

ELECTRONIC SUPPORTING INFORMATION

Identification and Validation of Novel Microtubule Suppressors with an Imidazopyridine Scaffold through Structure-Based Virtual Screening and Docking.

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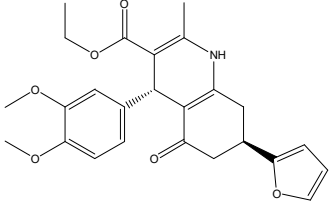
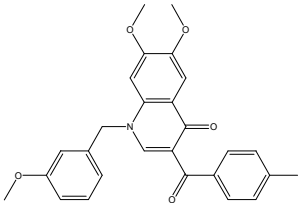
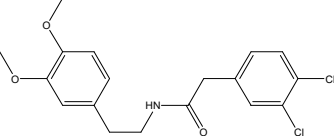
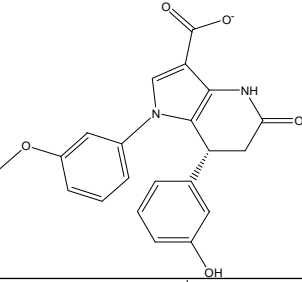
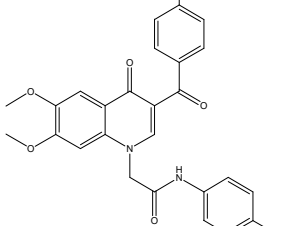
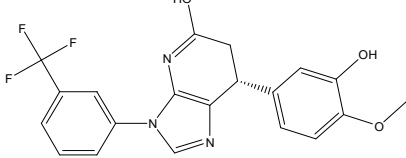
Figure S3: Chemical and geometric properties of the best 99 compounds from virtual screening and 50 ligands from complexes of known structure.

Figure S4: RMSD plots of the molecular dynamics simulations.

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Table S1: Chemical structure of shortlisted compounds and their binding energies by BUDE, AutoDock, and MOE.

Cp.	ZINC-ID	Structure	BUDE binding energy kj/mol	AutoDock binding energy Kcal/mol	MOE Binding Energy kcal/mol
1	Zinc02843810		-111.013	-9.79	-6.28
2	Zinc02691641		-92.82	-10.31	-7.2
3	Zinc03614688		-91.78	-7.87	-5.2
4	Zinc49543397		-104.3	-9.14	-8.01
5	Zinc02690781		-100.75	-10.08	-8.24
6	Zinc36360243		-100.9	-8.57	-10.88

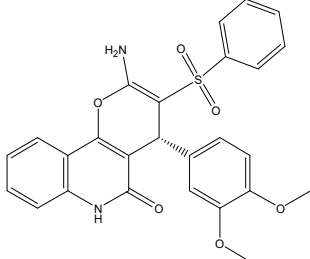
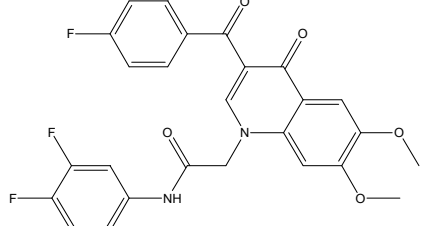
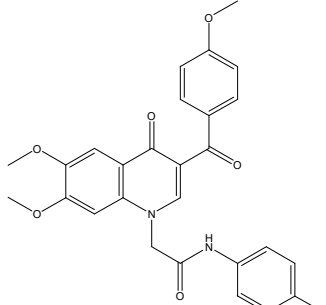
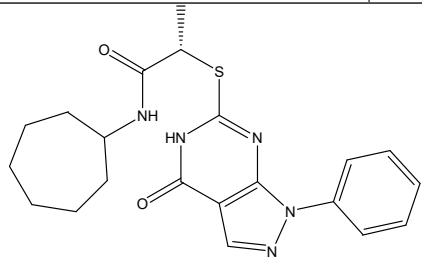
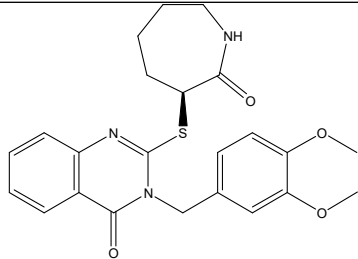
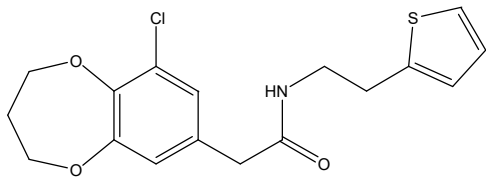
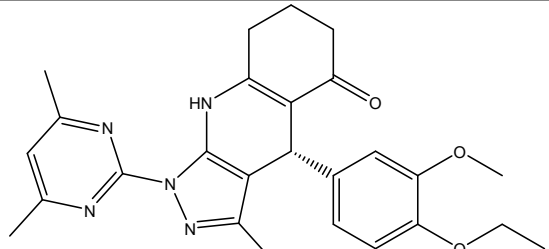
7	Zinc18200970		-125.35	-9.7	-6.9
8	Zinc02690805		-119.48	-9.65	-9.61
9	Zinc02690789		-108.21	-10.02	-8.93
10	Zinc07095120		-83.65	-9.79	-5.5
11	Zinc11112053		-83.66	-9.9	-6.32
12	Zinc23483881		-84.55	-9.9	-5.7
13	Zinc09373064		105.95	-10.91	-6.36

Table S2: Physical properties of shortlisted compounds calculated by MOE.

Compounds	ZINC ID	MwSt	Log p (O/W)	H-bond donors	H-bond acceptors	tPSA	Rotatable bond
1	Zinc02843810	437.49	2.6	1	4	87.00	7
2	Zinc02691641	443.49	4.6		5	65.07	5
3	Zinc03614688	368.26	4.08	1	3	47.56	3
4	Zinc49543397	377.37	3.16	2	3	103.62	4
5	Zinc02690781	474.48	3.79	1	5	84.94	8
6	Zinc36360243	403.36	3.9	2	5	79.87	4
7	Zinc18200970	490.53	3.4	2	5	116.95	5
8	Zinc02690805	496.44	3.8	1	5	84.94	8
9	Zinc02690789	490.48	3.4	1	6	94.17	9
10	Zinc07095120	411.53	4.05	2	4	88.38	6
11	Zinc11112053	439.53	3.7	1	5	80.23	6
12	Zinc23483881	351.85	3.3	1	3	47.56	6
13	Zinc09373064	459.53	3.2	1	6	91.16	5

Table S3 The effect of compound **6** on the phases of the MCF7 cell cycle.

compounds	%G0-G1	%S	%G2-M	%Pre G1
Control	53.91	42.71	3.38	1.48
6	36.28	29.66	34.06	27.36

Table S4 Hydrogen bonding of compounds **6,8** and **9** docked into the colchicine binding site.

Cp	Interacting moiety in compound	Amino acid involved	Distance Å	Type of interaction
6	OH-imidazopyridine OH-phenyl	C=O Thr α 179	2.5	H-bond
		NH Ala β 250	3.1	H-bond
8	C=O C=O	SH Cys β 241	3.1	H-bond
		OH Ser α 178	2.9	H-bond
9	OCH ₃ C=O	SH Cys β 241	3.6	H-bond
		OH Ser α 178	3.0	H-bond

Table S5 Average RMSD values (Å) of complexes of **6, 8, 9, 14, 15** and colchicine with tubulin over 1.5 μ s of simulation.

Å	6	8	9	14	15	colchicine
Average RMSD ligand	1.4 \pm 0.3	3.4 \pm 0.5	2.2 \pm 0.8	2.2 \pm 0.8	1.7 \pm 0.3	1.5 \pm 0.4
Average RMSD protein (AB)	2.5 \pm 0.2	2.5 \pm 0.2	2.4 \pm 0.2	2.6 \pm 0.3	2.6 \pm 0.3	2.5 \pm 0.2

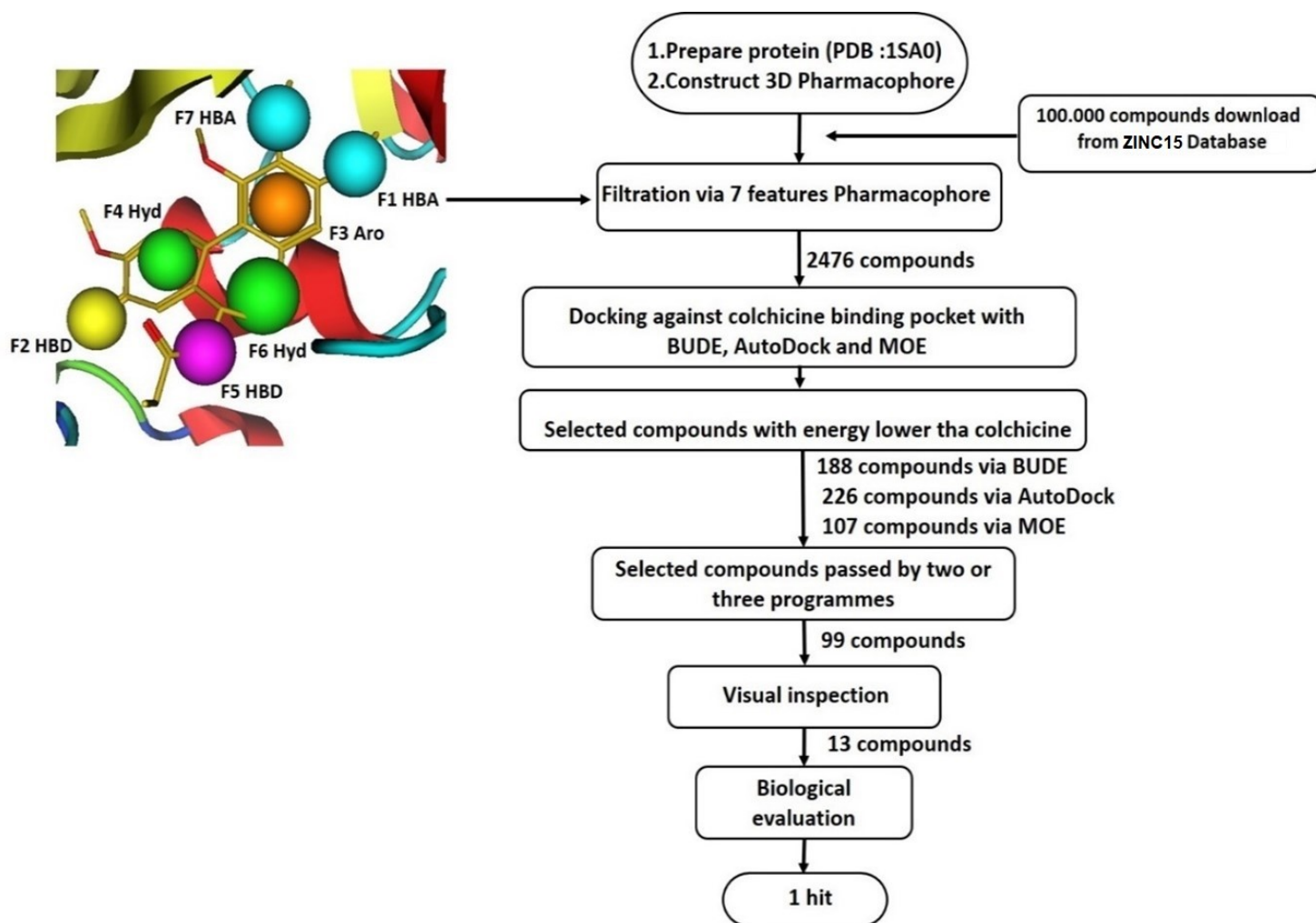


Figure S1 Schematic view of pharmacophore structure-based virtual screening.

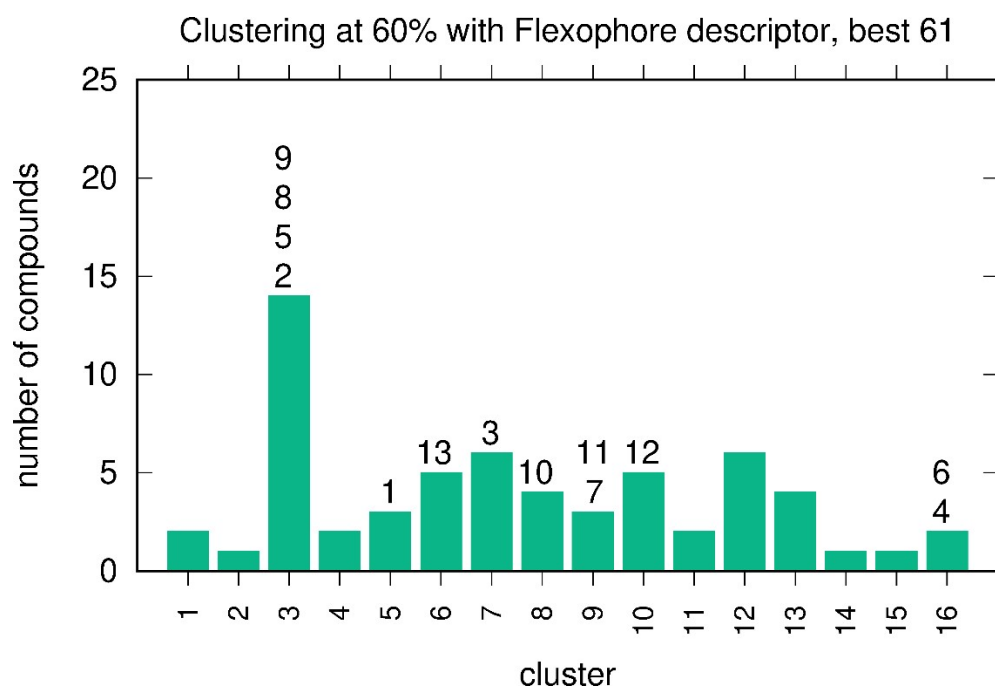
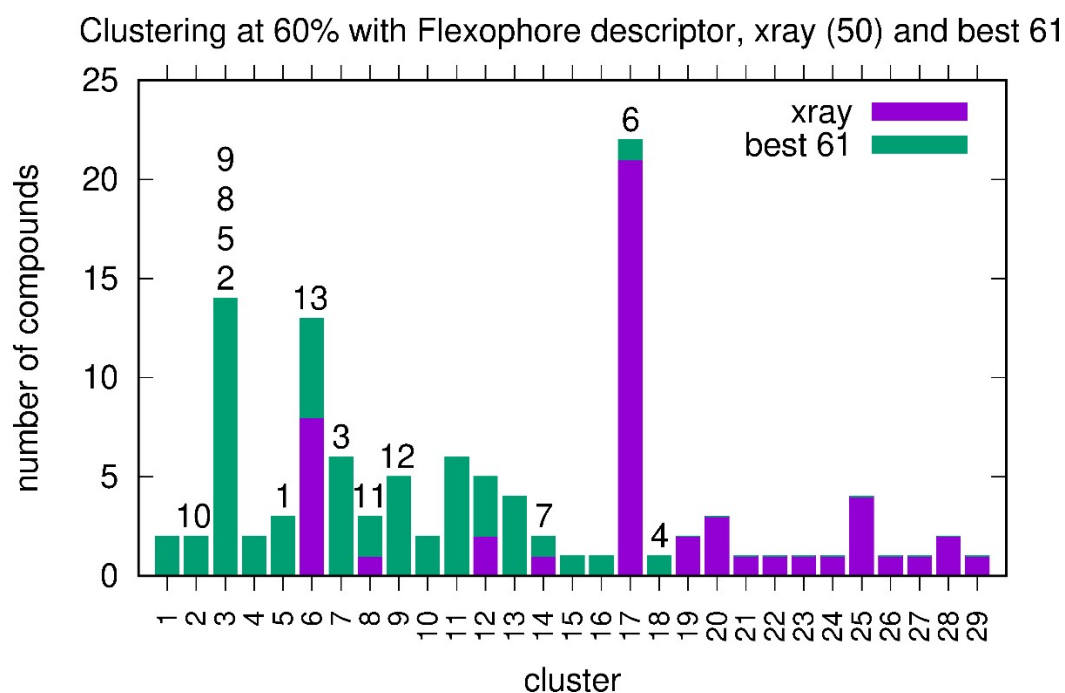
A)**B)**

Figure S2 A) Clustering of the best 61 compounds with the Flexophore descriptor at 60% similarity. **B)** Clustering of the best 61 compounds with the 50 ligands in the colchicine site from crystal structures reported to date, using the Flexophore descriptor at 60% similarity. The cluster locations of **1-13** are indicated.

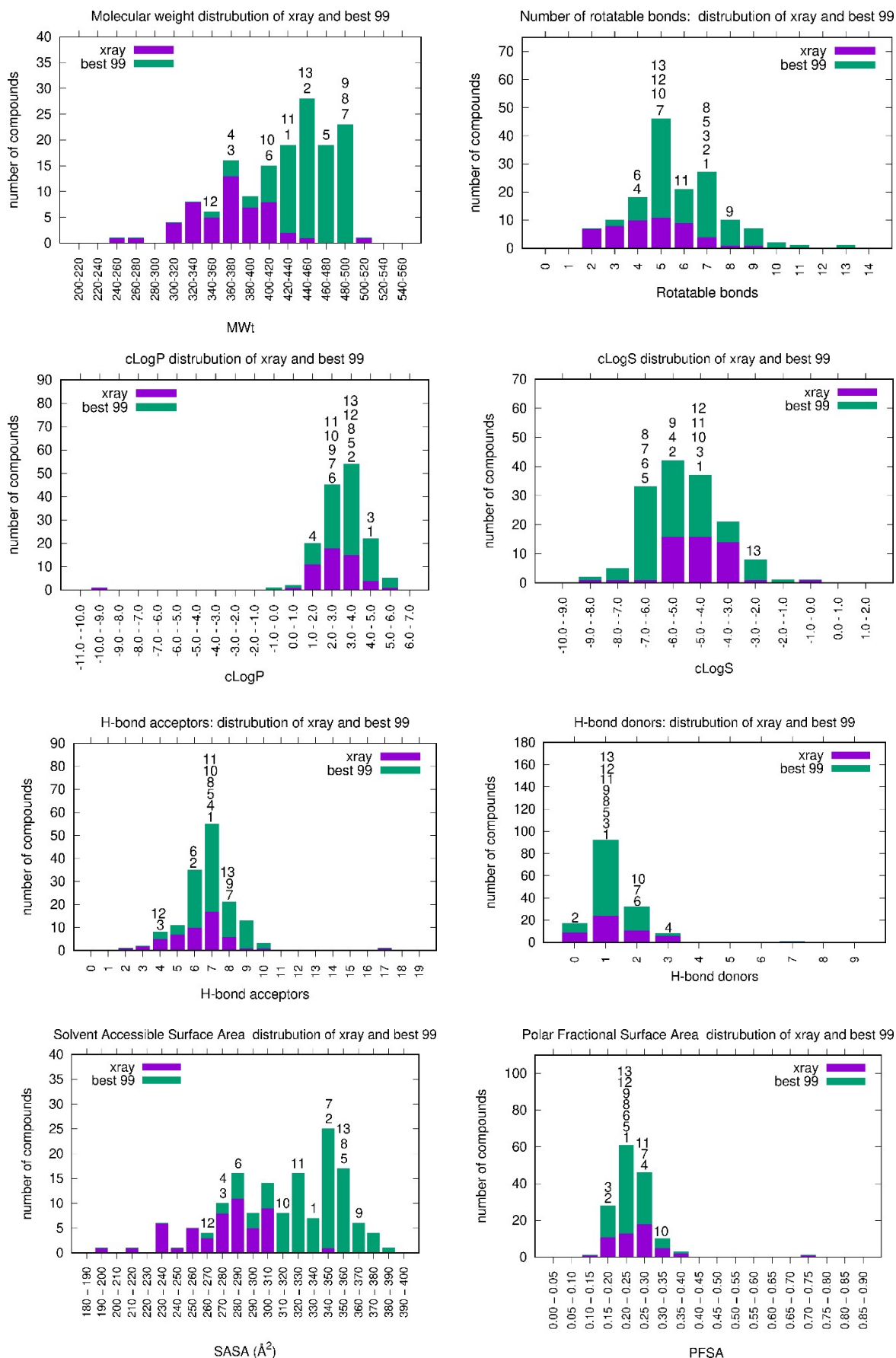


Figure S3 Histograms of ligand properties, comparing the 99 ligands from virtual screening with the 50 ligands from known crystal structures. Locations of the shortlisted compounds are indicated by the numbers.

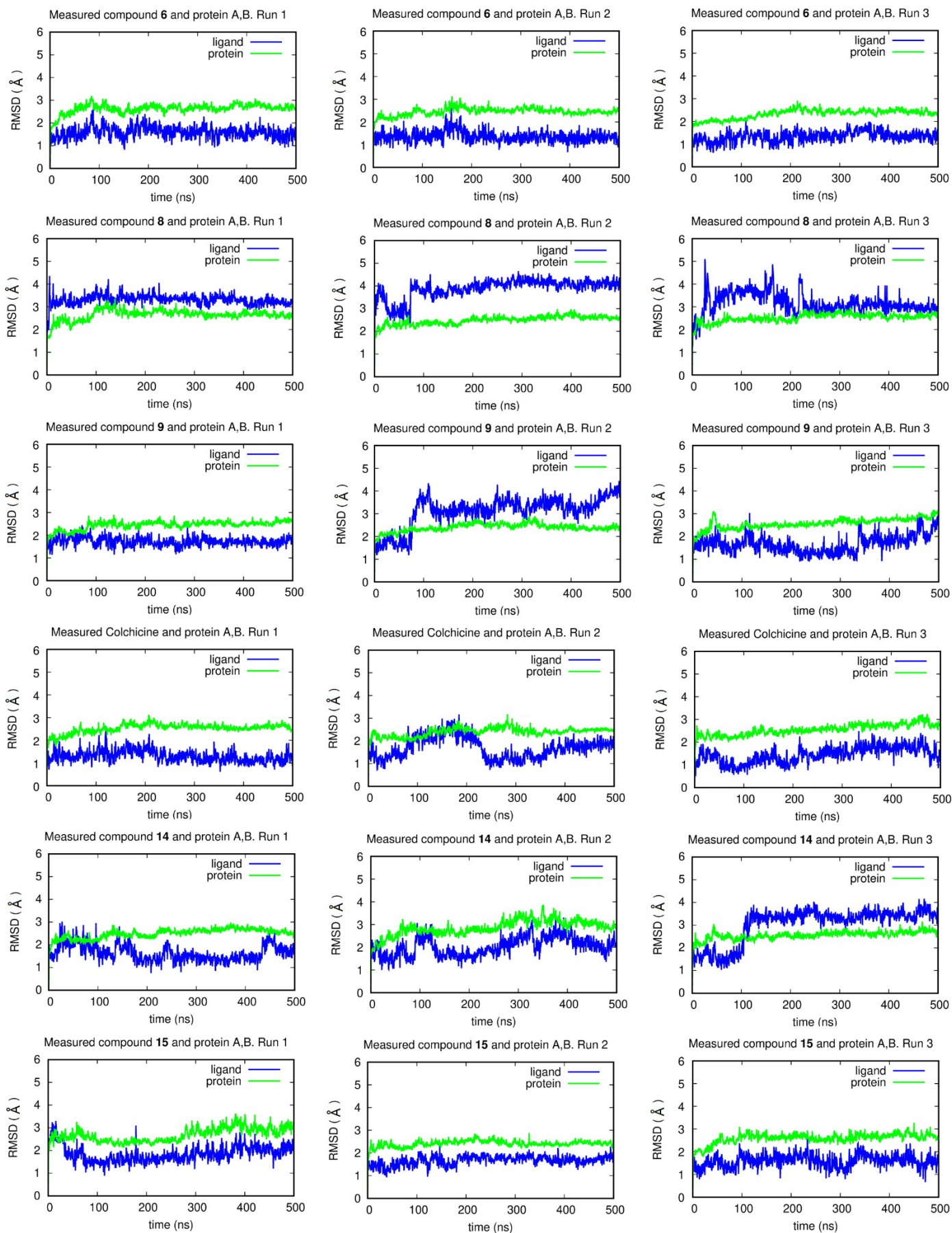
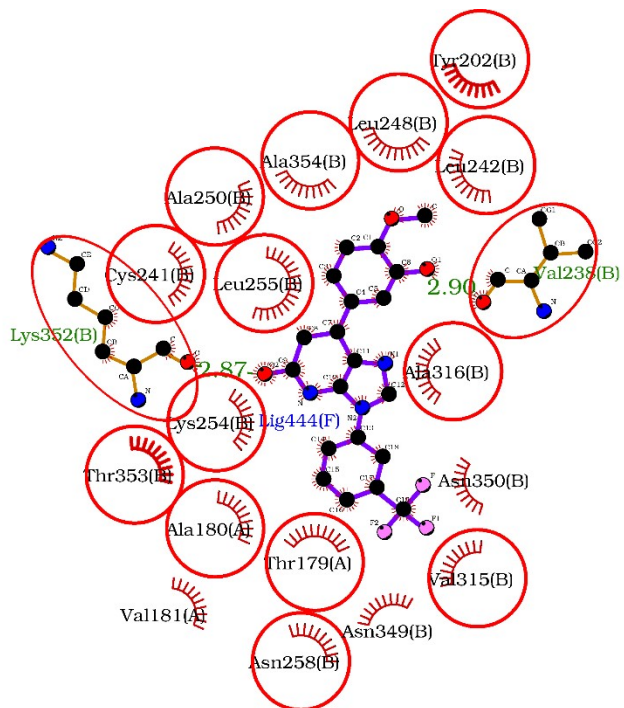
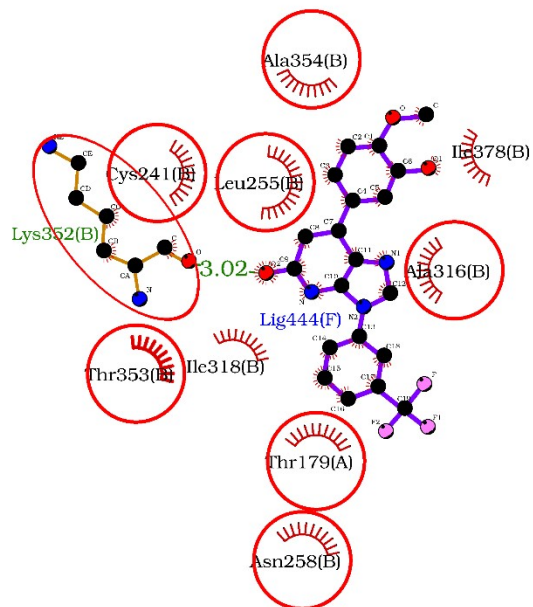


Figure S4 RMSD plots of all protein atoms of tubulin subunits A and B (α and β) superimposed on the time = 0 ns structure over the trajectories and measuring

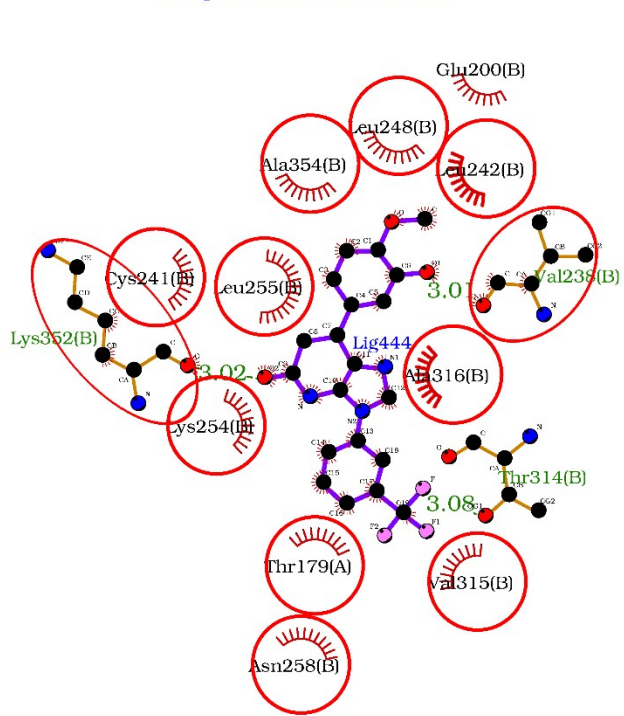
all atoms of the tubulin protein subunits A and B (green) and all atoms of the ligand (blue).



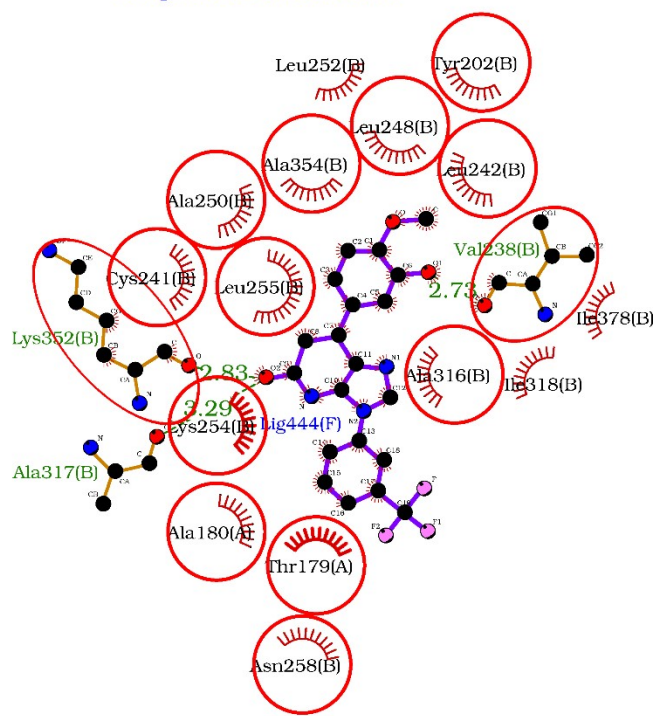
Compound 6 500 ns run 1



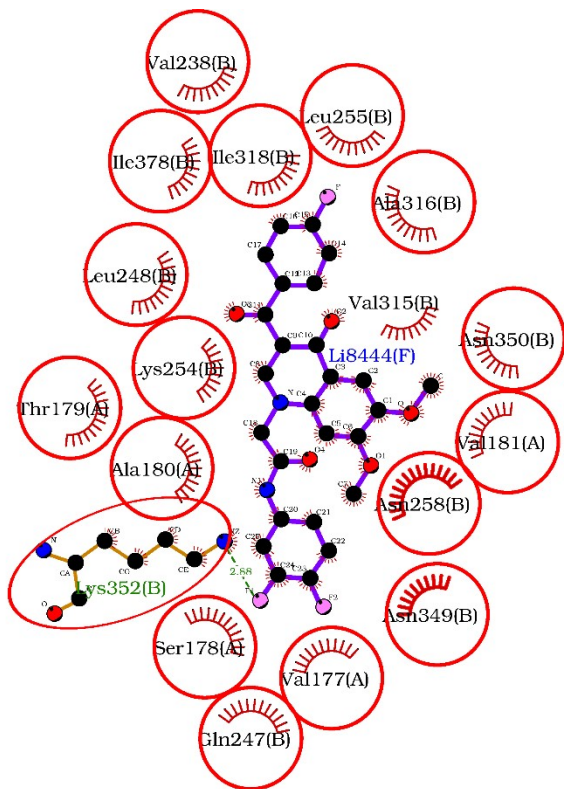
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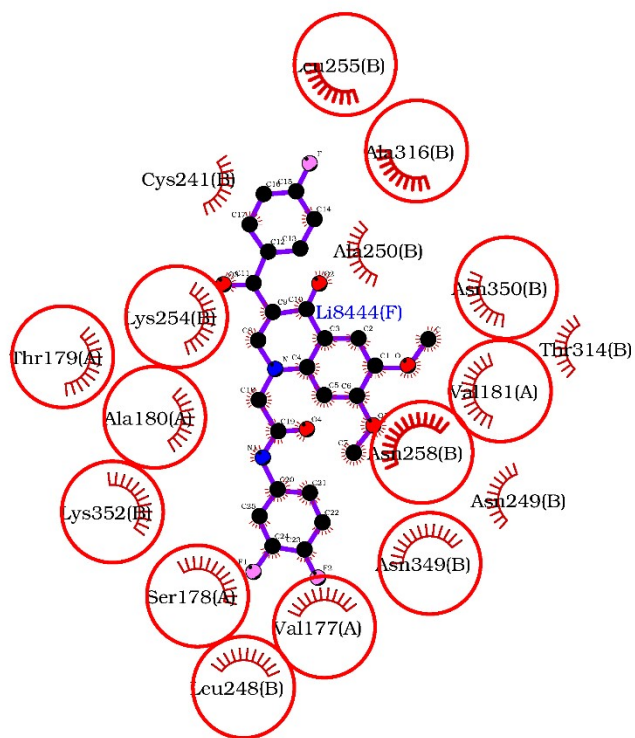
Compound 6 500 ns run 3



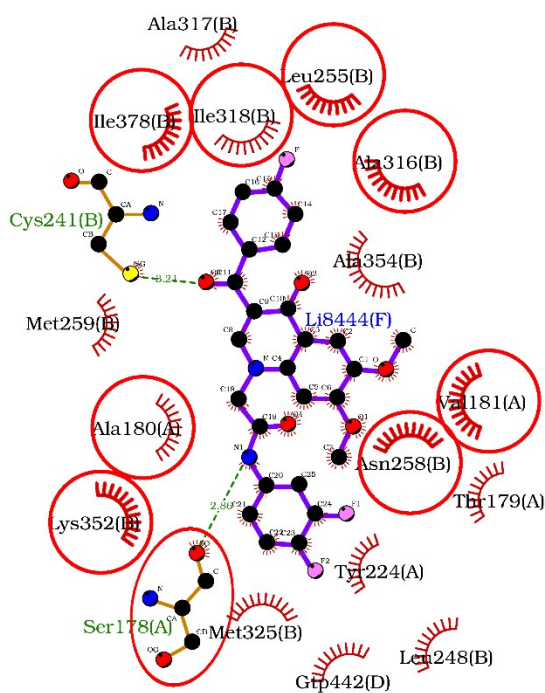
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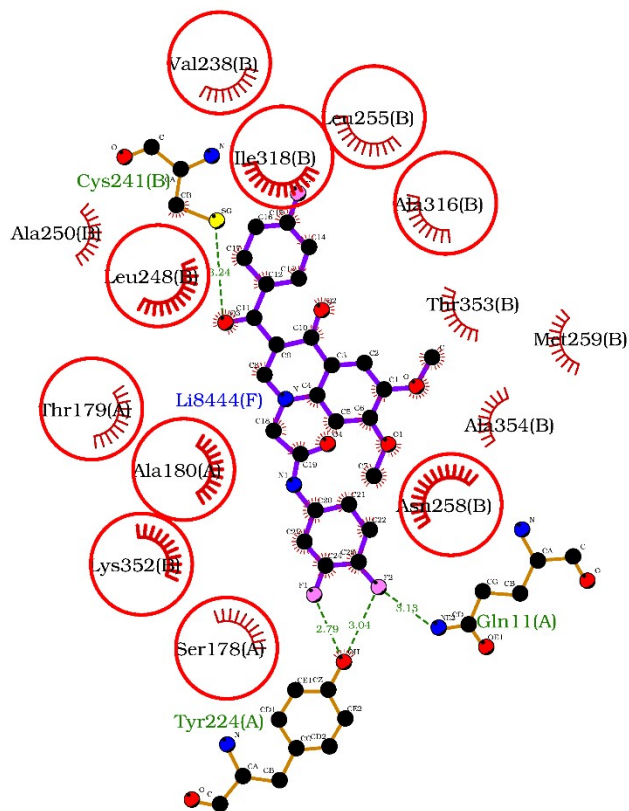
Compound 8 500 ns run 1



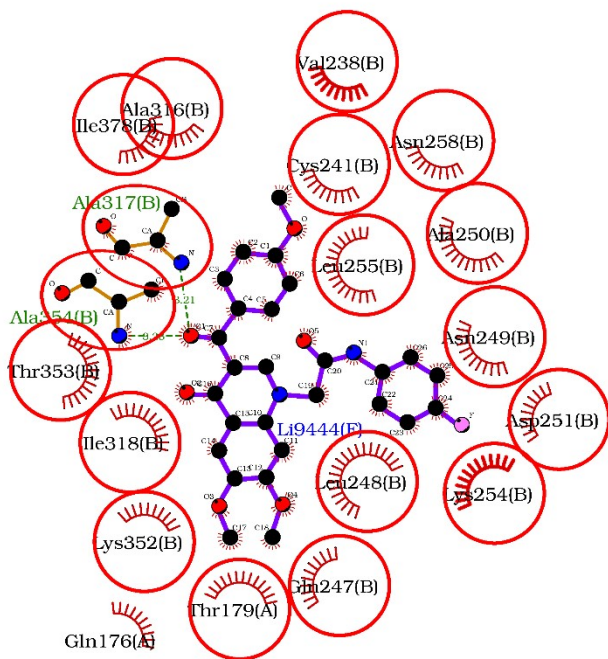
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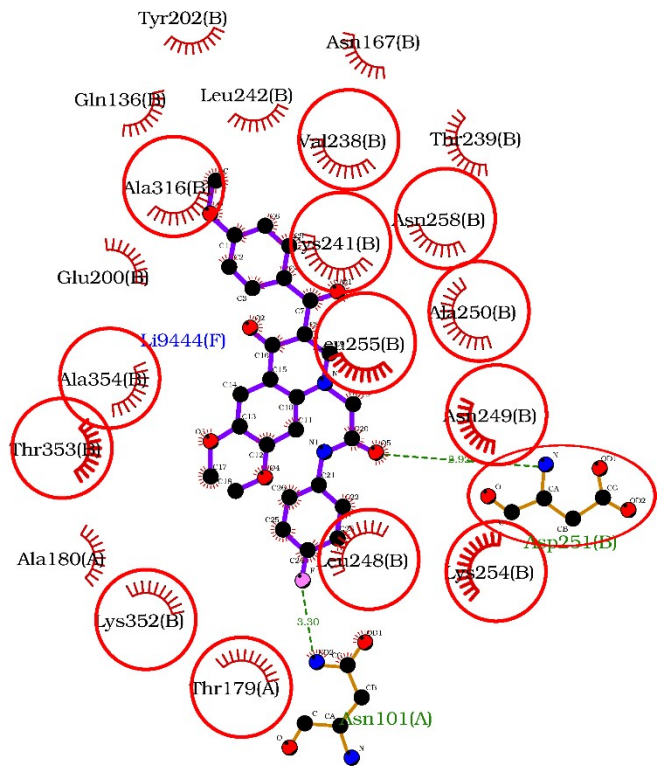
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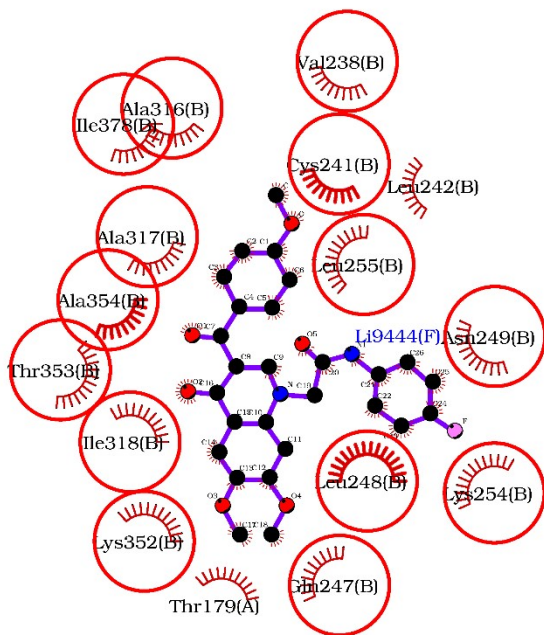
Compound 8 0 ns



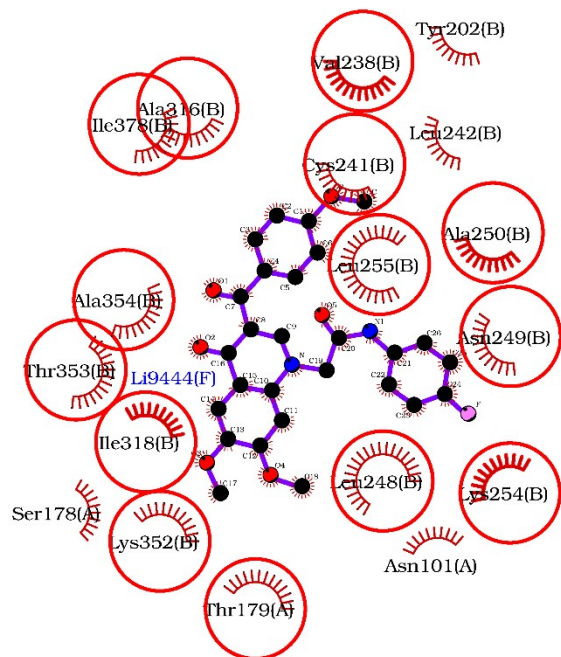
Compound 9 500 ns run 1



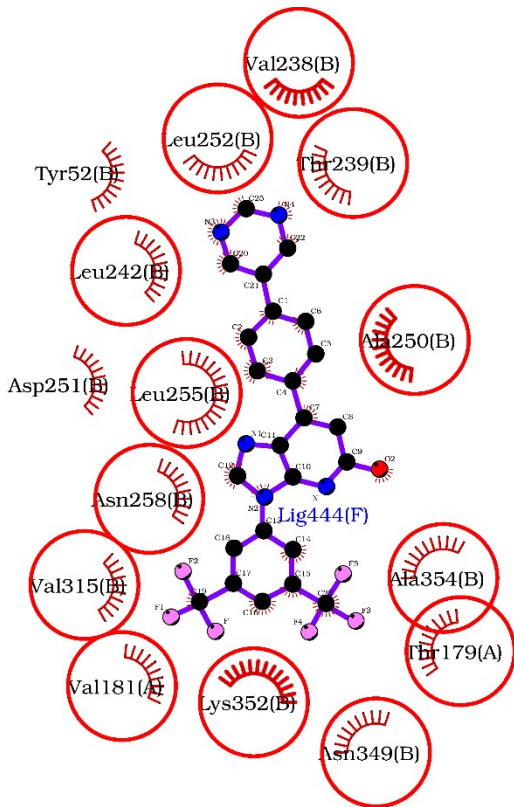
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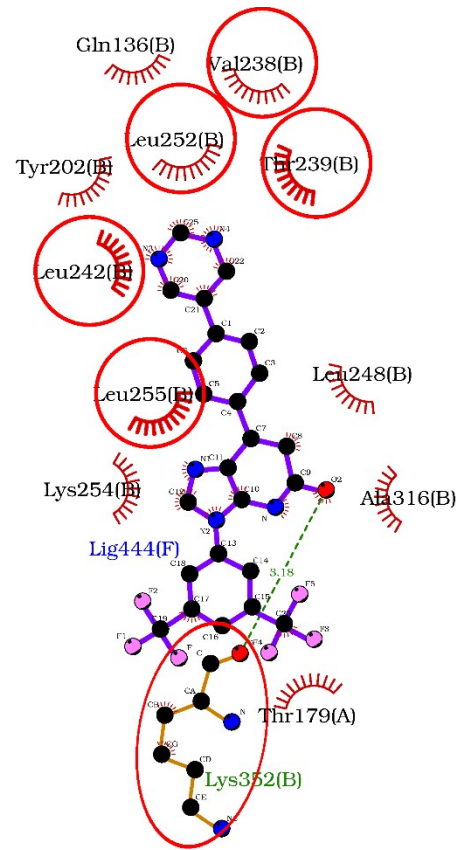
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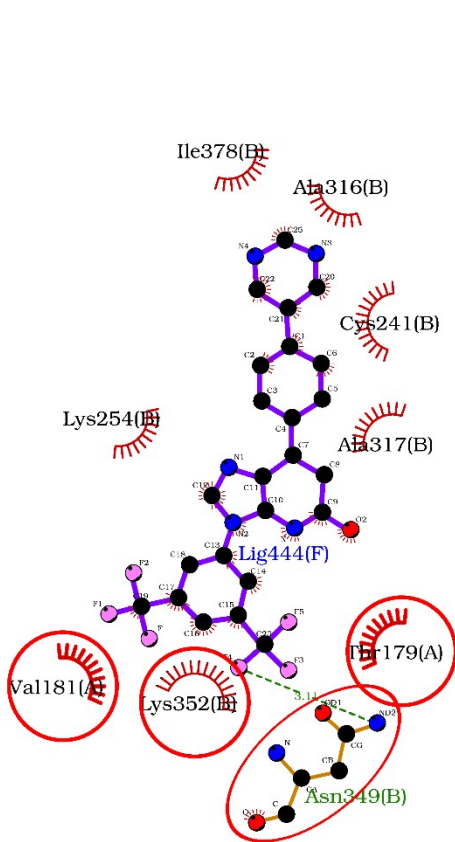
Compound 9 0 ns



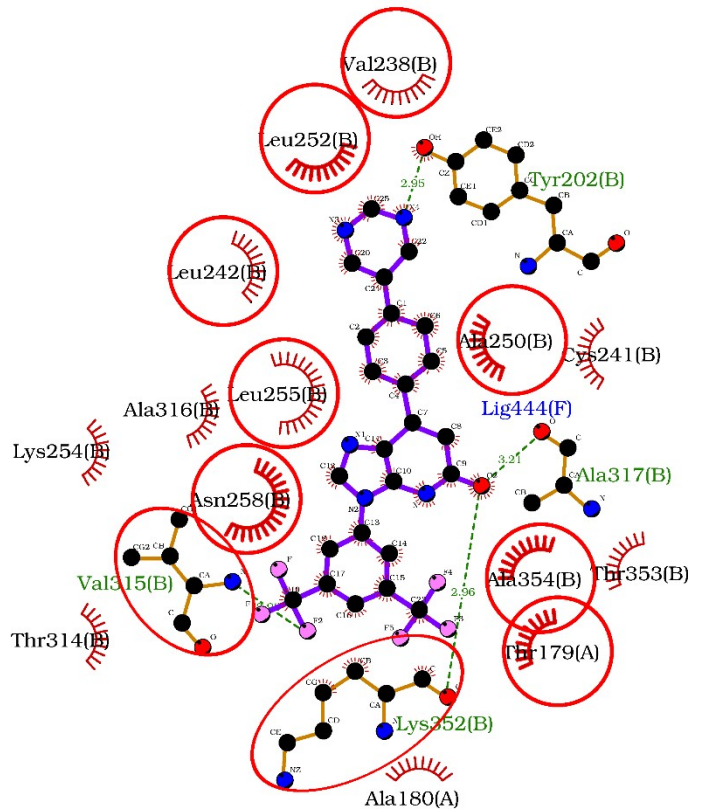
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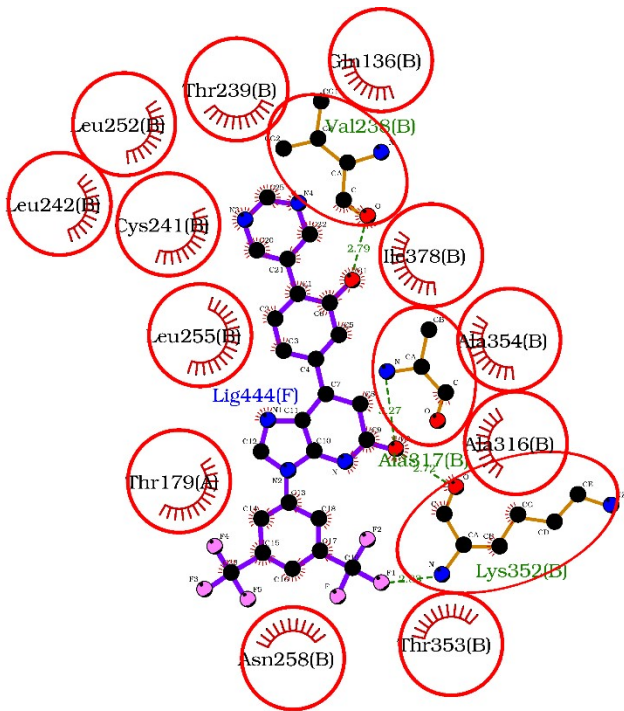
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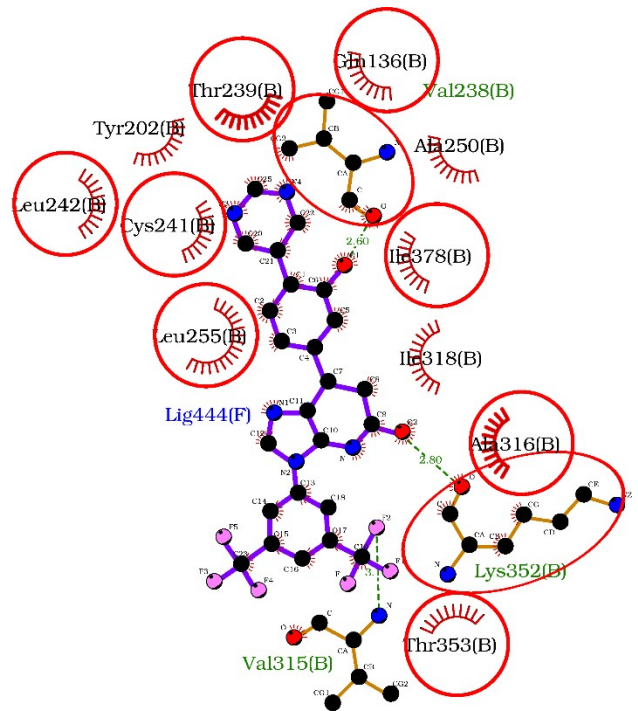
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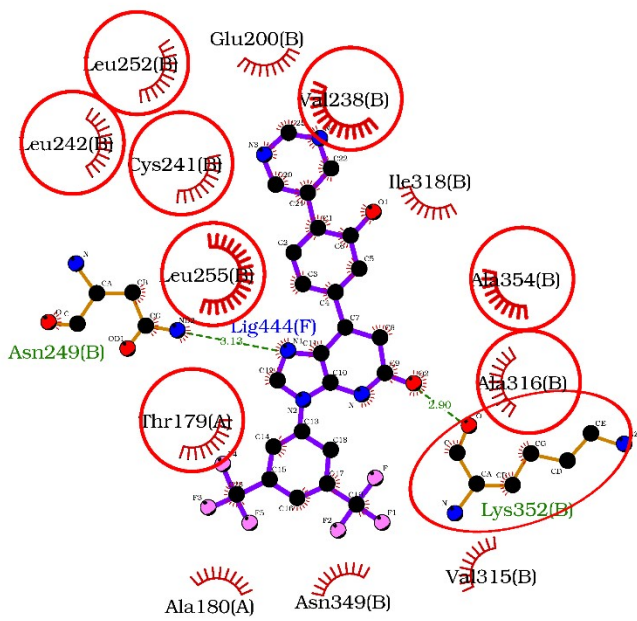
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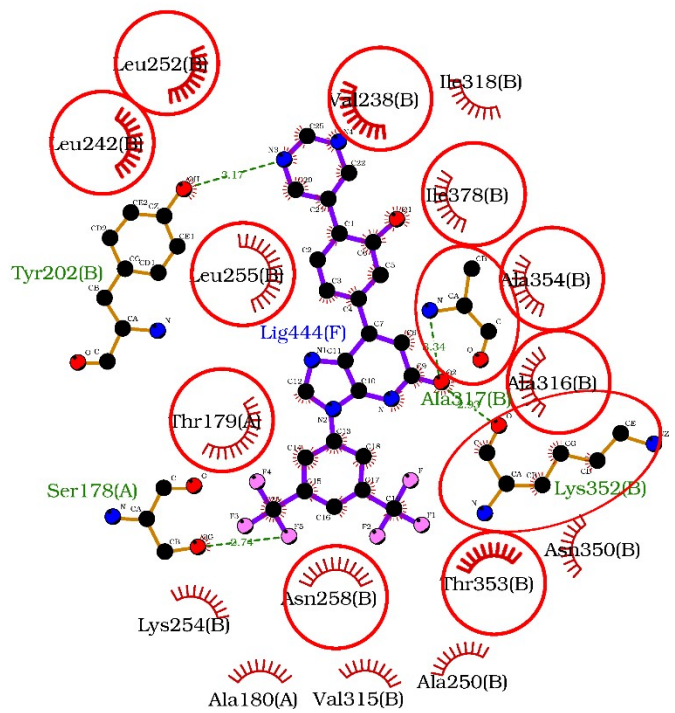
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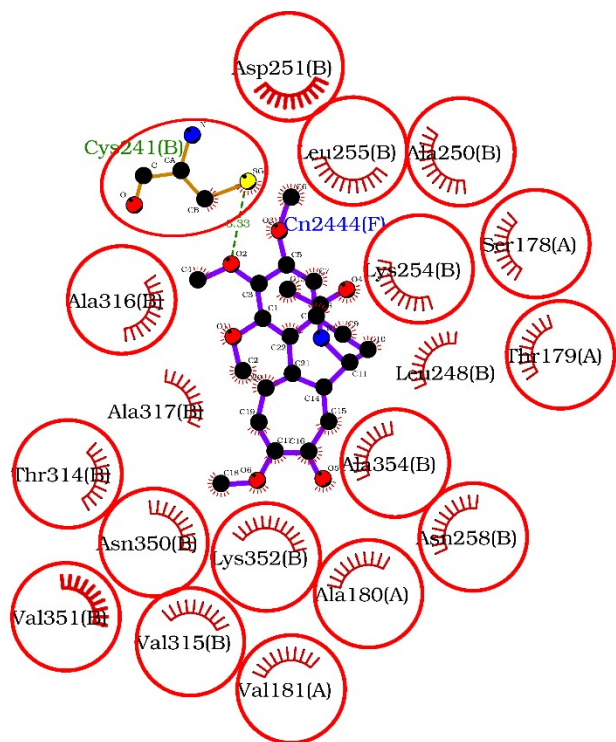
Compound 15 500 ns run 2



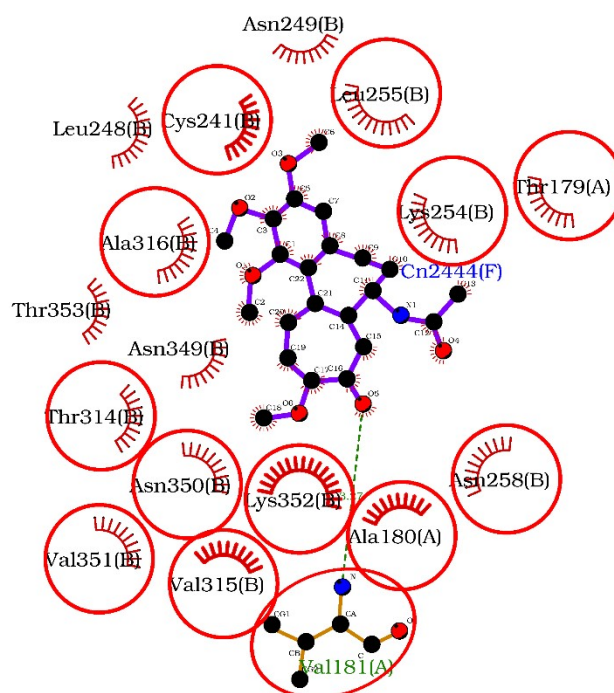
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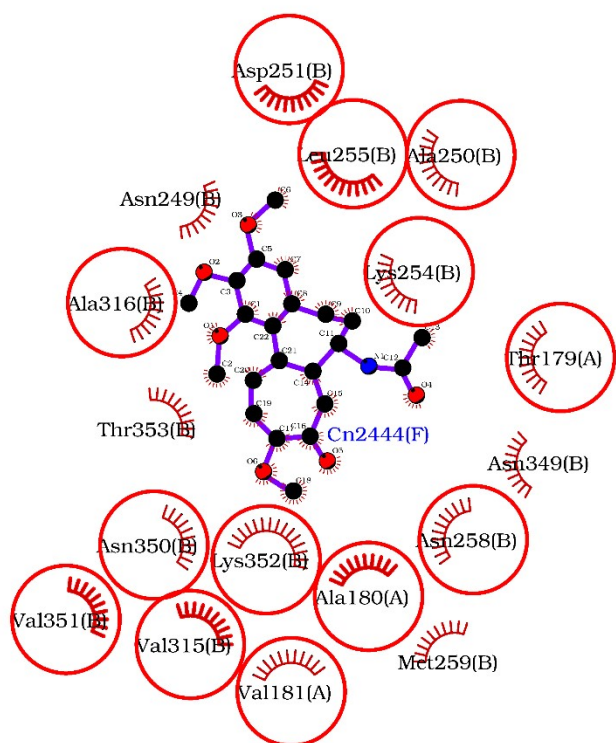
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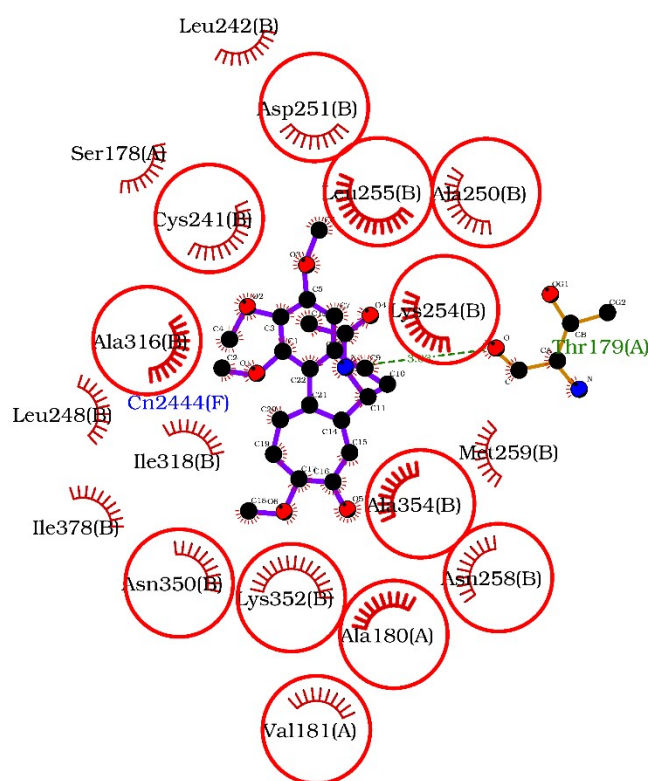
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Colchicine 500 ns run 2



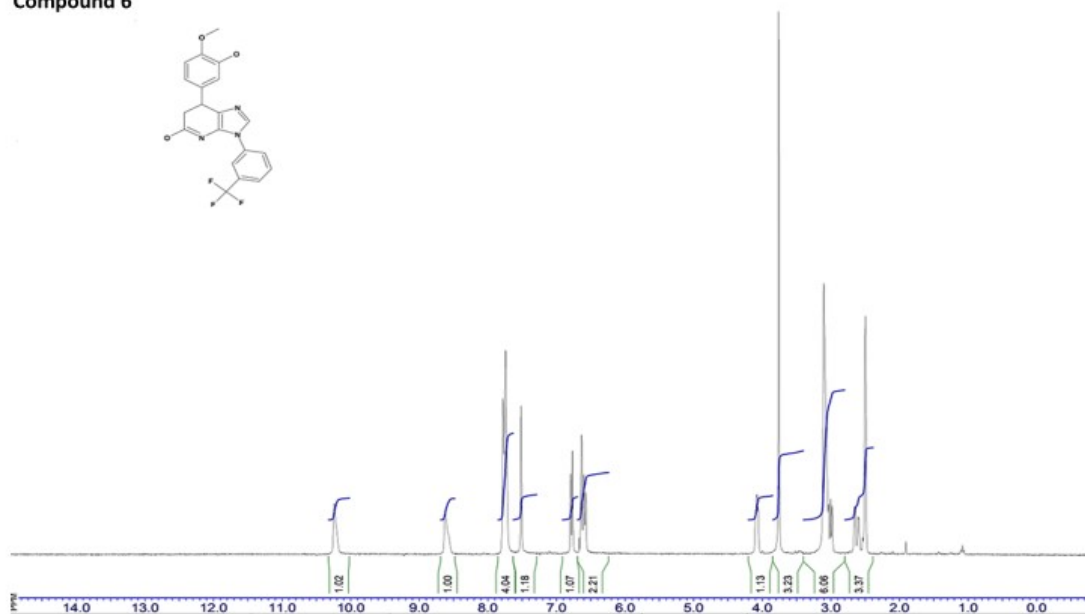
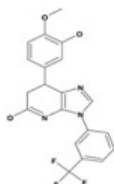
Colchicine 500 ns run 3



Colchicine 0 ns

Figure S5 Ligplot⁺ diagrams of the 500 ns structures for each simulation showing ligand-residue contacts at that moment, plus their respective starting structures.

Compound 6



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 Pulse sequence: s2pu1
 Ambient: temperature
 Mercury-300BB "NMR 300"
 Pulse 45.0 degrees
 OBSERVE C13, 75.4623880 MHz
 DECOUPLE H1, 300.6702830 MHz
 Power 34 dB
 Continuously on WALTZ-16 modulated,
 Line broadening 1.0 Hz, FT size 65536
 Total time 32 hr, 8 min, 22 sec

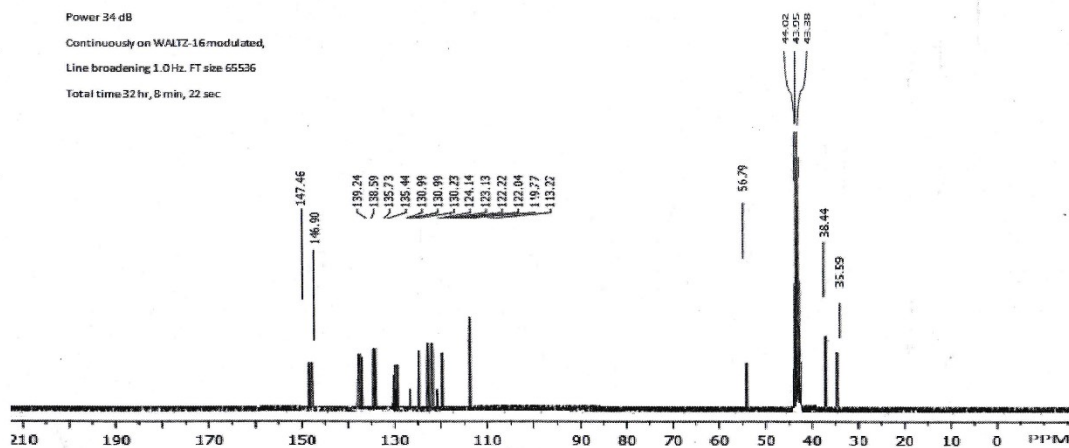
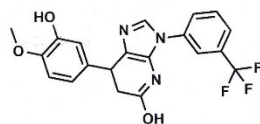


Figure S6 ¹H and ¹³C NMR spectra of compound 6.

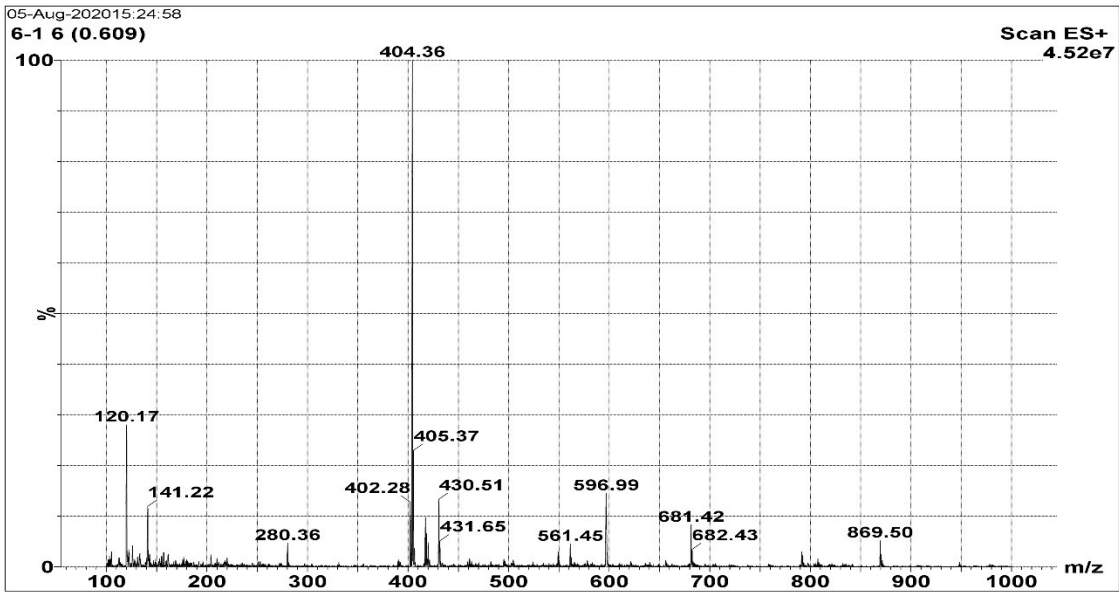
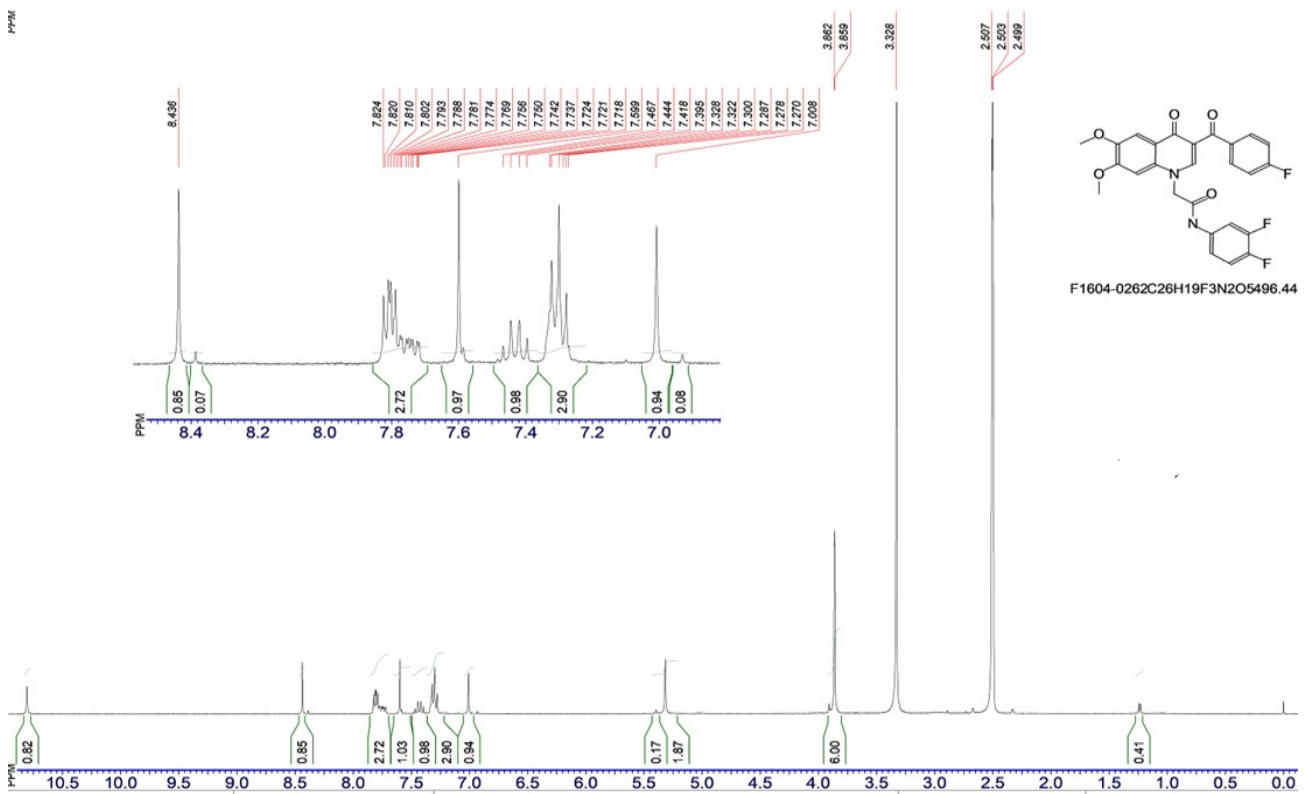


Figure S7 Mass spectrum of compound 6.



DMSO-C13/16Mar2022
Pulse sequence: s2pu1
Ambient: temperature
Mercury-300BB "NMR 300"
Pulse 45.0 degrees
OBSERVE C13, 75.4523880 MHz
DECOUPLE H1, 300.0702830 MHz
Power 34 dB
Continuously on WALTZ-16 modulated,
Line broadening 1.0 Hz, FT sbs 65536
Total time 34 hr, 25 min, 33 sec

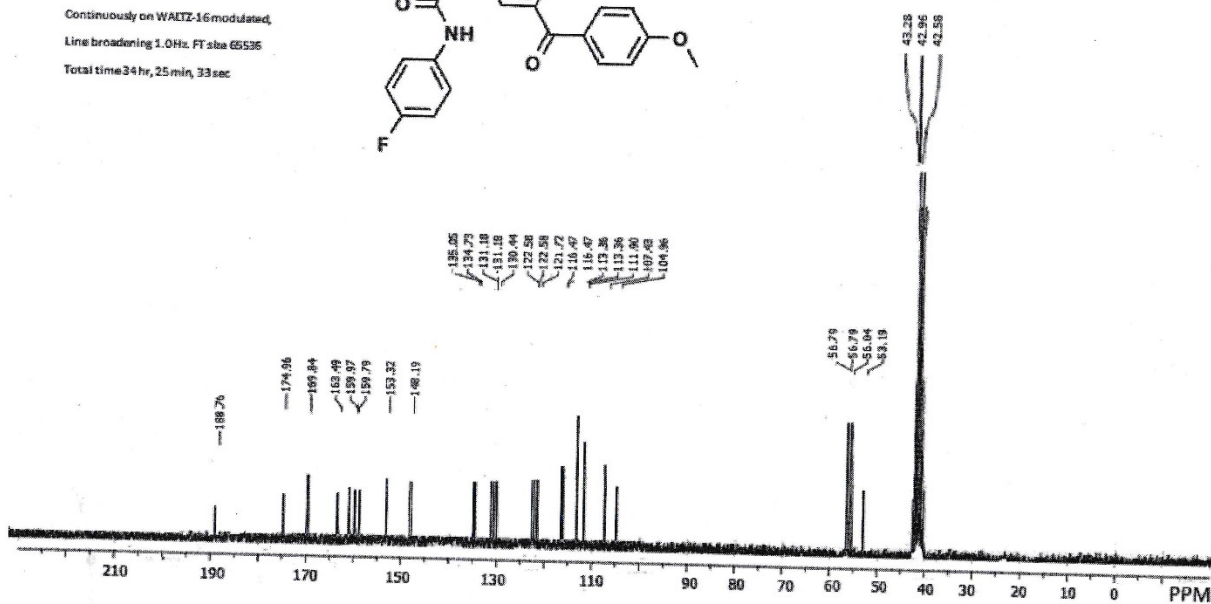
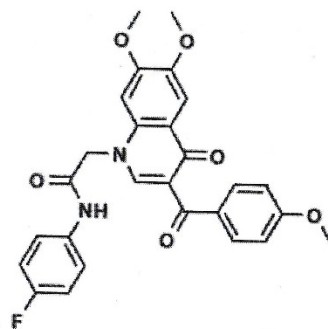


Figure S8 ¹H and ¹³C NMR spectrum of compound 8

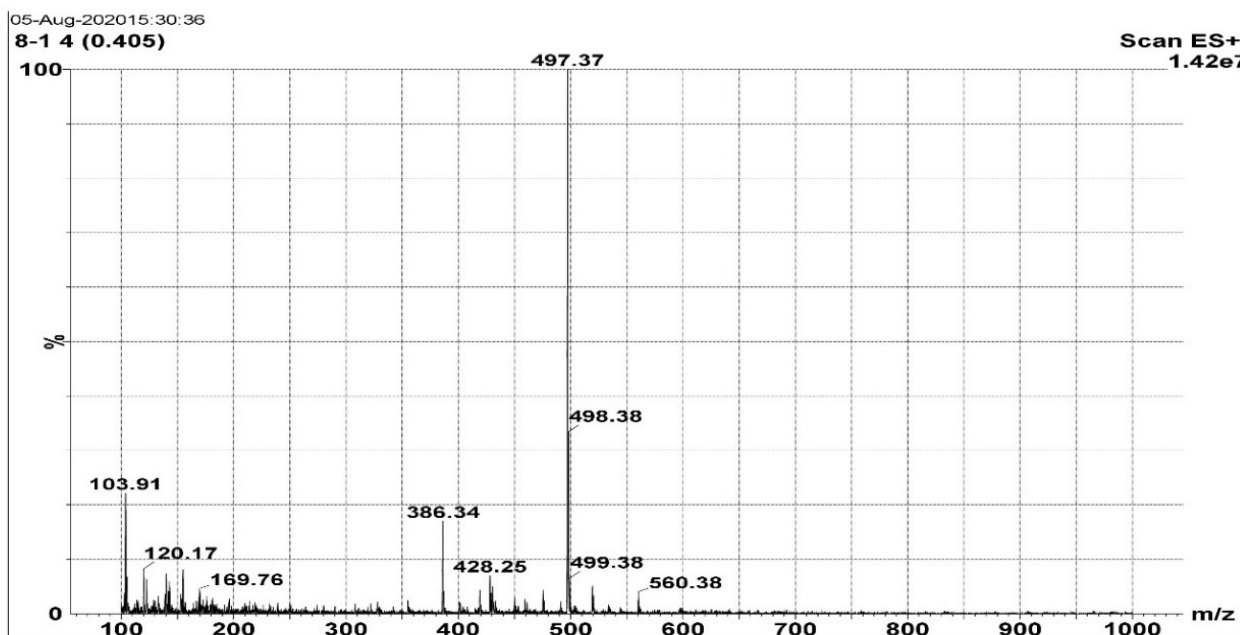
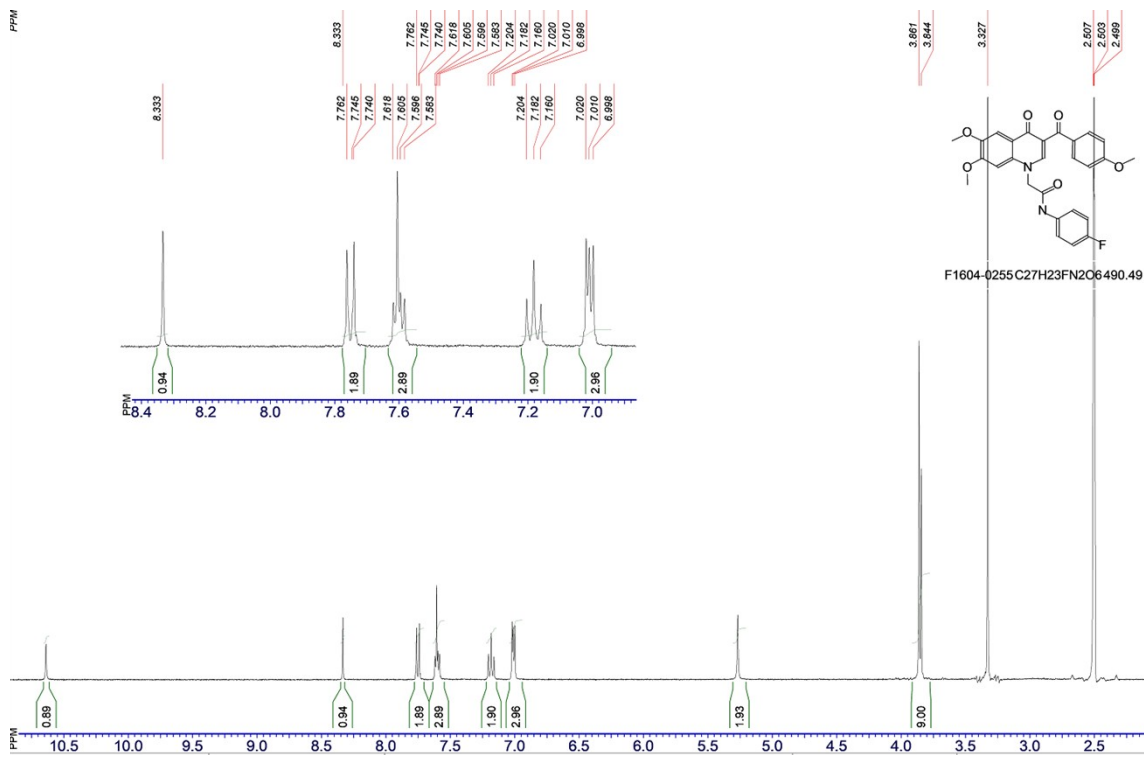


Figure S9 Mass spectrum of compound 8.



DMSO-C13/14Mar2022

Pulse sequence: s2pu1

Ambient: temperature

Mercury-300BB "NMR 300"

Pulse 45.0degrees

OBSERVE C13, 75.4523880 MHZ

DECOUPLE H1, 300.0702830 MHZ

Power 34 dB

Continuously on WALTZ-16 modulated,

Line broadening 1.0Hz. FT size 65536

Total time 35 hr, 20 min, 22 sec

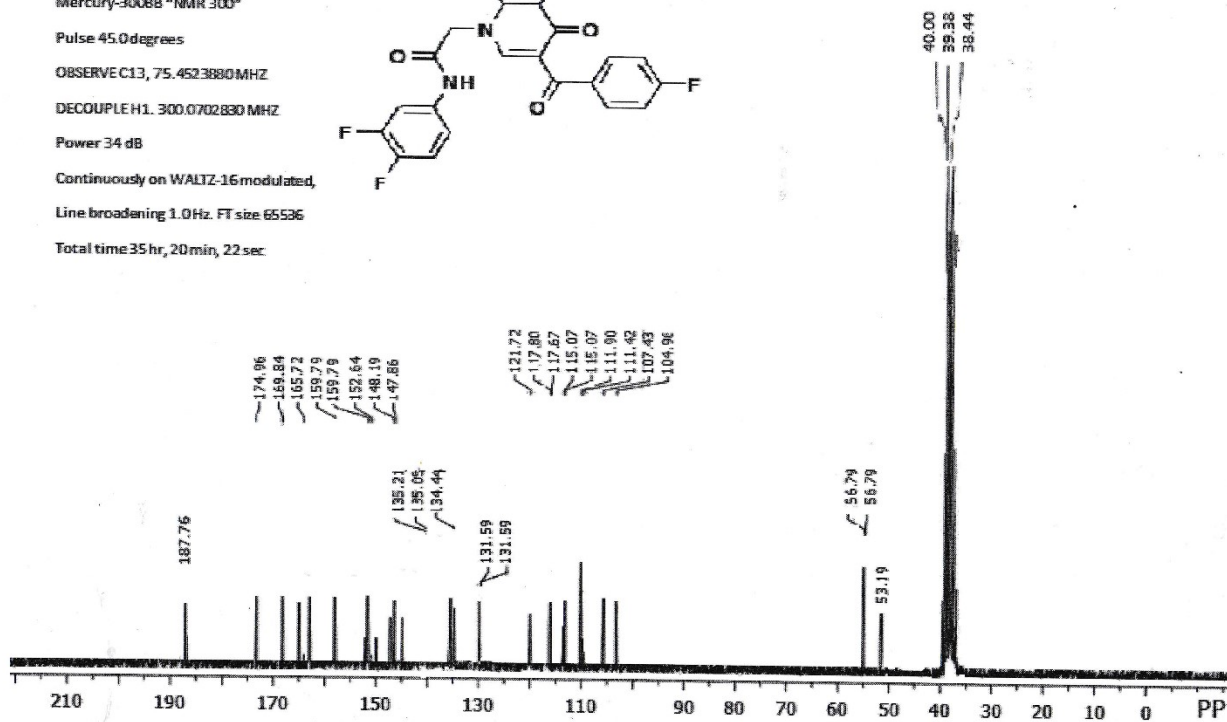
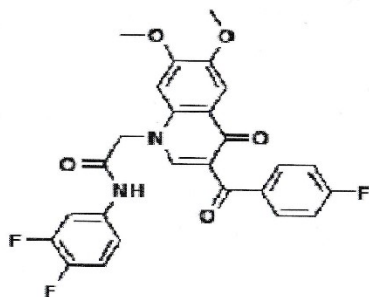
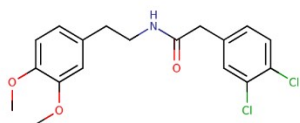


Figure S10 ^1H and ^{13}C NMR spectrum of compound 9

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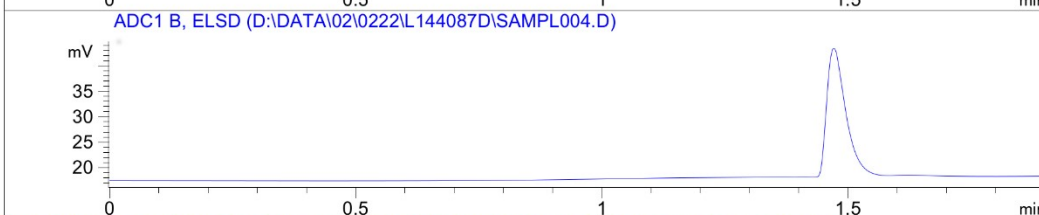
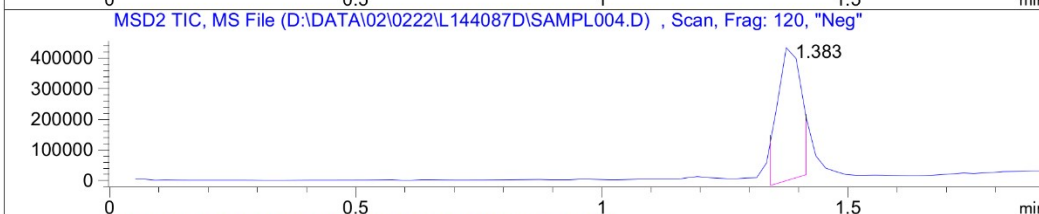
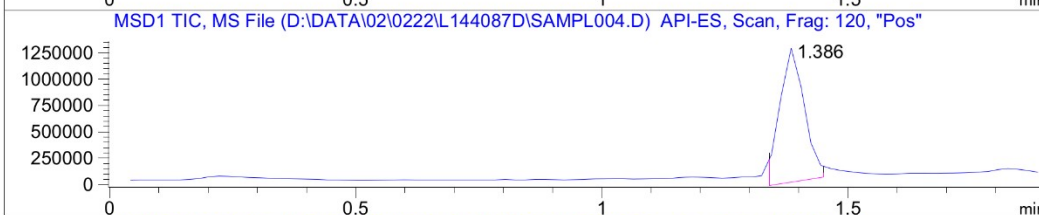
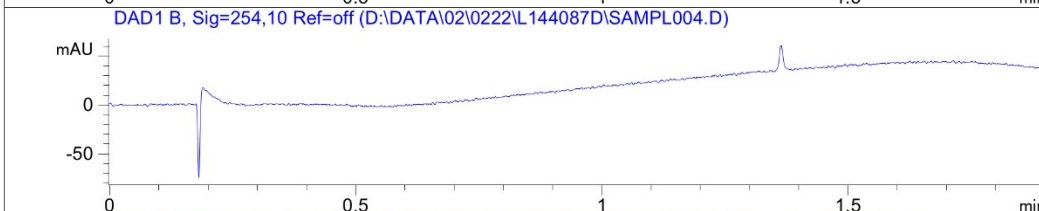
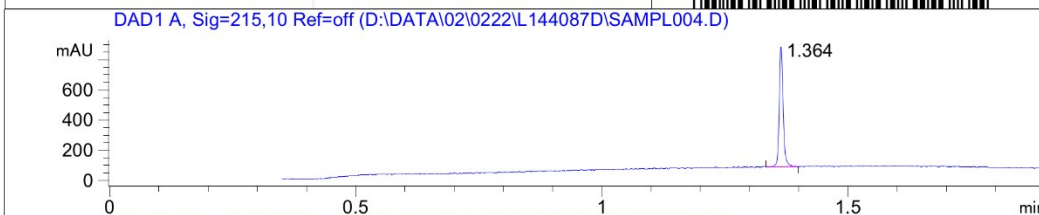


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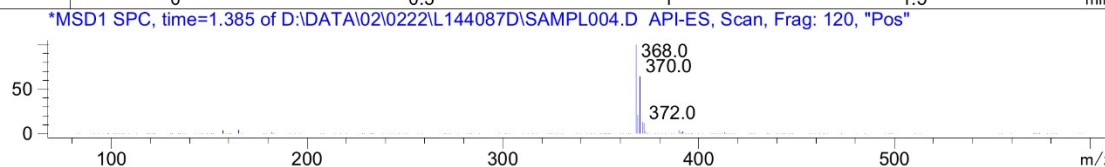


Mol Wt 368.25
Exact Mass 367.1

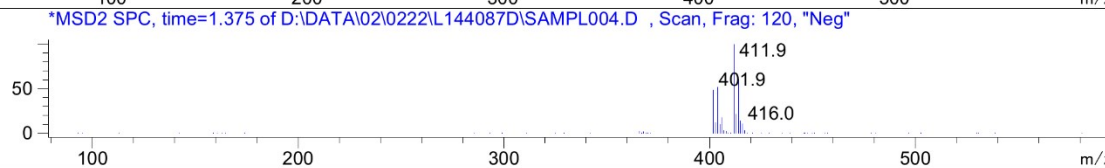
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RT 1.386



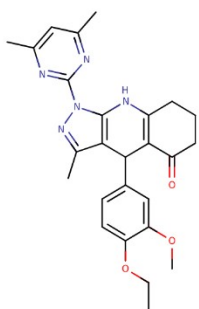
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T5105911

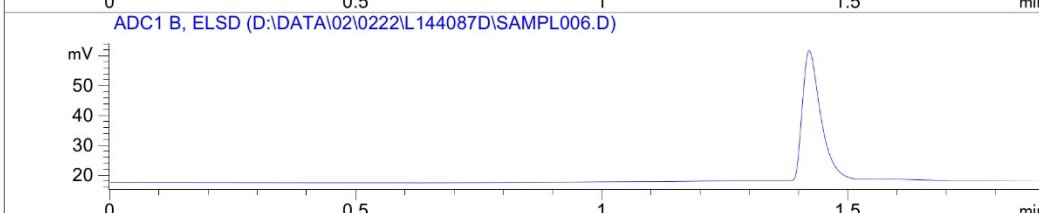
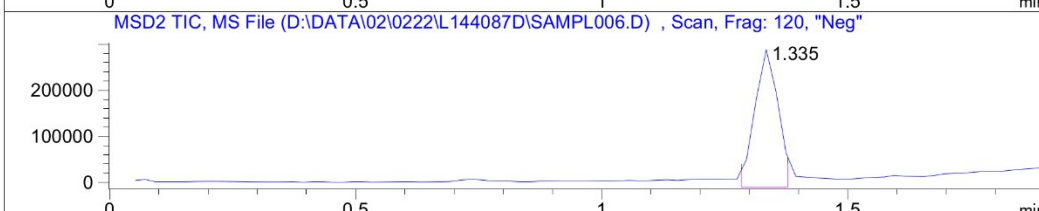
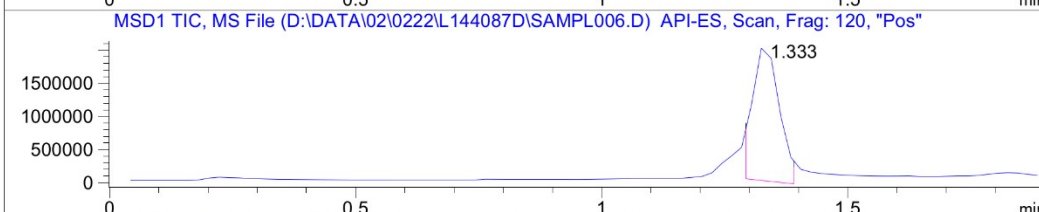
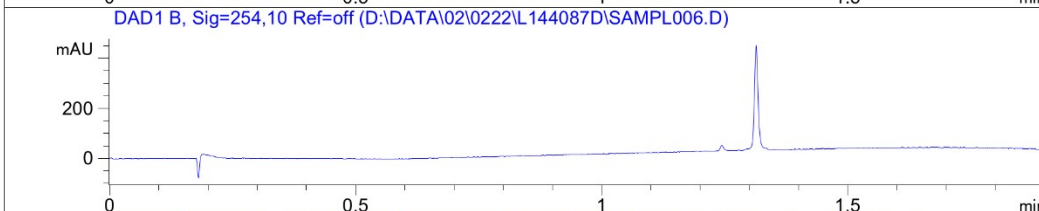
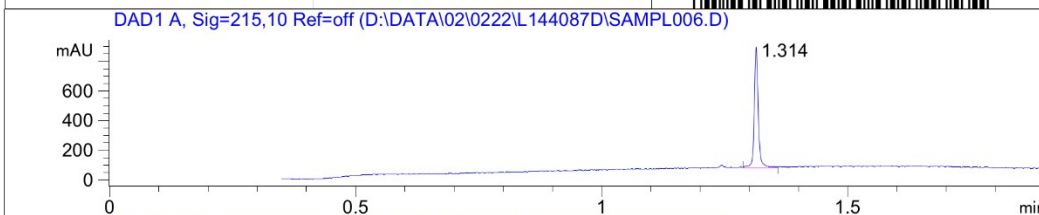


MaxPeak: 100.00%
Ret_Time: 1.314 min

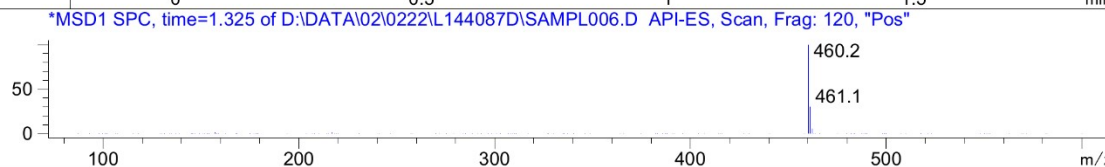


Mol Wt 459.54
Exact Mass 459.26

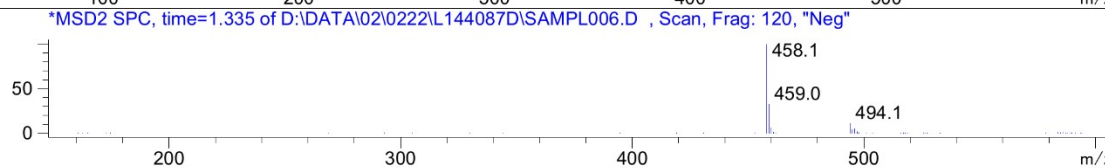
#	Time	Area%
1	1.314	100.00



RT 1.333

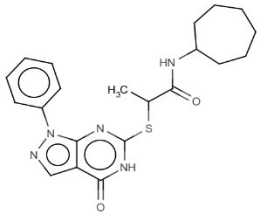


RT 1.335



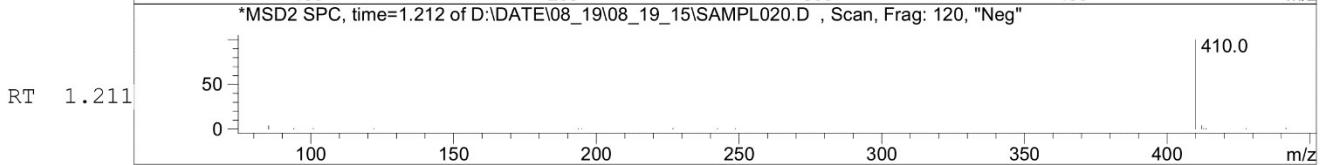
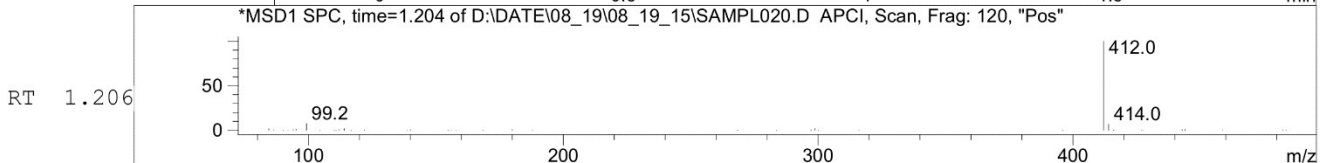
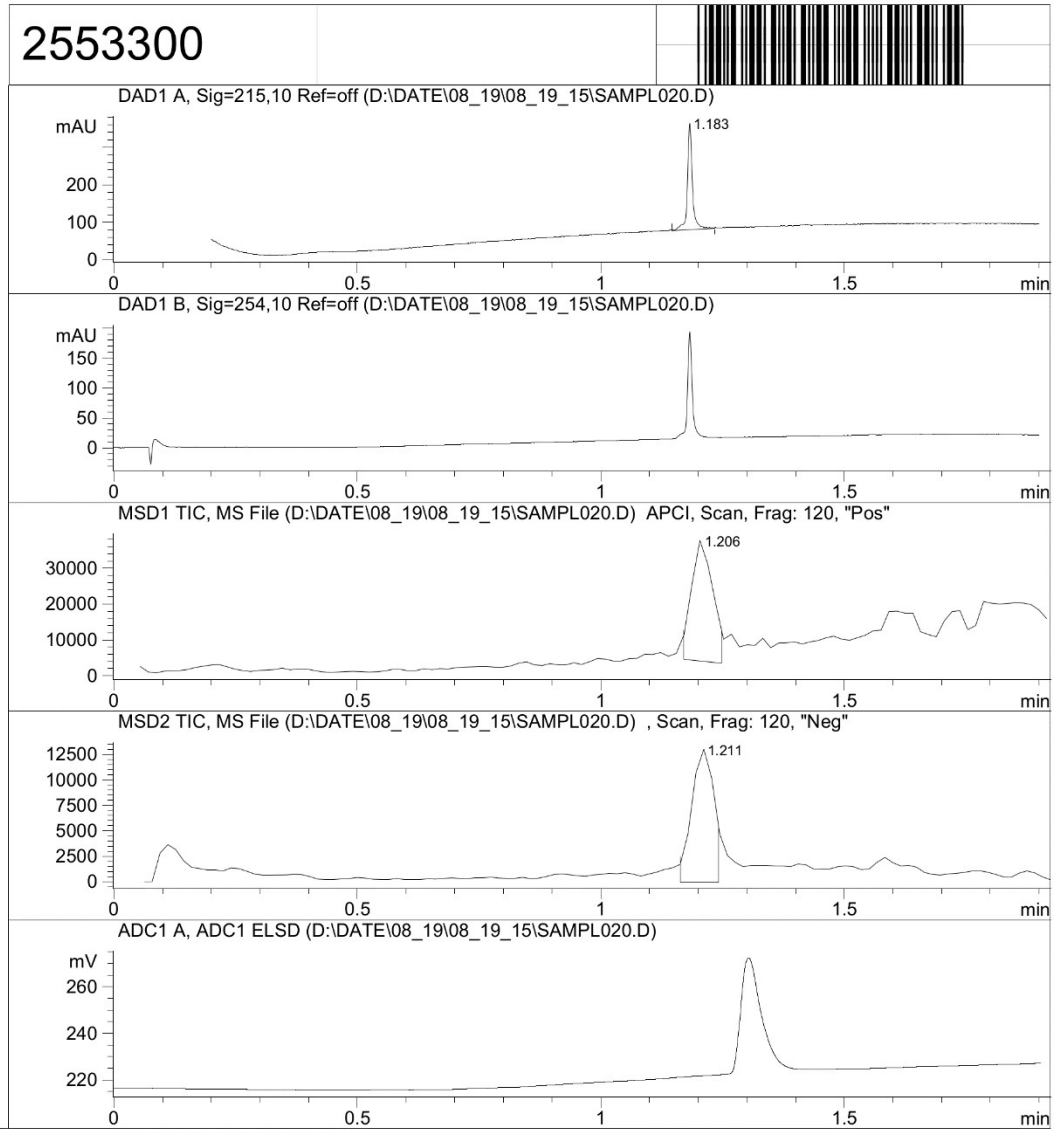
MaxPeak: 100.00%
Ret_Time: 1.183 min

2553300



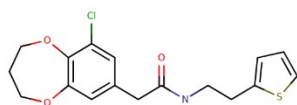
Mol Wt 411.522
Exact Mass 411.2

#	Time	Area%
1	1.183	100.00



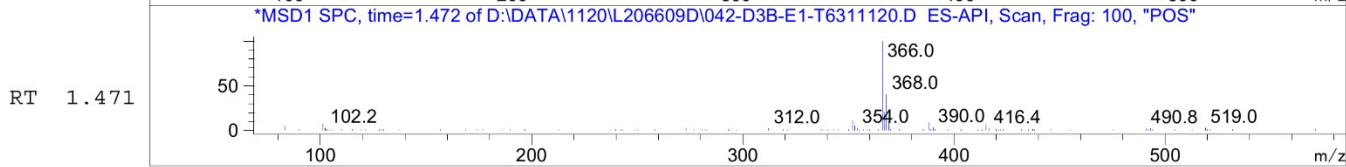
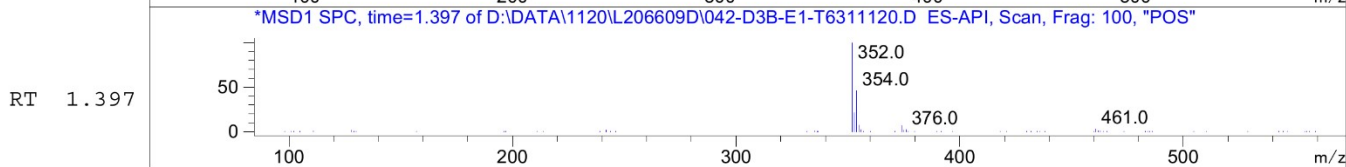
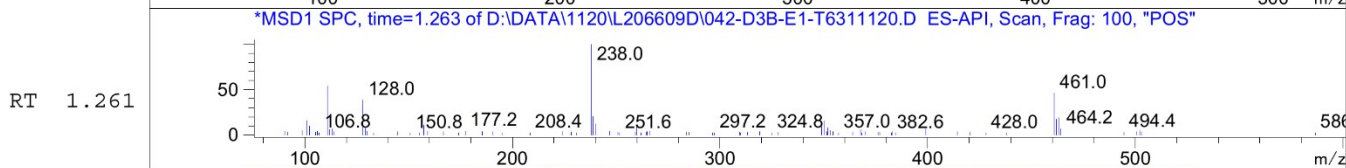
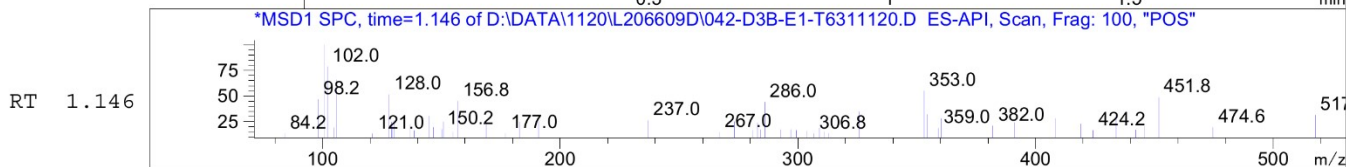
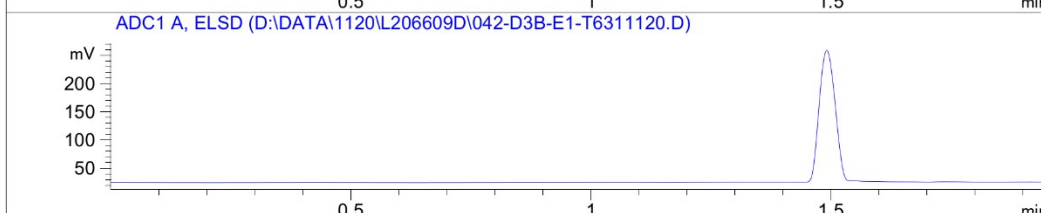
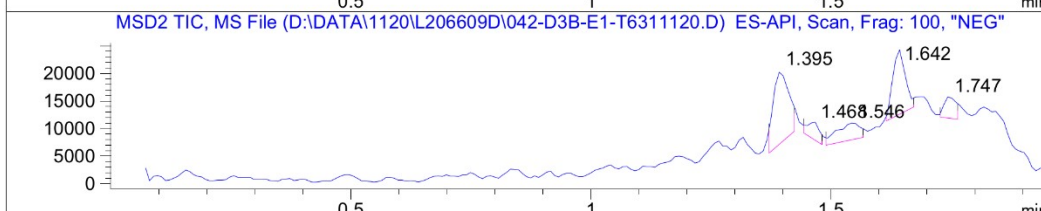
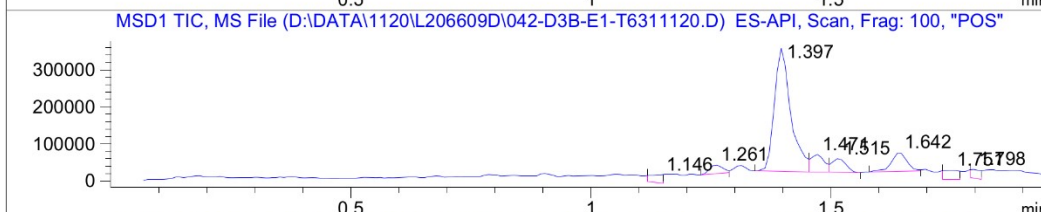
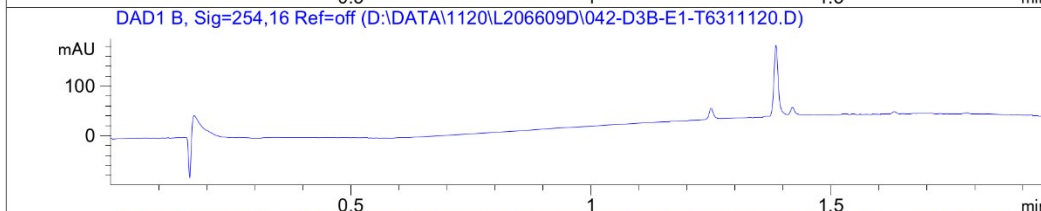
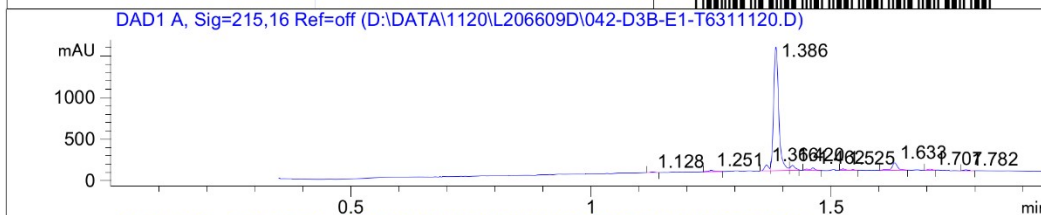
MaxPeak: 82.46%
Ret_Time: 1.386 min

T6311120



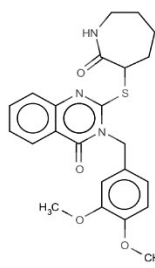
Mol Wt 351.85
Exact Mass 351.09

#	Time	Area%
1	1.128	0.41
2	1.251	0.95
3	1.366	3.41
4	1.386	82.46
5	1.420	3.06
6	1.462	1.86
7	1.525	1.11
8	1.633	5.79
9	1.707	0.55
10	1.782	0.42



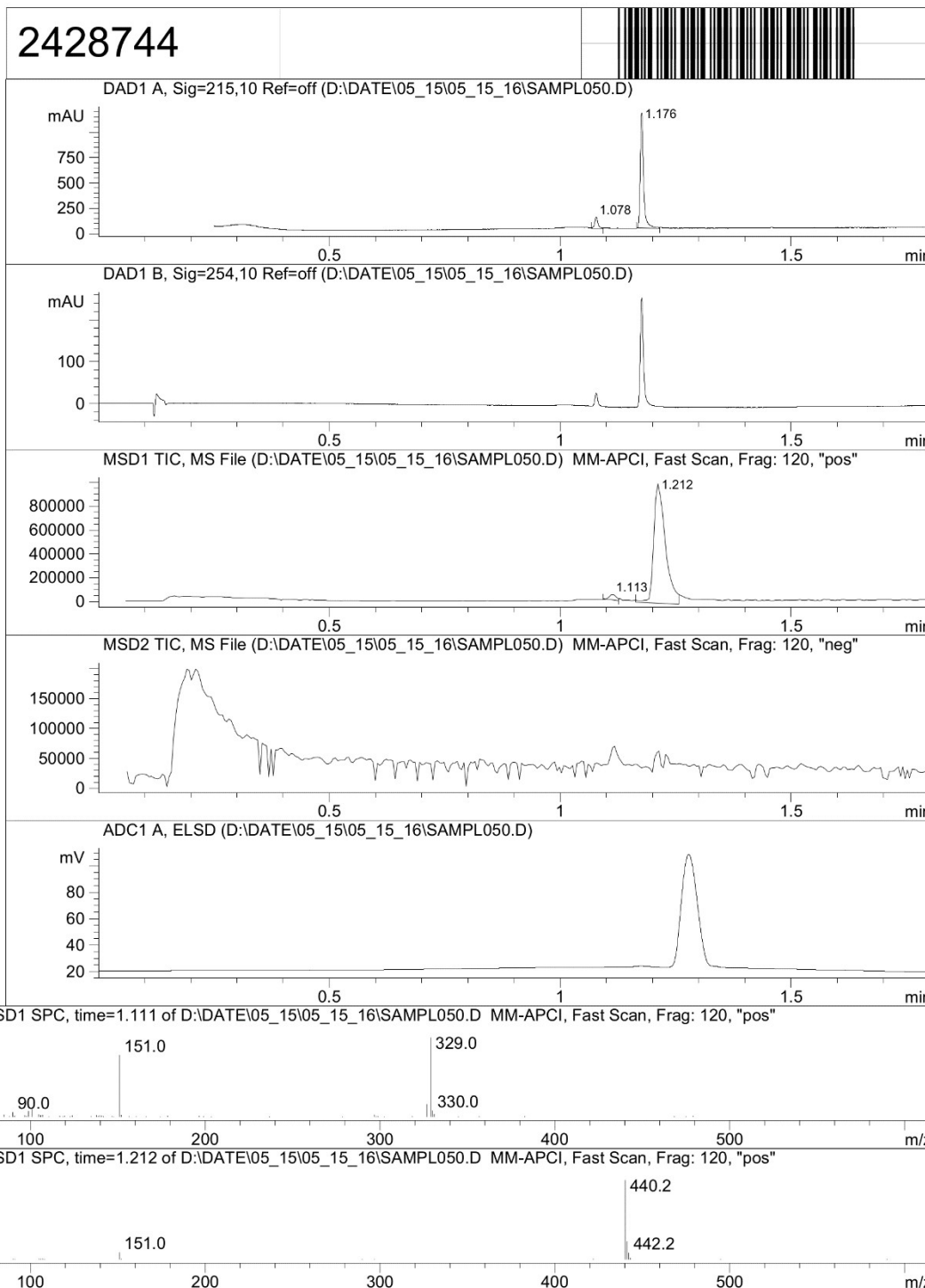
MaxPeak: 91.46%
Ret_Time: 1.176 min

2428744



Mol Wt 439.527
Exact Mass 439.18

#	Time	Area%
1	1.078	8.54
2	1.176	91.46



Inj.Date 5/16/2009

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Acq. Method C:\CHEM32\ -> ->

Figure S11 Spectra and analytical date of the short-listed compounds