Supporting Information

Generating selective leads for Mer kinase inhibitors – example of a comprehensive lead-generation strategy

J. Willem M. Nissink, Sana Bazzaz, Carolyn Blackett, Matthew A. Clark, Olga Collingwood, Jeremy S. Disch, Diana Gikunju, Kristin Goldberg, John P. Guilinger, Elizabeth Hardaker, Edward J. Hennessy, Rachael Jetson, Anthony D. Keefe, William McCoull, Lindsay McMurray, Allison Olszewski, Ross Overman, Alexander Pflug, Marian Preston, Philip B. Rawlins, Emma Rivers, Marianne Schimpl, Paul Smith, Caroline Truman, Elizabeth Underwood, Juli Warwicker, Jon Winter-Holt, Simon Woodcock, Ying Zhang

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Table SI1. Standard deviations for potency measurements in assays calculated for all compounds. ATP concentrations in potency assays are at K_m, unless stated otherwise.

Assay	ATP concentration	Standard deviation
Mertk	K_m	0.42
Mertk	1 mM	0.53
Axl	K_m	0.30
Tyro3	K_m	0.36
Flt3	K_m	0.52

Table SI2. Mertk activity in assay with increased ATP level (1mM). The Mertk data measured at K_m level of ATP is shown for comparison.

Cpd	Mertk pIC_{50} [ATP]=K_m	Mertk pIC_{50} [ATP]=1mM
1	8.8	8.6
2	6.6	6.1
3	7.0	6.0
4	9.1	8.6
5	7.3	6.5
6	7.3	6.4
7	6.4	5.5[a]
8	6.1	5.3
9	6.9	5.8
10	7.3	5.9
11	8.2	7.3
12	8.5	7.7
13	7.6	6.6
14	8.3	8.4
15	8.3	7.5
16	7.9	6.7[a]
17	7.2	6.5
18	7.6	6.2[a]
19	6.9	6.4
20	7.0	6.1

21	6.1	6.0
22	6.2	5.4
23	6.2	-

Mean of at least two independent measurements unless otherwise stated. See Table S1 for standard deviation of measurement. [a] Tested n=1.

Table SI3. Kinase selectivity profiles of DECL hits **12**-**14**. Inhibition values for panel of kinases, compound tested at 1μM (131, 131, 125 kinases tested for **12**, **13**, **14** respectively).

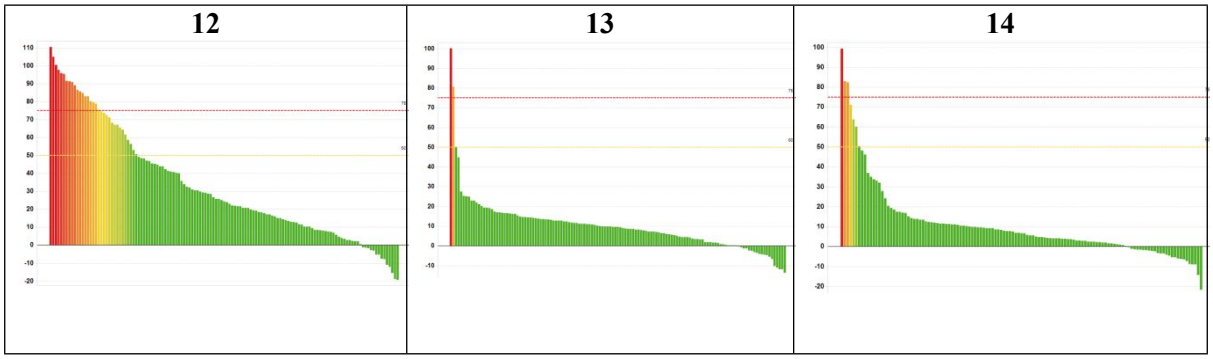


Table SI4. Experimental details for crystal structures.

PDB accession code	Compound #	MerTK protein construct	Experiment Type	Crystallisation conditions
7AVX	**1**	His6-thrombin-MerTK (E571-V864)	Co-Crystallisation	4 M NaCl, 0.1 M Tris pH 8.5
7AVY	**2**	MerTK (E571-V864)	Co-Crystallisation	1.8 M Li2SO4, 0.1 M PCTP pH 6.5
7AVZ	**5**	MerTK (E571-V864) K591R/K693R/K702R/K856R	Soak	4-4.5 M NaCl, 0.08 M Tris pH 8.5
7AW0	**9**	MerTK (E571-V864) K591R/K693R/K702R/K856R	Soak	4-4.5 M NaCl, 0.08 M Tris pH 8.5
7AW1	**11**	MerTK (E571-V864) K591R/K693R/K702R/K856R	Soak	4-4.5 M NaCl, 0.08 M Tris pH 8.5
7AW2	**14**	MerTK (E571-V864) K591R/K693R/K702R/K856R	Soak	4-4.5 M NaCl, 0.08 M Tris pH 8.5
7AW3	**12**	MerTK (E571-V864) K591R/K693R/K702R/K856R	Soak	4-4.5 M NaCl, 0.08 M Tris pH 8.5
7AW4	**13**	His6-thrombin-MerTK (E571-V864)	Co-Crystallisation	0.2 M MgCl2, 25 % PEG3350, 0.1 M PCPT pH 7.5

Table SI5. – Assay descriptions for Axl and Flt3 GLO assays.

In a few instances (as annotated in the tables), Axl and Flt potency was determined in GLO technology assays rather than Rapidfire LCMS assays. Correlation between Rapidfire MS and GLO assay potency data was observed to be good (data not shown). Experimental details for the GLO assays can be found below.

Axl ATP GLO

The ability of inhibitors to cause inhibition of AXL (GST-AXL(464-885) *in vitro* was assessed using the ADP GLO technology (Promega) that quantifies the conversion of ATP to ADP. AXL (25 nM) in assay buffer (20 mM HEPES pH 7.5, 0.005% Brij-35, 0.5 mM TCEP, 10 mM $Mg(OAc)_2$, 0.01% Pluronic-F127) was added to compound plates using a liquid dispenser, Certus. Assay plates were equilibrated for 30 min at rt before initiating the reaction by addition of Axltide (75 μM final) and ATP (140 μM final) substrates in assay buffer. The reaction was allowed to progress for 40 min at rt before the addition of ADP GLO R1 reagent and incubation for 60 min at rt, followed by the addition of ADP GLO R2 reagent and a further 30 min incubation at rt. The plates where then read on an Envision plate reader employing a Luminescent protocol. The dose-response curves were fit to the data using the non-linear regression analysis; 4 parameter logistic smart fit method in the Assay analyser and Condoseo applications of the Genedata Screener® software (Genedata, Inc., Basel, Switzerland).

Flt3 ATP GLO

The ability of inhibitors to cause inhibition of FLT3 (GST-TEV-6His-FLT3(564-993)-Thrombin-Avi-Flag) in vitro was assessed using the ADP GLO technology (Promega) that quantifies the conversion of ATP to ADP. FLT3 (30 nM) in assay buffer (20 mM HEPES pH 7.5, 0.005% Brij-35, 0.5 mM TCEP, 10 mM Mg(OAc)2, 0.01% Pluronic-F127) was added to compound plates using a liquid dispenser, BioRaptr. Assay plates were equilibrated for 30 min at 22 ^{o}C before initiating the reaction by addition of Axltide (30 μM final) and ATP (400 μM final) substrates in assay buffer. The reaction was allowed to progress for 90 min, at 22 ^{o}C, before the addition of ADP GLO R1 reagent and incubation for 60 min at rt, followed by the addition of ADP GLO R2 reagent and a further 60 min incubation at rt. The plates where then read on an Envision plate reader employing a Luminescent protocol. The dose-response curves were fit to the data using the non-linear regression analysis; 4 parameter logistic smart fit method in the Assay analyser and Condoseo applications of the Genedata Screener® software (Genedata, Inc., Basel, Switzerland).

Table SI6. Selection conditions for DECL experiments.

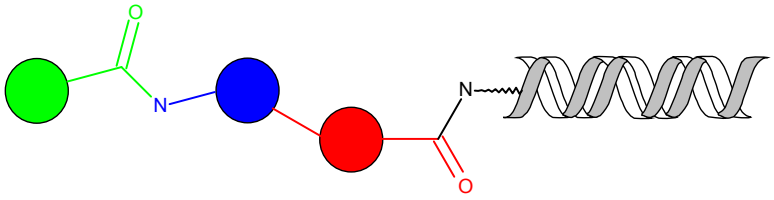
The following constructs and conditions were applied in DECL selection experiments.

Mer-long: construct 528-999; Mer-short: construct 571-864 ; NTC: non-target control

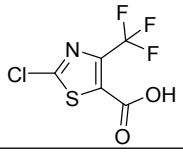
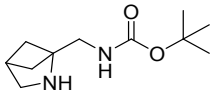
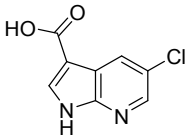
ATP was used at a concentration of 5 mM.

Construct	**State**	**Resin**	**Condition**
NTC		Ni-NTA	
6His-Mer(R528-M999)-Thrombin-Avi-Flag	Activated	Ni-NTA	
6His-Mer(R528-M999)-Thrombin-Avi-Flag	Partially dephosphorylated	Ni-NTA	
6His-Mer(R528-M999)-Thrombin-Avi-Flag	Dephosphorylated	Ni-NTA	
6His-thrombin-Mer(E571-V864)		Ni-NTA	
6His-thrombin-Mer(E571-V864)	Activated	Ni-NTA	
6His-Mer(R528-M999)-Thrombin-Avi-Flag	Partially dephosphorylated	Ni-NTA	+ ATP
6His-thrombin-Mer(E571-V864)		Ni-NTA	+ ATP
6His-thrombin-Mer(E571-V864)	Activated	Ni-NTA	+ ATP
6His-TEV-Kdr(V789-V1356)-LE-Flag		Ni-NTA	
NTC		Glutathione	
GST-Flt3(564-993)		Glutathione	
GST-Axl(464-885)		Glutathione	
GST-Axl(464-885)		Glutathione	+ ATP
GST-Lck(1-509)		Glutathione	

Table SI7. Prevalence table showing the building block structures comprising Family A at each cycle



Cycle A: Heteroaryl chloro/fluoro carboxylic acids
Cycle B: Boc protected diamines
Cycle C: Carboxylic acids

Cycle A	Cycle B	Cycle C
		
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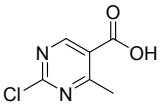		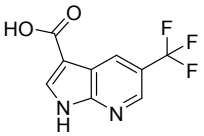
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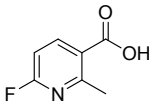		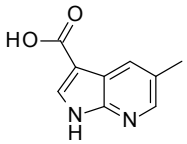
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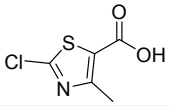
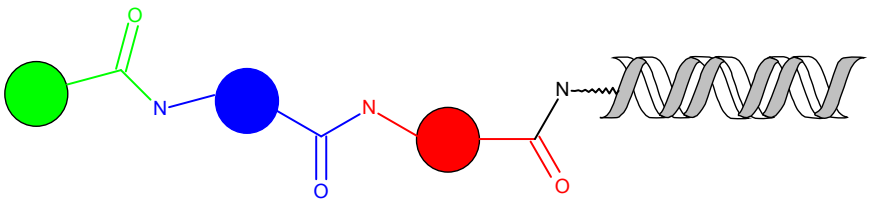
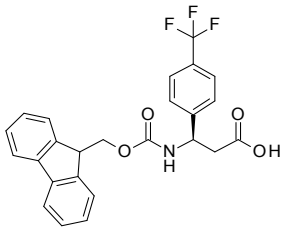
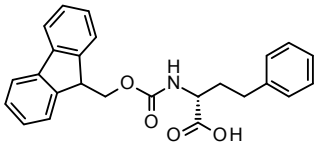
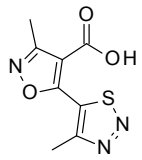
		
6%		

Family prevalence table illustrates the identity of the building block (BB) composition at each cycle of the library for Family A. Percentage below each BB indicates the frequency that a particular BB appeared in all of the members of the family. These structures represent tolerated variability of cycle A and cycle C building blocks of the active chemotype from the selection output data ie. an indication of SAR for structurally related active compounds.

Table SI8. Prevalence table showing the building block structures comprising Family B at each cycle



Cycle A: Fmoc protected amino acids
Cycle B: Fmoc protected amino acids
Cycle C: Carboxylic acids

Cycle A	Cycle B	Cycle C
		
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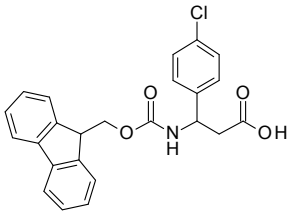	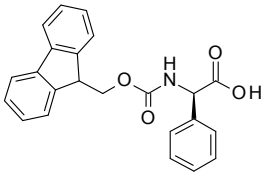	
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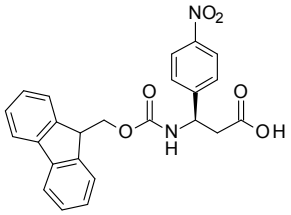	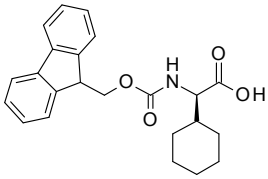	
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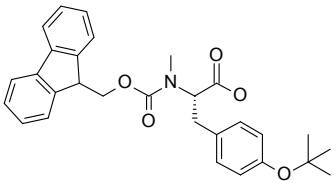
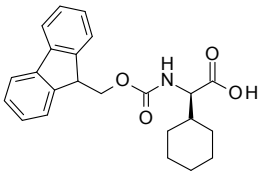
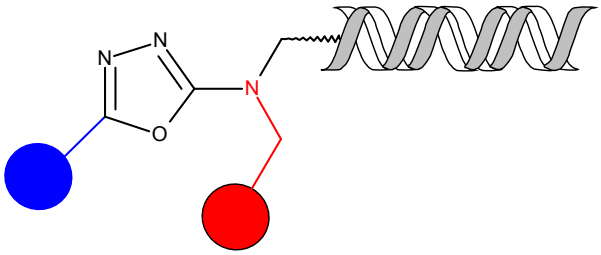
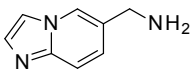
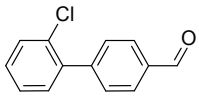
		
1%	9%	

Family prevalence table illustrates the identity of the building block (BB) composition at each cycle of the library for Family B. Percentage below each BB indicates the frequency that a particular building blocks appeared in all of the members of that family. These structures represent tolerated variability of cycle A and cycle B building blocks of the active chemotype from the selection output data. ie. an indication of SAR for structurally related active compounds

Table SI9. Prevalence table showing the building block structures comprising Family C at each cycle



Cycle A: Primary Amines
Cycle B: Aldehydes

Cycle A	Cycle B
	
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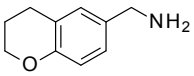	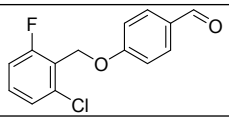
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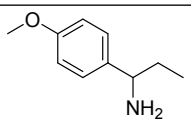	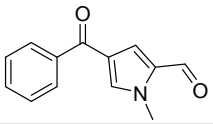
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	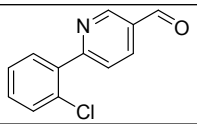
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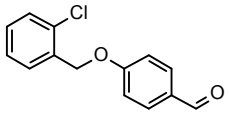
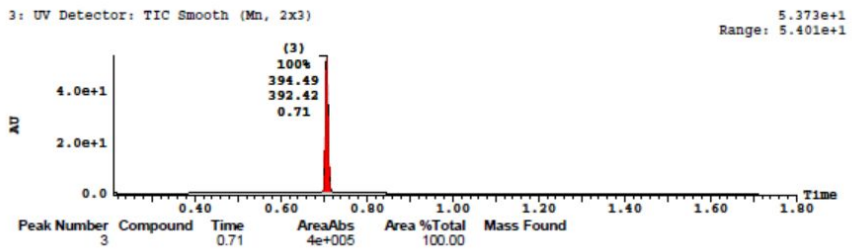
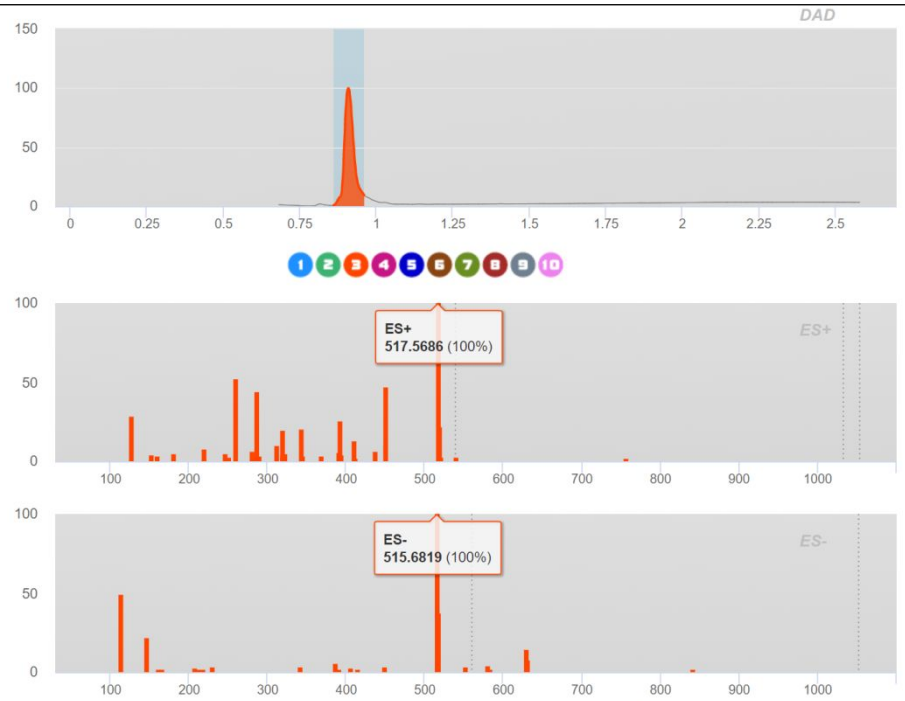
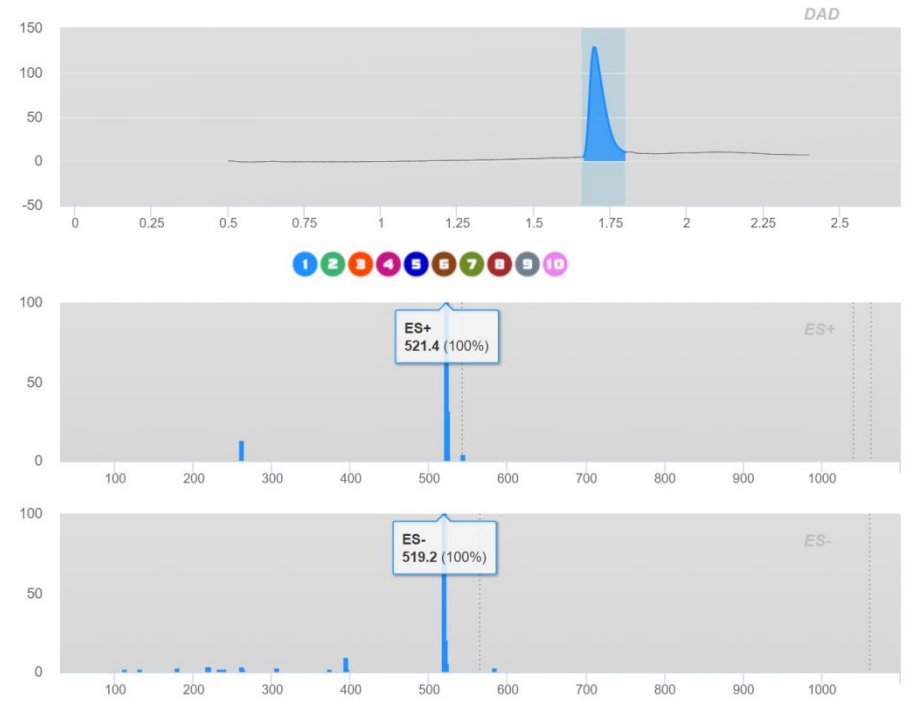
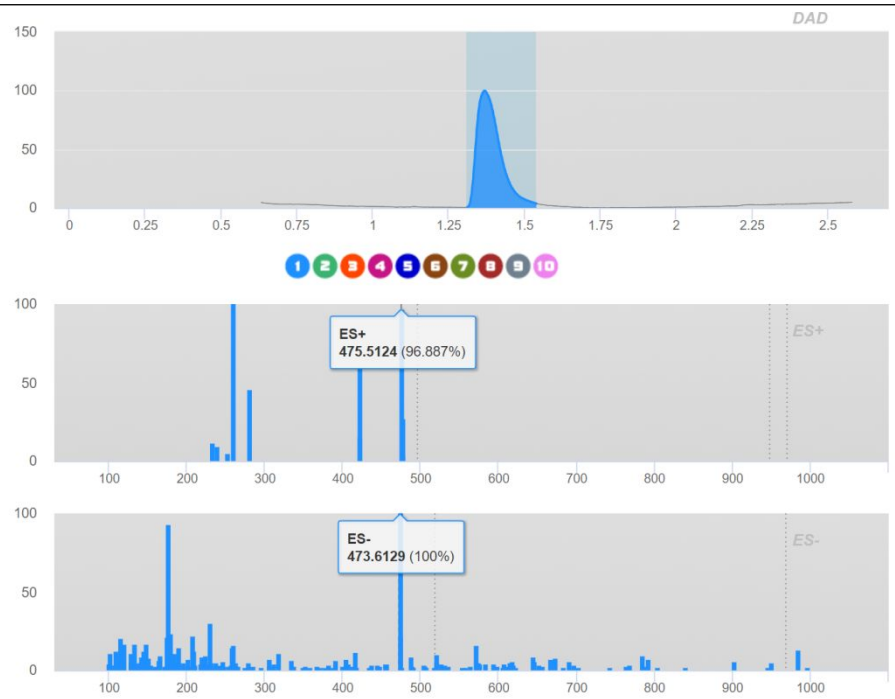
	
	6%

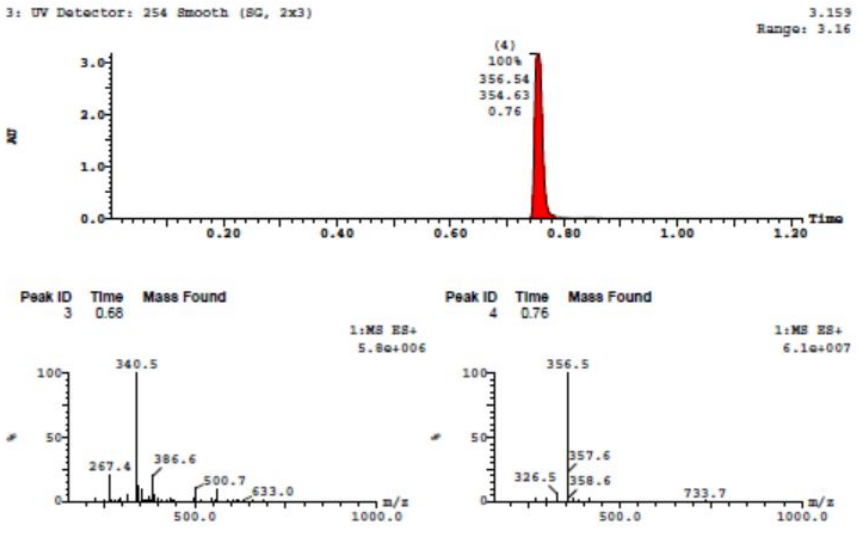
Family prevalence table illustrates the identity of the building block (BB) composition at each cycle of the library for Family C. Percentage below each BB indicates the frequency that a particular BB appeared in all of the members of that family. These structures represent tolerated variability of cycle A and cycle B building blocks of the active chemotype from the selection output data i.e. an indication of SAR for structurally related active compounds. The top 5 entries for cycle B are shown.

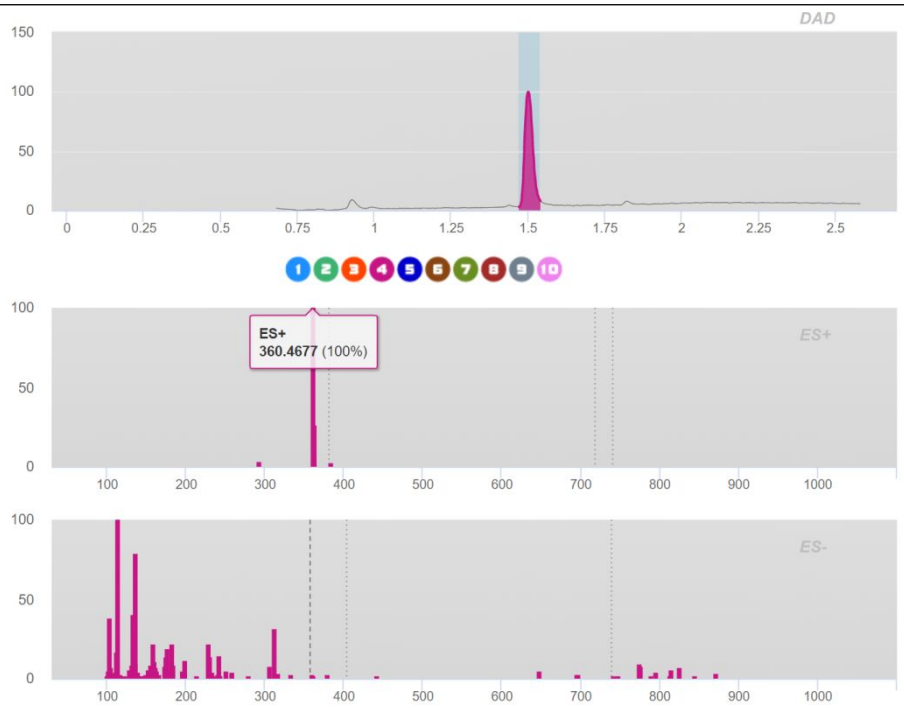
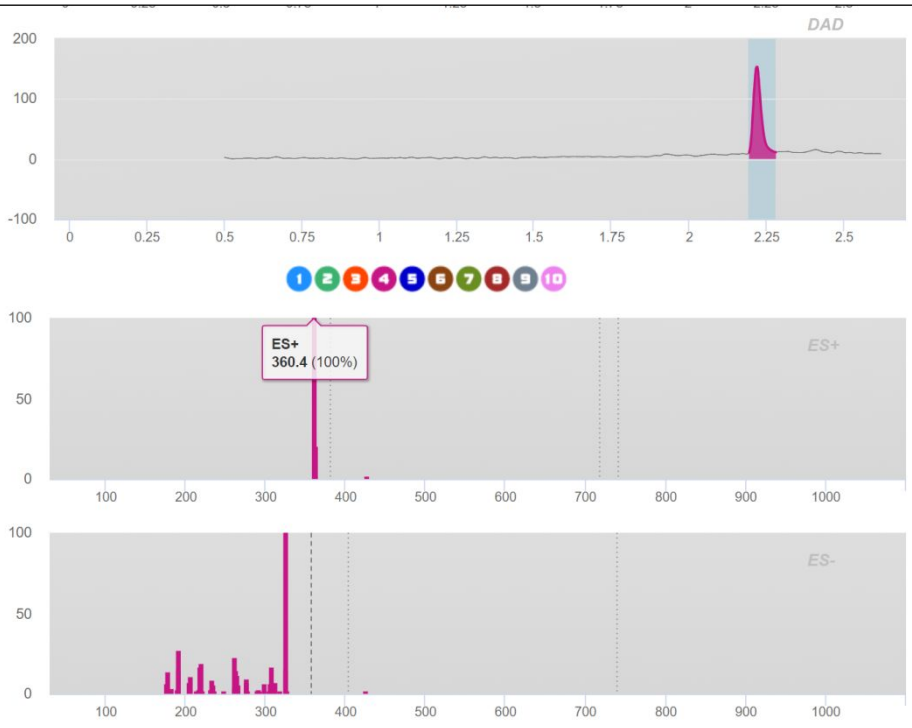
Figure SI10. HPLC traces for compounds. Purity was observed to be >95%.

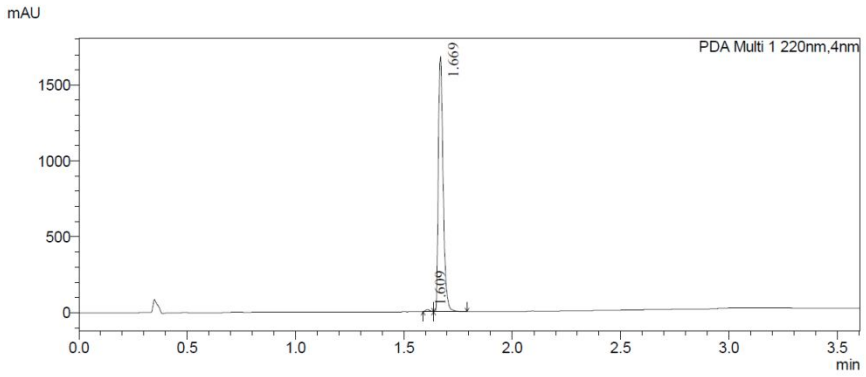
#		Notes
1	1 	^{1}H NMR (500 MHz, DMSO-d_6) 2.69 (s, 3H), 3.35 (s, 3H), 6.28 (d, *J* = 5.5 Hz, 1H), 7.15 – 7.22 (m, 1H), 7.24 – 7.37 (m, 4H), 7.39 – 7.47 (m, 2H), 7.47 – 7.55 (m, 2H), 7.59 (d, *J* = 2.6 Hz, 1H), 7.68 (dd, *J* = 1.2, 8.3 Hz, 2H), 7.91 (dd, *J* = 2.4, 13.1 Hz, 1H), 8.08 (d, *J* = 5.5 Hz, 1H), 10.89 (s, 1H), 12.02 (d, *J* = 2.2 Hz, 1H); HRMS (ES$^+$) for $C_{31}H_{24}N_5O_3F_2$ $(M+H)^+$: calcd 552.1847; found 552.1867.
2	**2** 	^{1}H NMR (500 MHz, DMSO-d_6) -0.1 – 0.05 (m, 2H), 0.26 – 0.35 (m, 2H), 0.64 – 0.76 (m, 1H), 1.31 – 1.46 (m, 2H), 1.53 (q, *J* = 6.8 Hz, 2H), 1.86 (d, *J* = 12.7 Hz, 2H), 1.97 – 2.13 (m, 1H), 2.83 (q, *J* = 12.3 Hz, 2H), 3.22 (d, *J* = 12.3 Hz, 2H), 3.86 (s, 3H), 3.90 (d, *J* = 6.3 Hz, 2H), 4.26 (t, *J* = 6.7 Hz, 2H), 7.23 (s, 1H), 7.86 (s, 1H), 8.35 (d, *J* = 10.3 Hz, 1H), 8.74 (s, 2H); HRMS (ES$^+$) for $C_{24}H_{31}N_6O_3$ $(M+H)^+$: calcd 451.2458; found 451.2471.
3	**3**	^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21 (d, *J* = 18.9 Hz, 4H),

		1.58 (s, 4H), 1.72 (s, 2H), 1.98 – 2.04 (m, 2H), 2.14 – 2.18 (m, 1H), 3.89 (s, 3H), 4.00 (s, 3H), 4.29 (t, *J* = 6.1 Hz, 2H), 6.10 (dd, *J* = 4.0, 2.5 Hz, 1H), 7.03 – 7.08 (m, 1H), 7.17 (dd, *J* = 4.1, 1.7 Hz, 1H), 7.28 (s, 1H), 7.86 (s, 1H), 8.38 (s, 1H), 8.51 (s, 1H), 9.07 (s, 2H), 9.75 (s, 1H), 10.48 (s, 1H); HRMS (ES^+) for $C_{27}H_{33}N_8O_3$ $(M+H)^+$: calcd 517.2676; found 517.2679.
4	**4** 	^1H NMR (300 MHz, DMSO-d_6) δ 1.08 (m, 2H), 1.48 (m, 12H), 1.76 (m, 5H), 1.92 (m, 2H), 2.32 (m, 4H), 2.40 (t, 2H), 3.95 (s, 3H), 4.15 (t, 2H), 4.28 (t, 2H), 7.15 (s, 1H), 7.65 (s, 1H), 8.40 (s, 1H), 8.86 (s, 2H), 9.55 (br s, 1H); HRMS (ES^+) for $C_{29}H_{41}N_6O_3$ $(M+H)^+$: calcd 521.3240; found 521.3256.

5		^{1}H NMR (500 MHz, DMSO-d_6) δ -0.07 – 0.02 (m, 2H), 0.22 – 0.32 (m, 2H), 0.89 – 0.94 (m, 1H), 0.97 (d, *J* = 6.4 Hz, 3H), 1.45 (ddq, *J* = 17.5, 11.9, 5.2 Hz, 2H), 1.61 (d, *J* = 11.5 Hz, 2H), 2.44 (dd, *J* = 18.8, 7.5 Hz, 1H), 2.57 (d, *J* = 8.2 Hz, 1H), 2.68 – 2.8 (m, 2H), 3.05 (s, 2H), 3.17 (t, *J* = 11.2 Hz, 4H), 3.58 (d, *J* = 7.2 Hz, 2H), 3.71 (d, *J* = 8.6 Hz, 2H), 3.88 (dq, *J* = 11.2, 7.6, 5.7 Hz, 1H), 6.17 (d, *J* = 8.0 Hz, 1H), 7.24 – 7.37 (m, 2H), 7.56 (s, 1H), 9.02 (s, 1H) ; HRMS (ES$^+$) for $C_{24}H_{33}N_6O_2F_2$ $(M+H)^+$: calcd 465.2633; found 475.2621.
6	**6** 	^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (s, 2H), 1.54 (s, 5H), 2.84 (d, *J* = 5.1 Hz, 4H), 2.87 – 2.92 (m, 4H), 3.72 – 3.87 (m, 4H), 7.13 (t, *J* = 7.3 Hz, 1H), 7.33 – 7.45 (m, 2H), 7.51 (d, *J* = 8.9 Hz, 2H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.92 (d, *J* = 8.9 Hz, 2H), 8.07 (s, 1H), 8.43 (s, 1H), 9.62 (s, 1H); HRMS (ES$^+$) for $C_{25}H_{31}N_6O_3S$ $(M+H)^+$: calcd 495.2178; found 495.2202

7	**7** 	^{1}H NMR (500 MHz, DMSO-d_6) δ 3.21 (s, 3H), 4.65 (s, 2H), 6.62 (s, 2H), 7.29 (dd, J = 39.6, 6.8 Hz, 7H), 7.72 (s, 1H), 8.25 (s, 1H), 9.12 (s, 1H), 9.59 (s, 1H) ; HRMS (ES$^+$) for $C_{18}H_{19}N_4O_3S$ $(M+H)^+$: calcd 371.1178; found 371.1188.
8		^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 – 1.43 (2H, m), 1.45 – 1.58 (2H, m), 1.58 – 1.69 (2H, m), 1.82 – 1.93 (2H, m), 2.17 (3H, s), 2.69 (3H, d), 3.72 (3H, s), 4.08 – 4.20 (1H, m), 6.71 (1H, dd), 6.77 (1H, d), 7.31 (1H, d), 8.10 (1H, d), 8.32 (1H, s), 8.42 (1H, s), 8.92 (1H, d); HRMS (ES$^+$) for $C_{19}H_{26}N_5O_2$ $(M+H)^+$: calcd 356.2087; found 356.2099

9		^{1}H NMR (400 MHz, DMSO-d_6) δ 1.5 – 2.2 (m, 8H), 2.95 (br s, 3H), 7.50 (q, 1H), 7.75 (br s, 1H), 8.0 (s, 1H); HRMS (ES$^+$) for $C_{18}H_{20}N_5O_2F_2$ $(M+H)^+$: calcd 360.1636; found 360.1648
10		^{1}H NMR (500 MHz, DMSO-d_6) δ 1.7 – 1.81 (m, 2H), 1.82 – 1.93 (m, 2H), 2.03 (dtd, *J* = 14.4, 7.1, 6.4, 4.4 Hz, 2H), 2.61 – 2.7 (m, 1H), 3.33 (s, 3H), 4.17 (d, *J* = 7.0 Hz, 2H), 5.34 (s, 2H), 7.04 – 7.16 (m, 2H), 7.45 (ddd, *J* = 15.0, 8.4, 6.6 Hz, 1H), 7.66 (s, 1H), 7.94 (s, 1H); HRMS (ES$^+$) for $C_{18}H_{20}N_5OF_2$ $(M+H)^+$: calcd 360.1636; found 2360.1648
11		Compound obtained as part of library work; no NMR recorded and confirmed by MS: (ESI) $[M+H]^+$ 593. Purity >95% was confirmed by HPLC

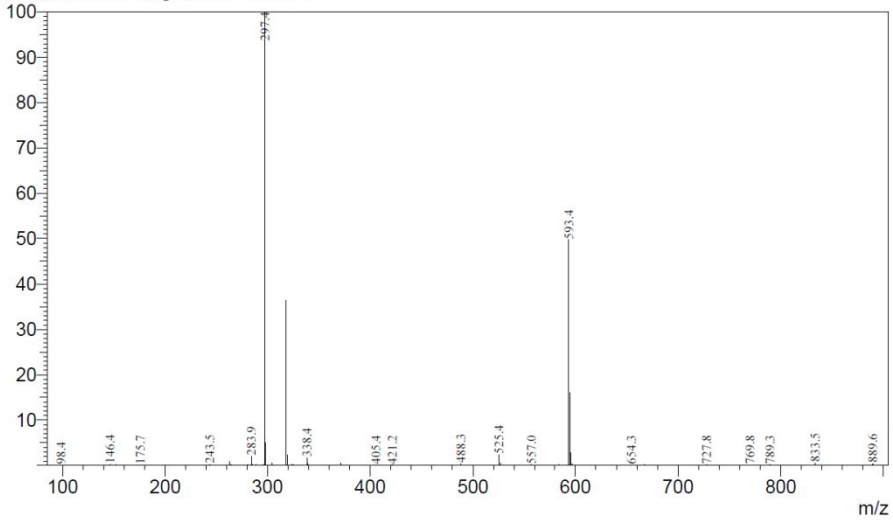


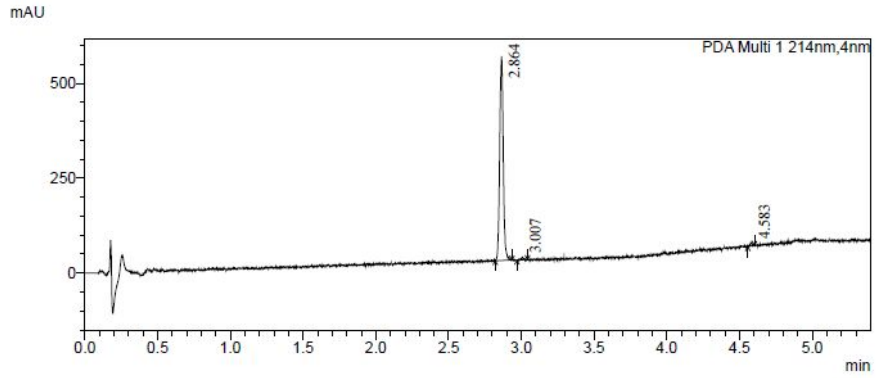
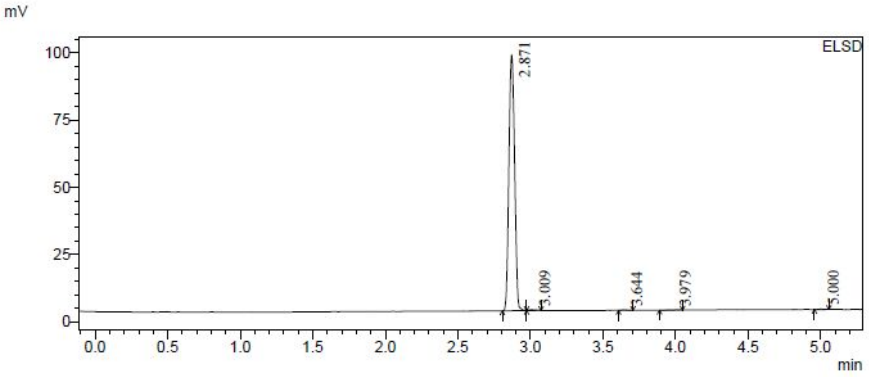
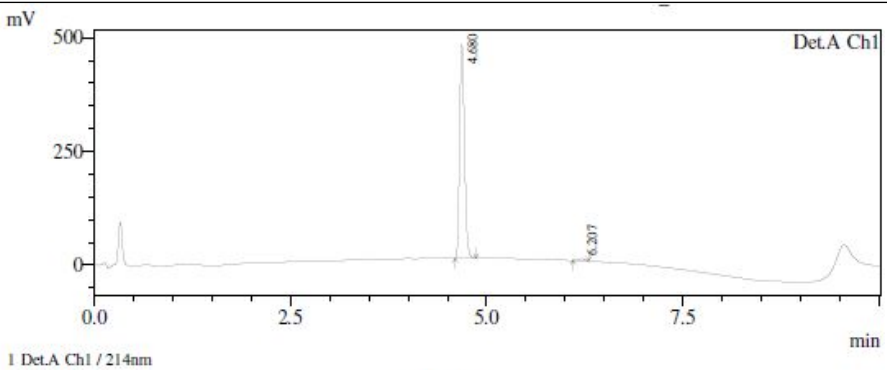
Peak Table

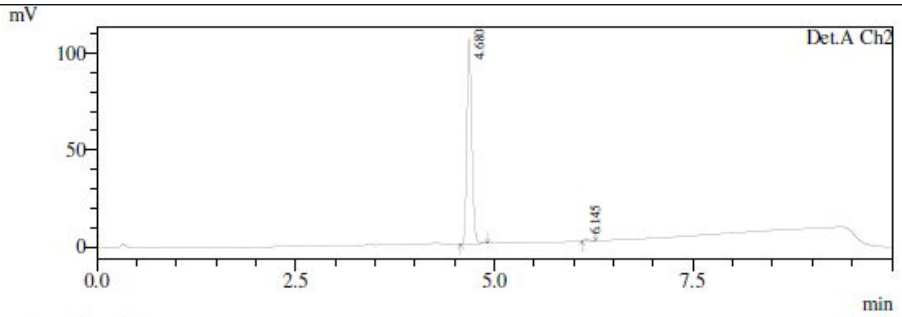
PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.609	12715	0.760	18972	0.734
2	1.669	1659324	99.240	2564158	99.266
Total		1672039	100.000	2583131	100.000

MassPeaks:418
Spectrum Mode:Averaged 1.657-1.673(207-209) BasePeak:297.4(673214)
BG Mode:Calc Segment 1 - Event 1



12	<Chromatogram>  Peak Table PDA Ch1 214nm Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 2.864 | 532655 | 97.329 | 912907 | 98.343 2 | 3.007 | 7415 | 1.355 | 6970 | 0.751 3 | 4.583 | 7203 | 1.316 | 8413 | 0.906 Total | | 547273 | 100.000 | 928290 | 100.000  Peak Table ELSD Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 2.871 | 94281 | 99.375 | 260503 | 99.316 2 | 3.009 | 296 | 0.312 | 850 | 0.324 3 | 3.644 | 98 | 0.103 | 302 | 0.115 4 | 3.979 | 69 | 0.072 | 299 | 0.114 5 | 5.000 | 131 | 0.138 | 342 | 0.130 Total | | 94874 | 100.000 | 262296 | 100.000	white solid. 98.3% (UV 214 nm). LC-MS $(M+H)^+$ = 499.35. HPLC RT_{min} = 2.864 min. ^{1}H NMR (400 MHz, MeOD) δ 1.74 (dd, 2H, *J* = 5.0, 2.0 Hz), 2.16 (m, 2H), 2.88 (s, 3H), 2.98 (t, 1H, *J* = 3.2 Hz), 3.48 (s, 2H), 4.25 (s, 2H), 7.90 (s, 1H), 8.27 (d, 1H, *J* = 2.4 Hz), 8.38 (d, 1H, *J* = 2.4 Hz).
13	 1 Det.A Ch1 / 214nm <Result> PeakTable Detector A Ch1 214nm Peak# | Ret. Time | Area | Height | Area % 1 | 4.680 | 2019104 | 471420 | 98.739 2 | 6.207 | 25792 | 4509 | 1.261 Total | | 2044896 | 475929 | 100.000	white solid. 96.4% (UV 214 nm). LC-MS $(M+H)^+$ = 615. ^{1}H NMR (400 MHz, DMSO) δ 8.88 (m, 2H), 8.12 – 7.43 (m, 5H), 7.40 – 7.02 (m, 3H), 5.28 (s, 1H), 4.47 (s, 1H), 3.20-2.81 (m, 5H), 2.65 – 2.55 (m, 3H), 2.47 – 2.28 (m, 3H), 1.99-1.89 (m, 2H), 1.57 (m, 2H).



1 Det.A Ch2 / 254nm

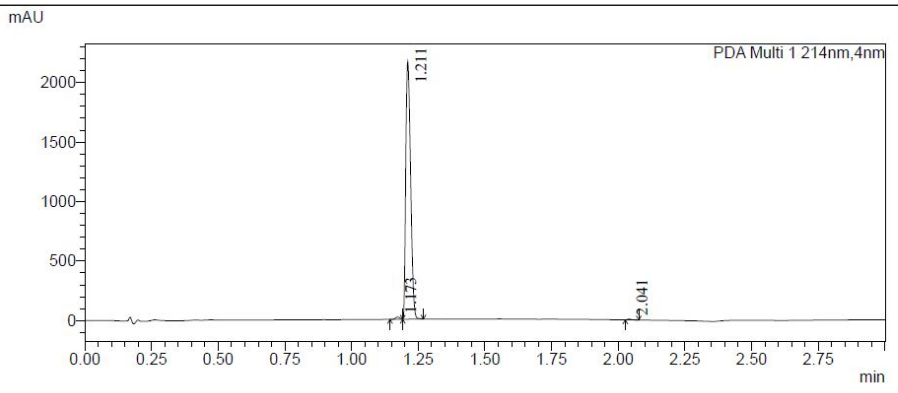
<Result>

PeakTable

Detector A Ch2 254nm

Peak#	Ret. Time	Area	Height	Area %
1	4.680	453205	106289	99.199
2	6.145	3660	777	0.801
Total		456865	107066	100.000

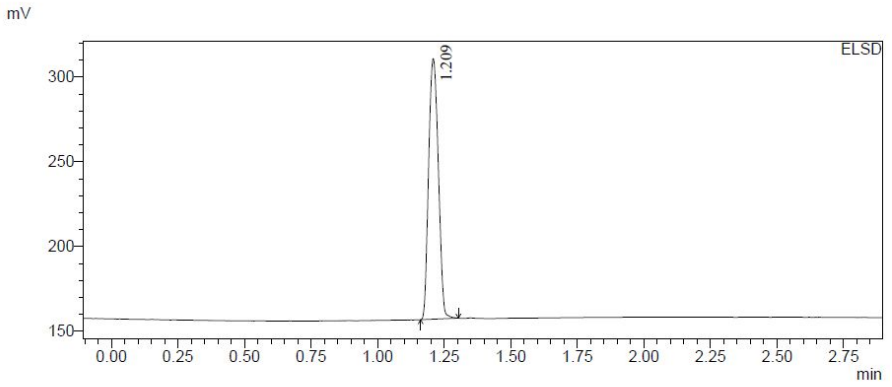
14



Peak Table

PDA Ch1 214nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.173	19301	0.886	21642	0.781
2	1.211	2152883	98.792	2743243	99.002
3	2.041	7014	0.322	6025	0.217
Total		2179198	100.000	2770909	100.000



Peak Table

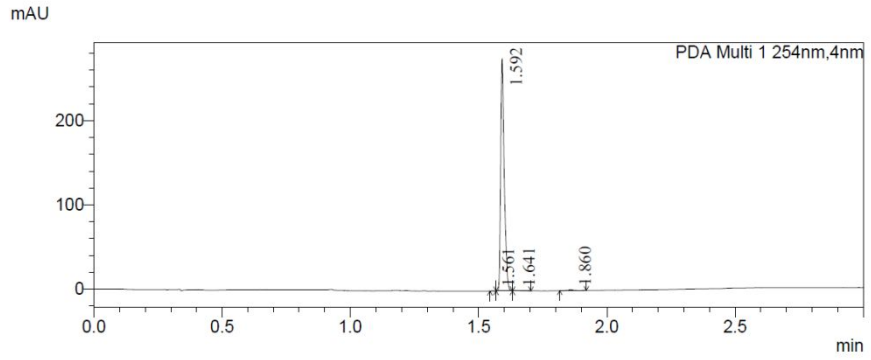
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.209	152394	100.000	390056	100.000
Total		152394	100.000	390056	100.000

white solid. 99.0% (UV 214 nm). LC-MS $(M+H)^+$ = 416. HPLC RT_{min} = 1.211 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (1H, s), 8.32 (1H, d, *J* = 5.6 Hz)), 8.15 (1H, d, *J* = 2.0 Hz), 8.03 - 7.90 (4H, m), 7.66 - 7.56 (3H, m), 7.51 - 7.41 (3H, m), 4.85 (2H, s), 3.19 (3H, s).

15

<Chromatogram>

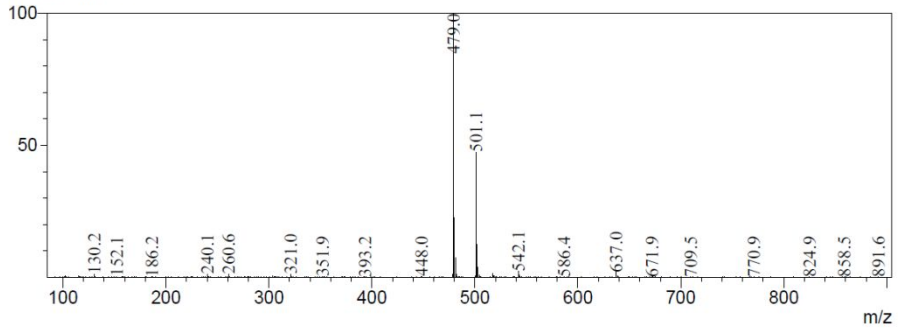


Peak Table
PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.561	178	0.079	167	0.062
2	1.592	223294	99.157	266303	99.004
3	1.641	641	0.285	1070	0.398
4	1.860	1079	0.479	1441	0.536
Total		225192	100.000	268982	100.000

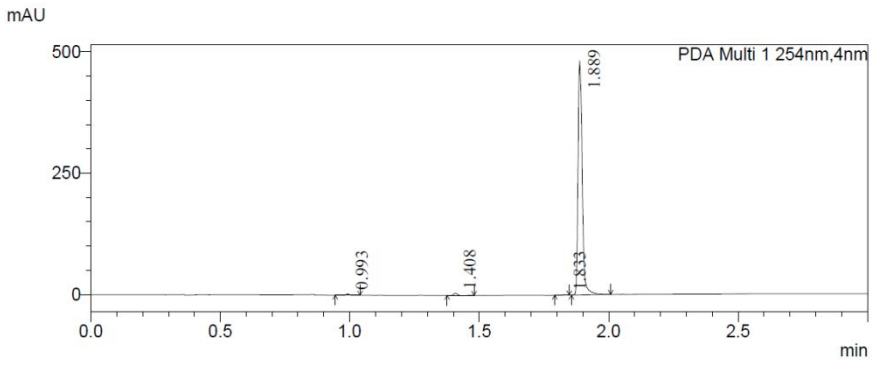
Mass Spectrum

Line#:1 R.Time:1.588(Scan#:197)
MassPeaks:822



16

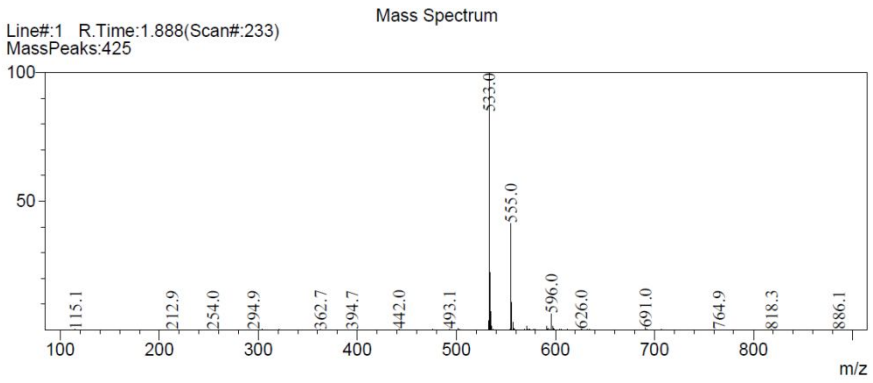
<Chromatogram>



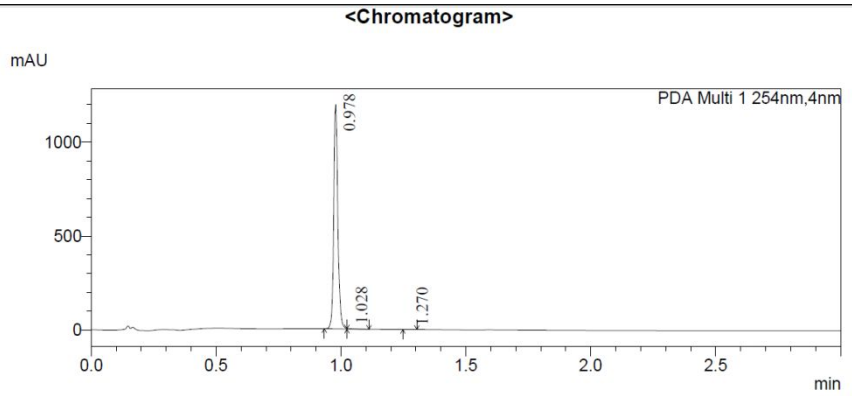
Peak Table
PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.993	1761	0.421	1849	0.336
2	1.408	3676	0.880	4133	0.750
3	1.833	739	0.177	627	0.114
4	1.889	411692	98.522	544619	98.801
Total		417868	100.000	551228	100.000

White solid. ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.59 (2H, d), 2.04 (2H, s), 2.72 (3H, d), 2.86 (1H, s), 3.46 (2H, s), 4.19 (2H, d), 8.24 - 8.39 (3H, m), 8.63 - 8.73 (2H, m), 12.70 (1H, s). HRMS for $C_{21}H_{18}N_6O_2F_6S$ $(M+H)^+$: calcd 533.1194; found 533.1212

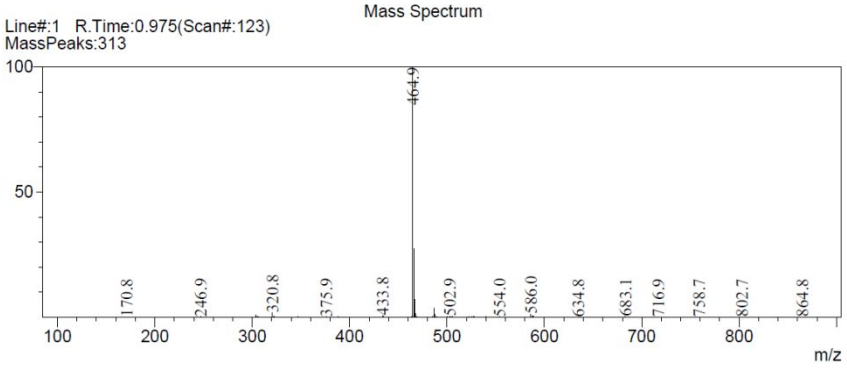

Mass Spectrum
Line#:1 R.Time:1.888(Scan#:233)
MassPeaks:425
100
50
533.0
555.0
115.1
212.9
254.0
294.9
362.7
394.7
442.0
493.1
596.0
626.0
691.0
764.9
818.3
886.1
100
200
300
400
500
600
700
800
m/z

17

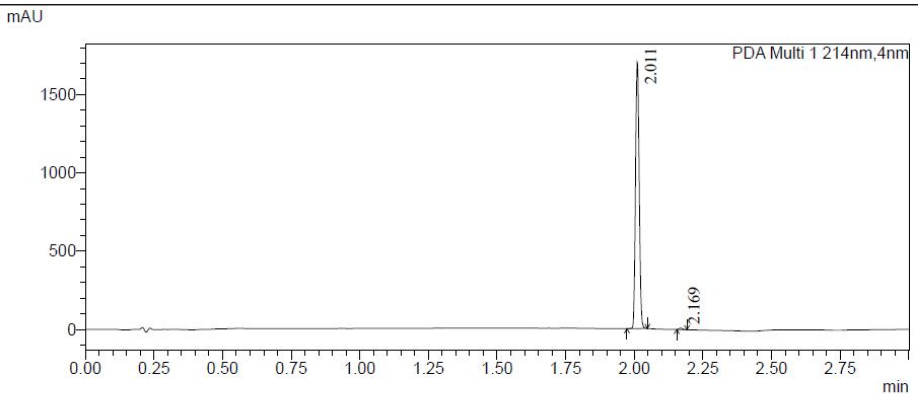
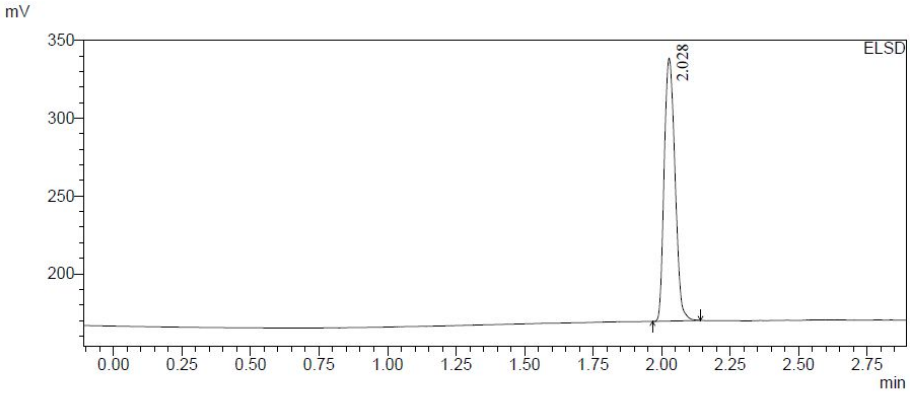


Peak Table
PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.978	1195078	99.683	1338354	99.596
2	1.028	2456	0.205	3440	0.256
3	1.270	1340	0.112	1986	0.148
Total		1198874	100.000	1343780	100.000



^{1}H NMR (DMSO-*d6*, 400 MHz) δ 1.59 (2H, m), 2.04 (2H, s), 2.72 (3H, d), 2.86 (1H, s), 3.45 (2H, s), 4.16 (2H, d), 7.17 (1H, m), 8.00 - 8.09 (2H, m), 8.29 (1H, m), 8.38 (2H, dd), 12.17 (1H, s). HRMS for $C_{20}H_{19}N_6O_2F_3S$ $(M+H)^+$: calcd 465.1321; found 465.1302

18	 Peak Table PDA Ch1 214nm Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 2.011 | 1542774 | 99.380 | 1594277 | 99.466 2 | 2.169 | 9624 | 0.620 | 8567 | 0.534 Total | | 1552397 | 100.000 | 1602843 | 100.000  Peak Table ELSD Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 2.028 | 165962 | 100.000 | 464520 | 100.000 Total | | 165962 | 100.000 | 464520 | 100.000	white solid. 99.5% (UV 214 nm). LC-MS $(M+H)^+$ = 432. HPLC RT_{min} = 2.011 min. ^1H NMR (400 MHz, CD_3OD) δ 8.03 – 7.96 (2H, m), 7.63 – 7.56 (2H, m), 7.53 (1H, dd, *J* = 6.2, 1.8 Hz), 7.45 – 7.34 (3H, m), 7.11 – 7.05 (2H, m), 6.77 – 6.70 (1H, m), 4.60 (2H, s), 4.19 – 4.12 (2H, m), 3.09 (3H, s), 2.78 (2H, t, *J* = 6.4 Hz), 2.08 – 1.93 (2 H, m).

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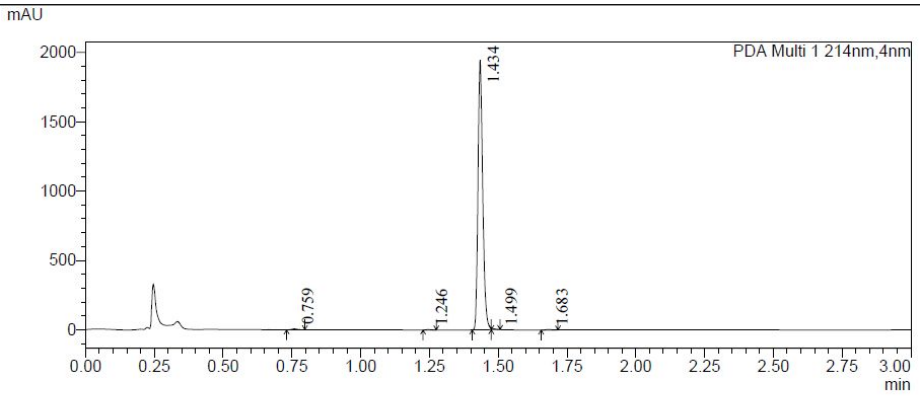
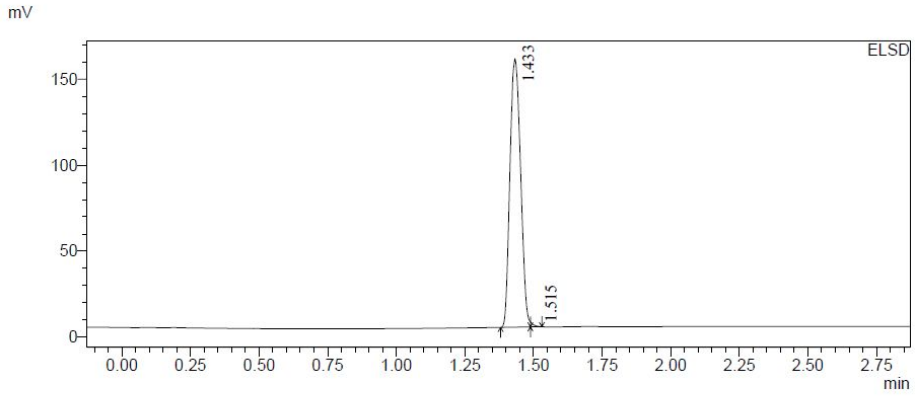


Peak Table

PDA Ch1 214nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.759	6443	0.329	8870	0.377
2	1.246	2756	0.141	3099	0.132
3	1.434	1944837	99.284	2325420	98.943
4	1.499	1166	0.060	7847	0.334
5	1.683	3667	0.187	5029	0.214
Total		1958870	100.000	2350265	100.000



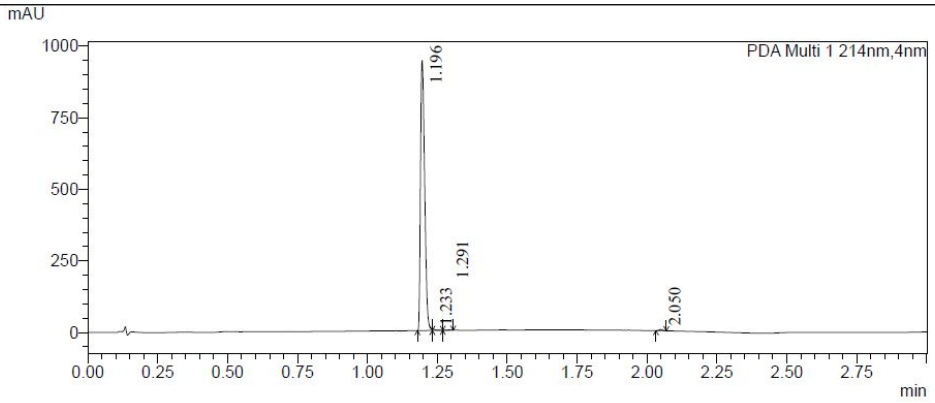
Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.433	153170	99.731	422176	99.571
2	1.515	413	0.269	1820	0.429
Total		153583	100.000	423996	100.000

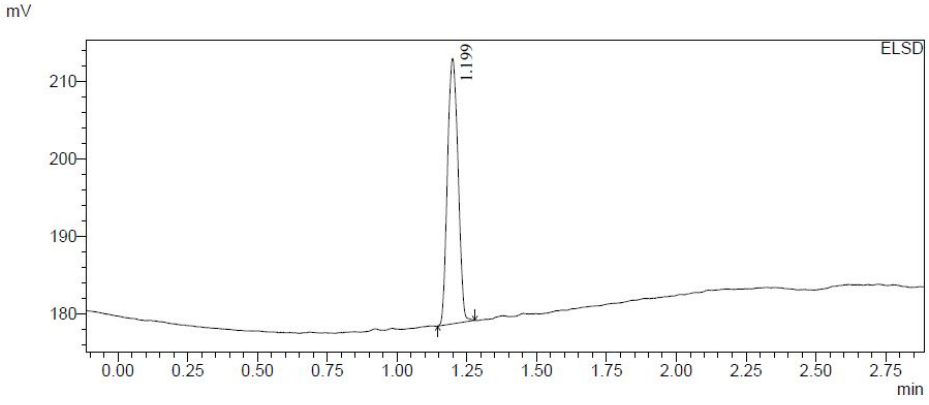
white solid. 98.9% (UV 214 nm). LC-MS $(M+H)^+$ = 417. HPLC RT_{min} = 1.434 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.14 (3H, s), 4.72 (2 H, s), 7.28 (1H, dd, *J* = 9.2, 1.6 Hz), 7.46 – 7.56 (2H, m), 7.55 – 7.70 (4 H, m), 7.87 (1H, d, *J* = 8.4 Hz), 7.97 (1H, s), 8.38 (1H, dd, *J* = 8.2, 2.2 Hz), 8.65 (1H, s), 9.18 – 9.24 (1H, m).

20



Peak Table
PDA Ch1 214nm

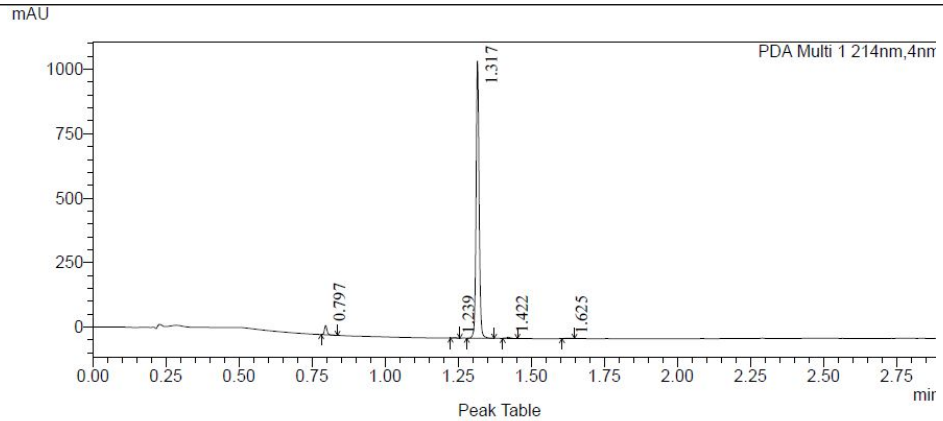
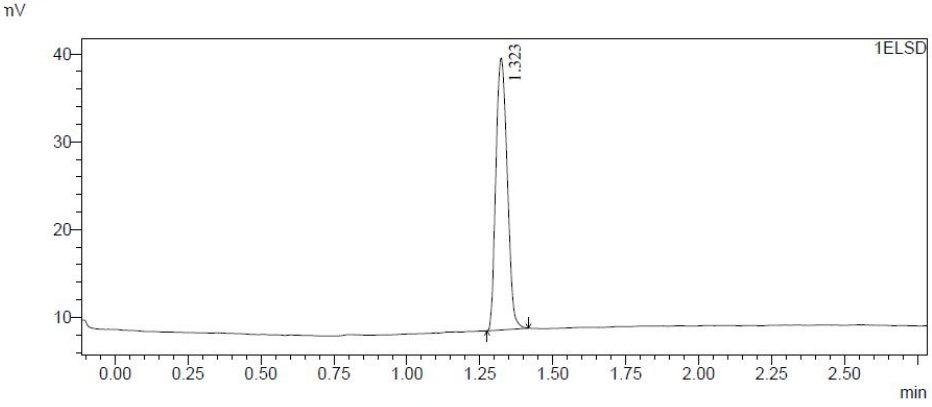
Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.196	885102	98.872	970790	99.179
2	1.233	4731	0.528	3126	0.319
3	1.291	896	0.100	1131	0.116
4	2.050	4468	0.499	3782	0.386
Total		895197	100.000	978829	100.000



Peak Table
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.199	33869	100.000	90493	100.000
Total		33869	100.000	90493	100.000

white solid. 99.2% (UV 214 nm). LC-MS $(M+Na)^+$ = 390. HPLC RT_{min} = 1.196 min. ^1H NMR (400 MHz, $CDCl_3$) δ 1.50-1.75 (6H, m), 1.88-1.99 (3 H, m), 3.10-3.15 (3 H, s), 4.75-4.86 (2H, s), 4.86-4.95(1H, t, *J* = 5.8 Hz), 7.01-7.08 (2H, d, *J* = 8.8 Hz), 7.75-7.83 (2H, d, *J* = 8.8 Hz), 7.92-8.03 (2H, m) , 8.16-8.21 (1H, s) , 8.35-8.36 (1H, s) , 8.88-9.01 (1H, s).

21	 Peak Table PDA Ch1 214nm Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 0.797 | 35666 | 3.224 | 21870 | 2.697 2 | 1.239 | 1823 | 0.165 | 1253 | 0.155 3 | 1.317 | 1062929 | 96.094 | 781565 | 96.400 4 | 1.422 | 4318 | 0.390 | 4152 | 0.512 5 | 1.625 | 1400 | 0.127 | 1912 | 0.236 Total | | 1106135 | 100.000 | 810752 | 100.000  Peak Table ELSD Peak# | Ret. Time | Height | Height% | Area | Area% 1 | 1.323 | 30700 | 100.000 | 83984 | 100.000 Total | | 30700 | 100.000 | 83984 | 100.000	white solid. 96.4% (UV 214 nm). LC-MS $(M+H)^+$ = 409. HPLC RT_{min} = 1.317 min. ^{1}H NMR (400 MHz, MeOD) δ 3.25-3.27 (3H, s), 4.76-4.85 (2 H, s), 7.44-7.48 (1 H, dd, *J* = 9.2, 1.2 Hz), 7.58-7.70 (2H, m), 7.87-7.93(1H, s), 8.45-8.48(1H, s), 8.58-8.62 (1H, s), 8.93-8.98 (1H, s).

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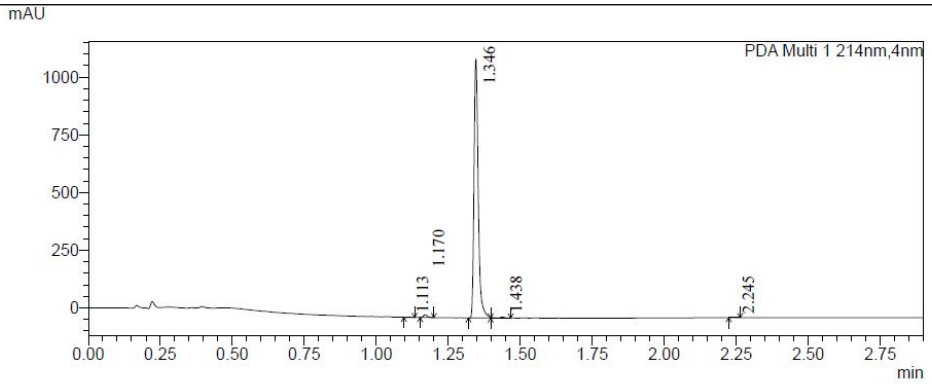
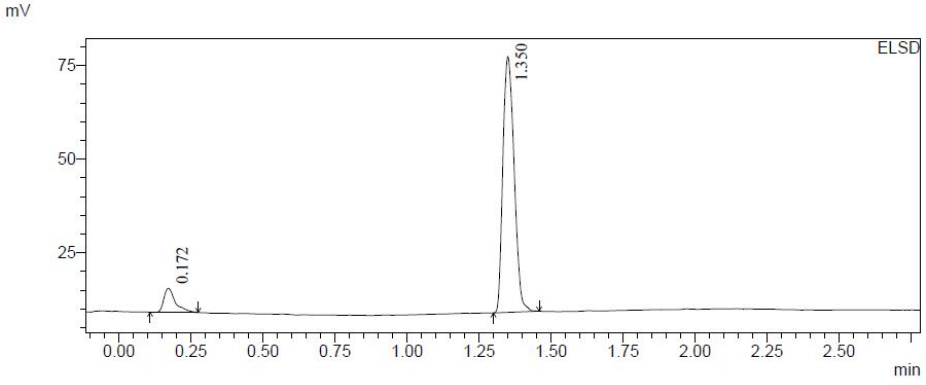


Peak Table
PDA Ch1 214nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.113	1141	0.101	939	0.081
2	1.170	11804	1.040	11140	0.959
3	1.346	1117119	98.449	1142775	98.392
4	1.438	3697	0.326	5633	0.485
5	2.245	959	0.085	966	0.083
Total		1134720	100.000	1161453	100.000



Peak Table
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.172	6319	8.659	17526	8.600
2	1.350	66659	91.341	186265	91.400
Total		72979	100.000	203791	100.000

white solid. 98.4% (UV 214 nm). LC-MS $(M+H)^+$ = 363. HPLC RT_{min} = 1.346 min. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.52 (3 H, s), 2.97 (6 H, s), 3.08 (3 H, s), 4.64 (2 H, s), 6.60 - 6.67 (2 H, m), 7.25 (1 H, d, J = 9.6 Hz), 7.54 - 7.65 (3 H, m), 7.97 (1 H, s), 8.61 (1 H, s).

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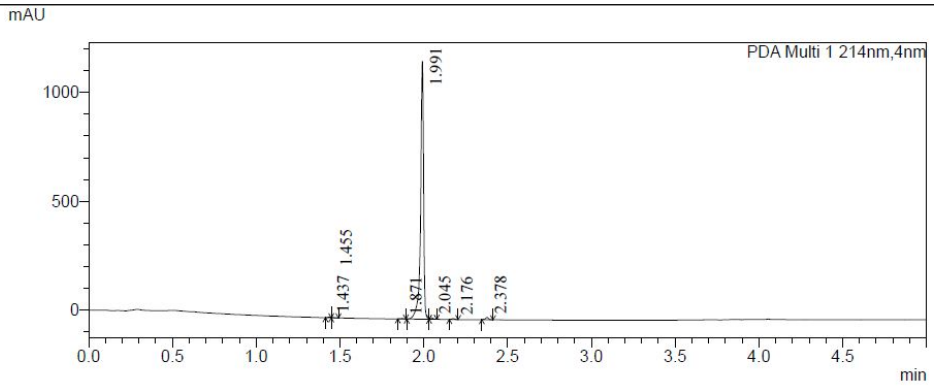
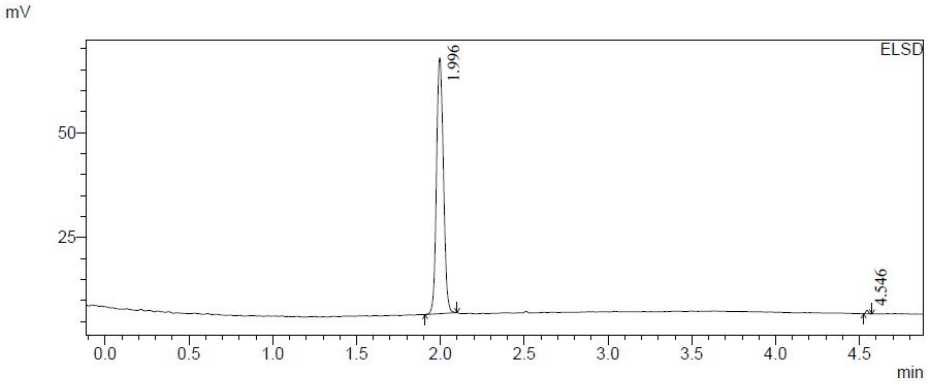


Peak Table
PDA Ch1 214nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.437	2604	0.215	3121	0.204
2	1.455	2077	0.172	1917	0.125
3	1.871	2066	0.171	2799	0.183
4	1.991	1182295	97.691	1496589	97.833
5	2.045	7043	0.582	8221	0.537
6	2.176	3814	0.315	4272	0.279
7	2.378	10338	0.854	12817	0.838
Total		1210237	100.000	1529736	100.000



Peak Table
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.996	60608	98.705	175229	99.452
2	4.546	795	1.295	965	0.548
Total		61403	100.000	176194	100.000

white solid. 97.8% (UV 214 nm). LC-MS $(M+H)^+$ = 374. HPLC RT_{min} = 1.991 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (3 H, t, J = 7.0 Hz), 3.17 (3 H, s), 4.69 – 4.80 (4 H, m), 7.24 – 7.45 (3 H, m), 7.56 – 7.65 (2 H, m), 7.71 – 7.81 (2 H, m), 7.96 (1 H, d), 8.61 – 8.67 (1 H, m).