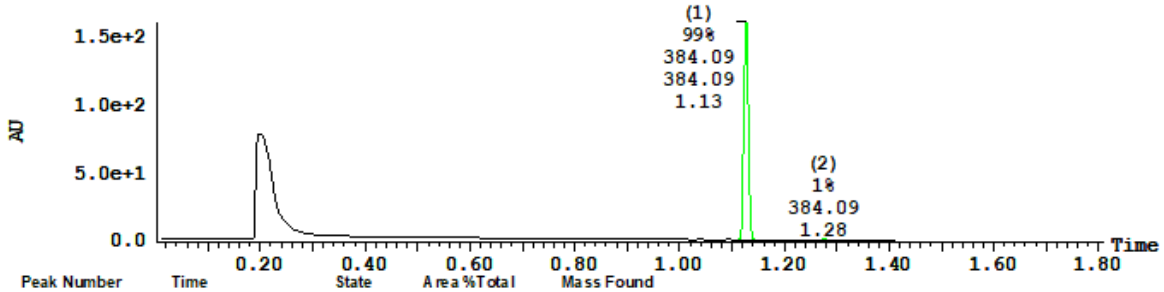
Compound 13

Openlynx Report - CLF Page 1

Vnumber:50551347#868473#A8 Sample ID:1012918829 Description:9700394710;A8
Vial:7:8,A Date:09-May-2019 Time:17:43:50
Instrument:ACQ-SQD#LBA695 Method:N:\UPLC2_Nugenesis_P30_N30@B5601B5601.olp

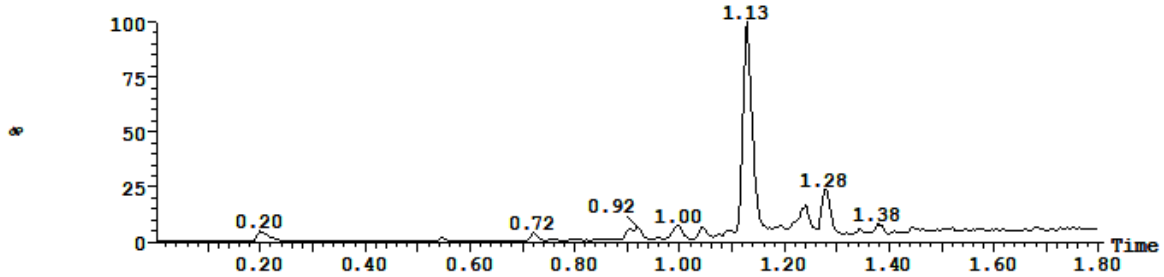
Printed: Fri May 10 11:41:56 2019

3: UV Detector: TIC 1.591e+2
Range: 1.62e+2

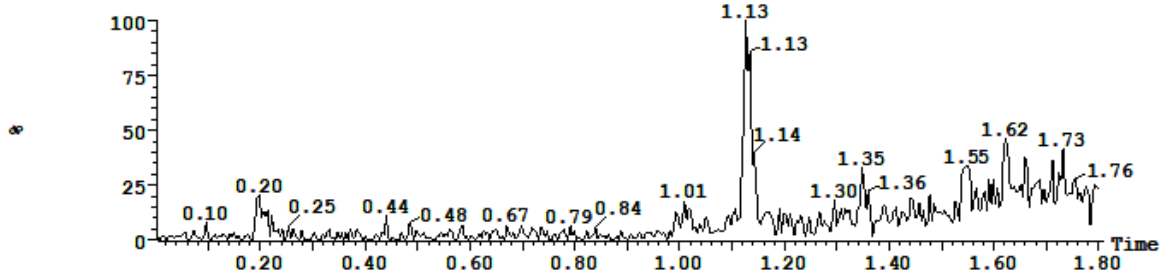


Peak Number	Time	State	Area %Total	Mass Found
1	1.13	OK	99.29	384.09, 384.09
2	1.28	Diversity fail	0.71	384.09

1: MS ES+ :TIC 1.0e+008



2: MS ES- :TIC 5.1e+005



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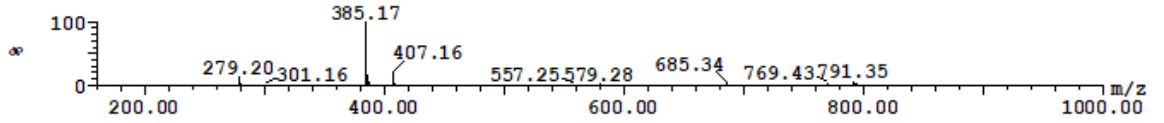
2.7e+007



Vnumber:50551347#868473#A8
Vial:7:8,A
Instrument:ACQ-SQD#LBA695

Sample ID:1012918829
Date:09-May-2019
Method:N:\UPLC2_Nugenesis_P30_N30@B5601B5601.olp

Description:9700394710;A8
Time:17:43:50

Printed: Fri May 10 11:41:56 2019

Peak ID	Time	Mass Found	BPM	State
1	1.13	443.09,383.09	443	OK

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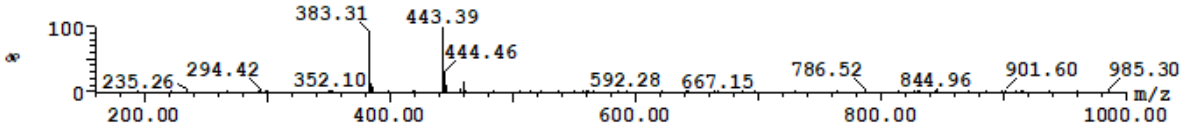
2:MS ES-
7.5e+004



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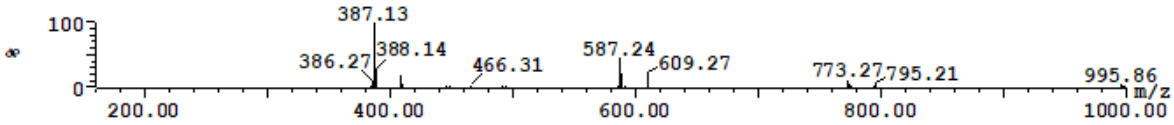
1:MS ES+
3.6e+006



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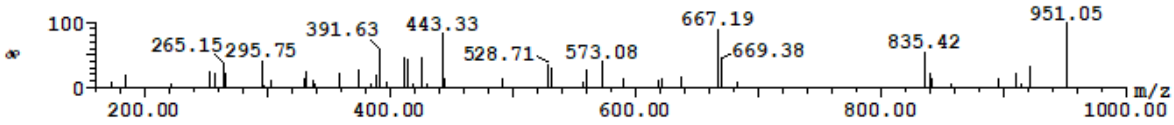
2:MS ES-
2.8e+003



Compound 14

Openlynx Report - CLF

Page 1

Vnumber:44624930#358483#H7
Vial:8:7,H
Instrument:ACQ-SQD#LBA695

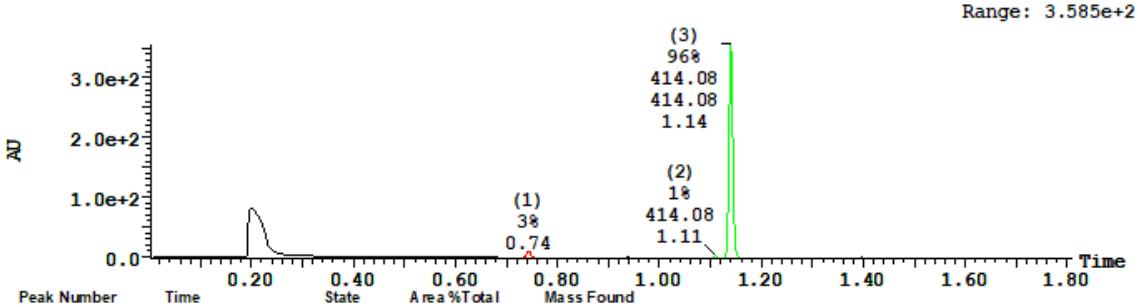
Sample ID:590015244 4
Date:10-Sep-2016
Method:N:\UPLC2_Nugenesis_P30_N30@B5601B5601.olp

Description:2600116311;H7
Time:14:30:40

Printed: Wed Sep 14 13:46:59 2016

3: UV Detector: TIC

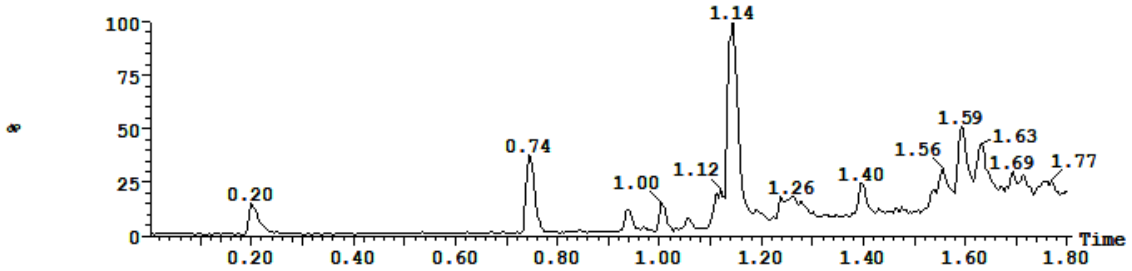
3.566e+2
Range: 3.585e+2



Peak Number	Time	State	Area %Total	Mass Found
1	0.74	Diversity fail	2.96	
2	1.11	OK	0.94	414.08
3	1.14	OK	96.11	414.08, 414.08

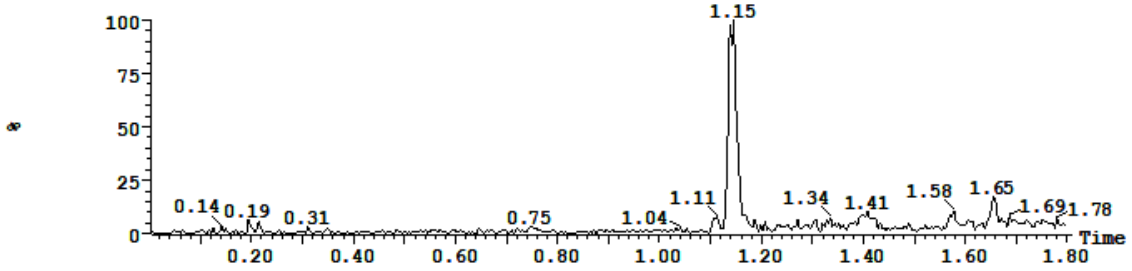
1: MS ES+ :TIC

3.1e+008



2: MS ES- :TIC

1.5e+007



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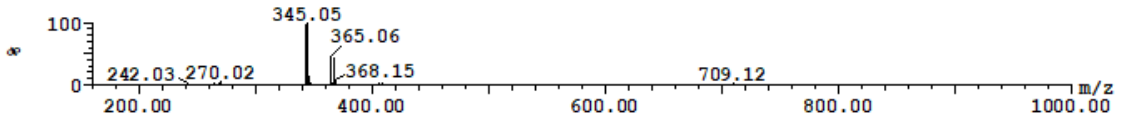
1:MS ES+
1.8e+007



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Date:10-Sep-2016
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Description:2600116311;H7
Time:14:30:40

Printed: Wed Sep 14 13:46:59 2016

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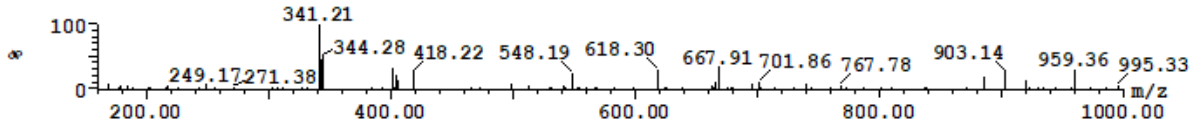
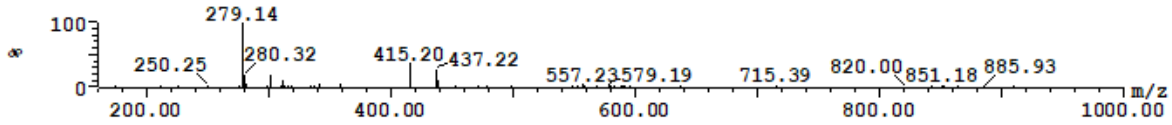
2:MS ES-
4.0e+004



Peak ID	Time	Mass Found	BPM	State
2	1.11	437.08,415.08	279	OK

2:(Time: 1.11) Combine (272:279-(252:264+287:299))

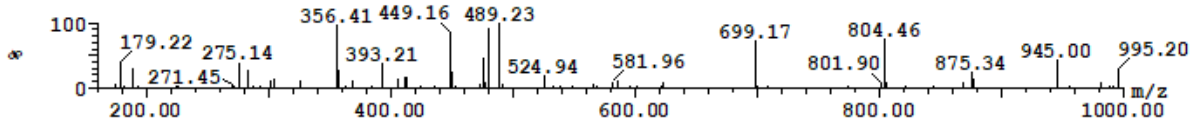
1:MS ES+
5.5e+006



Peak ID	Time	Mass Found	BPM	State
2	1.11		489	Diversity fail

2:(Time: 1.11) Combine (271:279-(252:264+286:299))

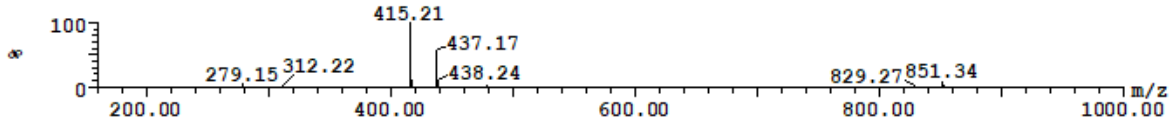
2:MS ES-
1.7e+004



Peak ID	Time	Mass Found	BPM	State
3	1.14	437.08,415.08	415	OK

3:(Time: 1.14) Combine (279:287-(260:272+301:314))

1:MS ES+
7.2e+007



Peak ID	Time	Mass Found	BPM	State
3	1.14	473.08,413.08	473	OK

3:(Time: 1.14) Combine (279:286-(259:272+301:313))

2:MS ES-
3.0e+006