

## ***Supplementary Materials***

### **Design and synthesis of water-soluble and potent MMP-13 inhibitors with activity in human osteosarcoma cells**

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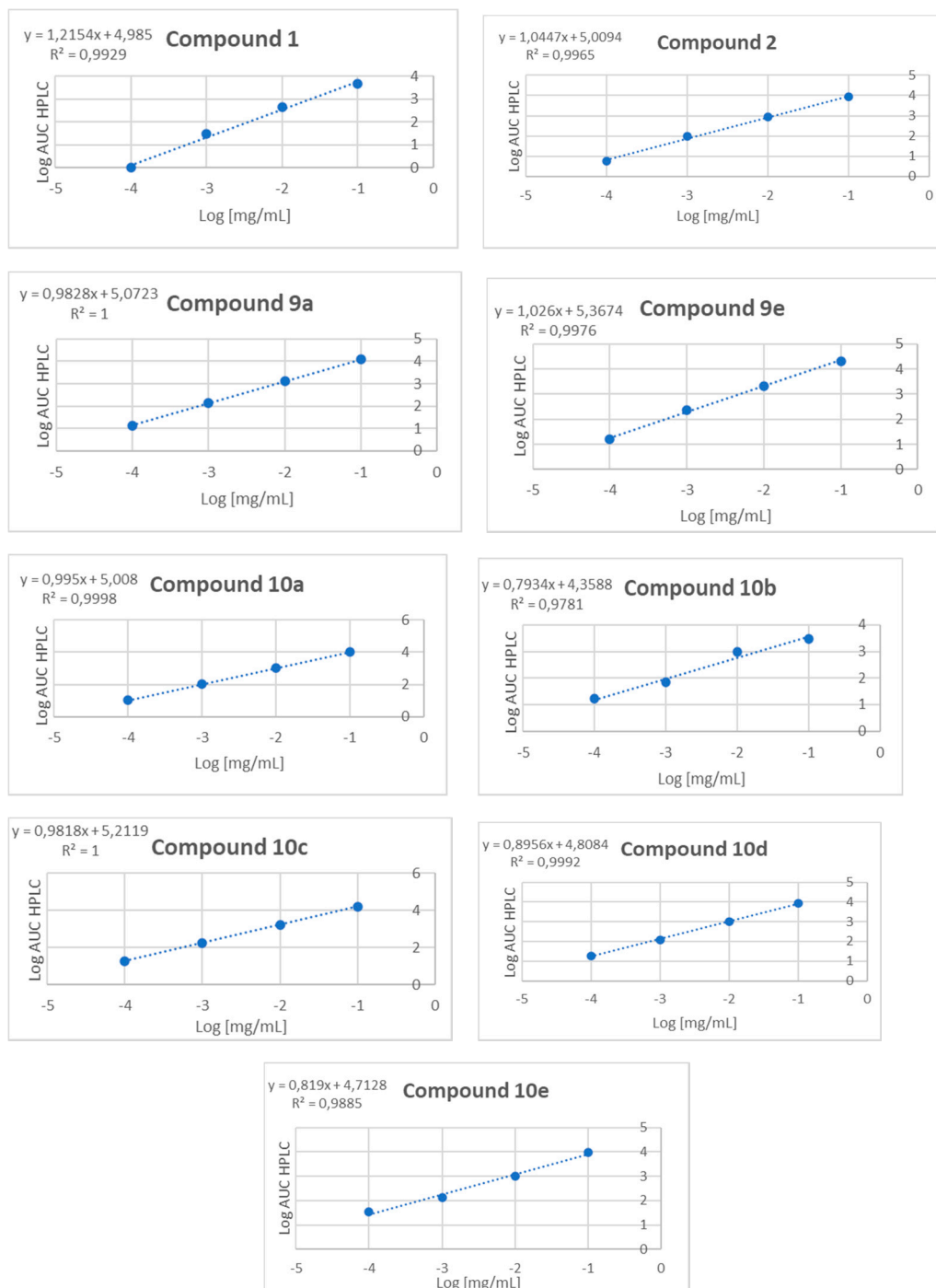
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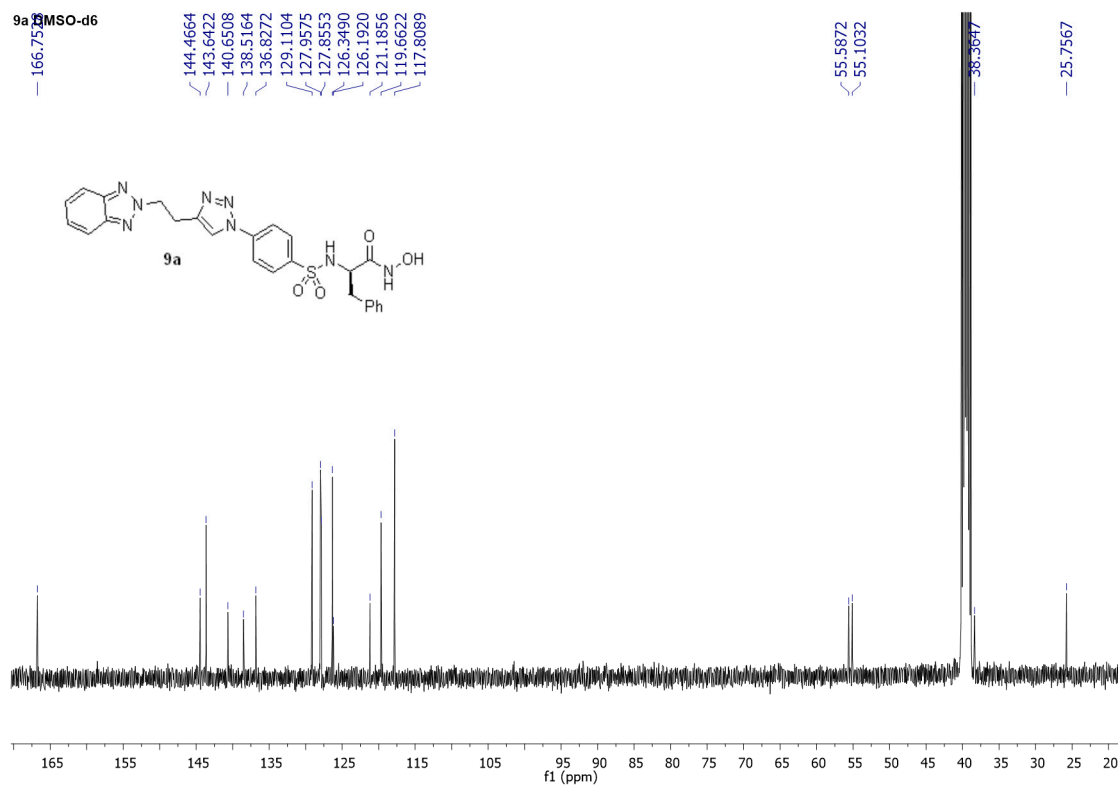
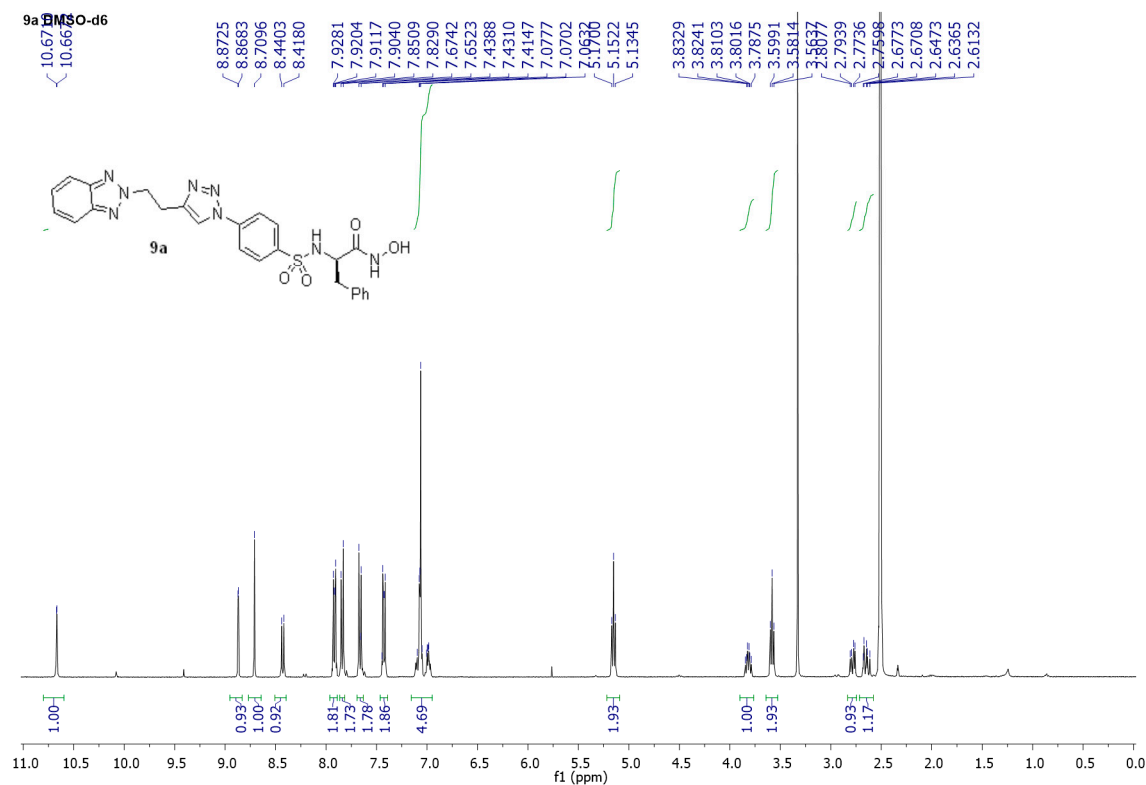
## PART A. Calibration curves of compounds for solubility studies.

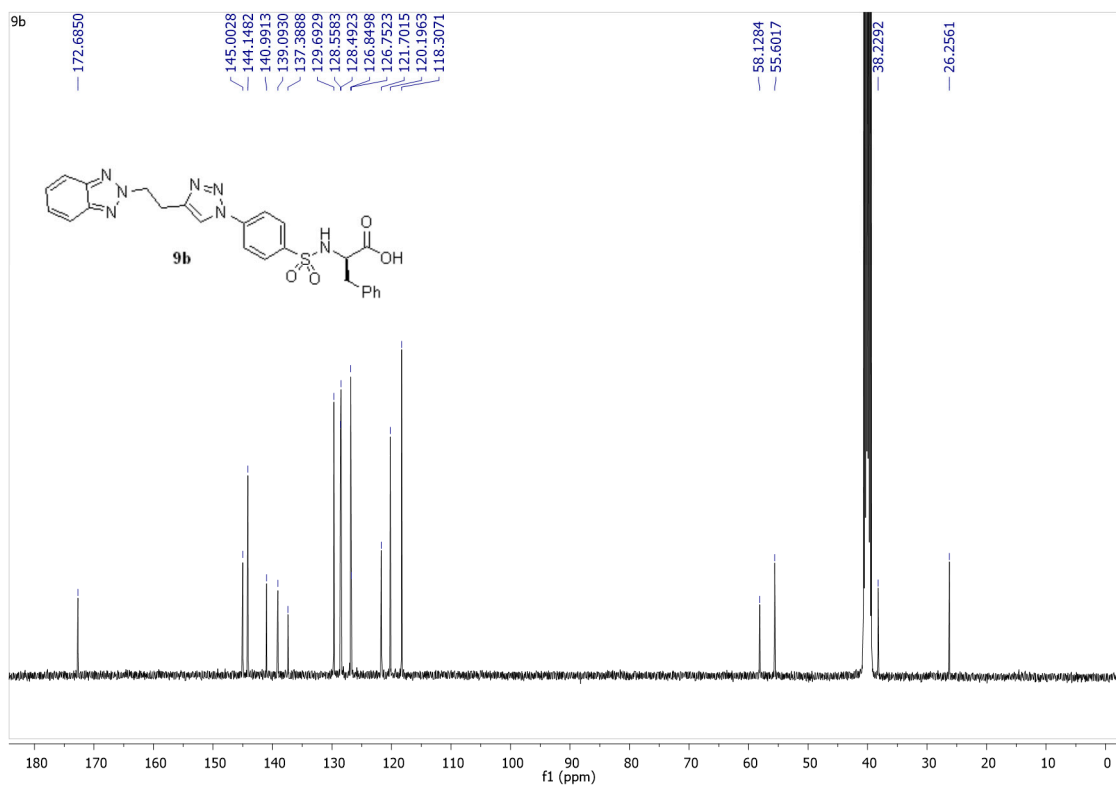
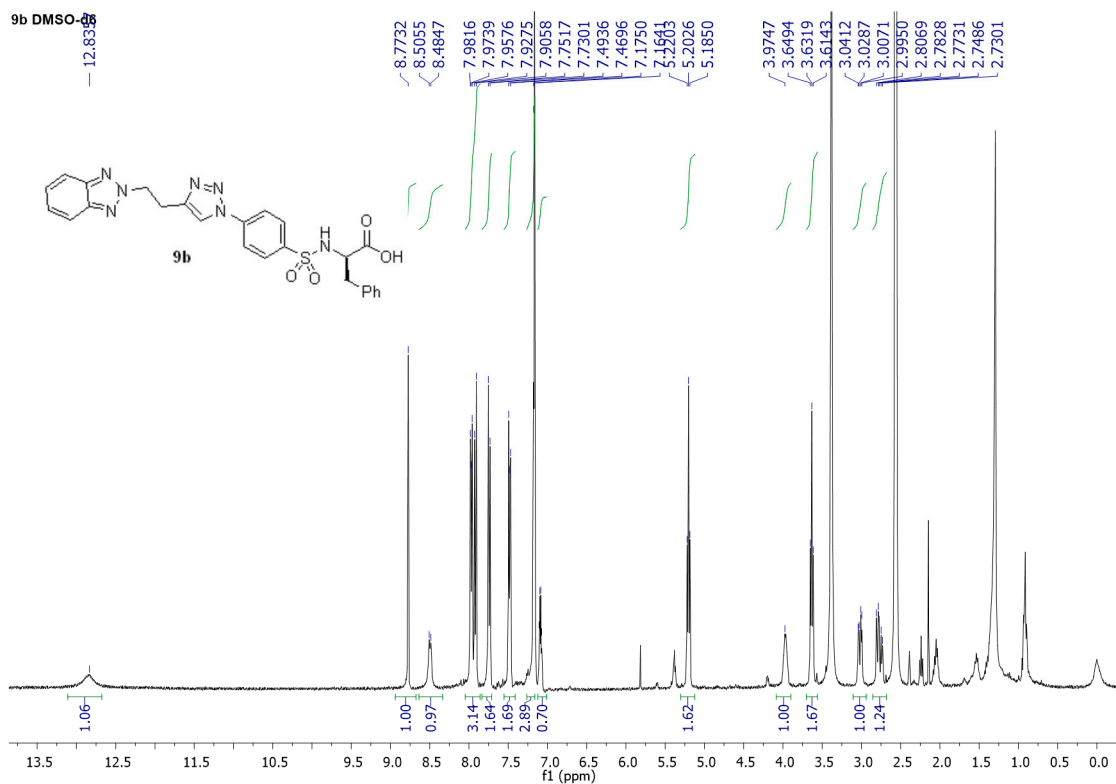
To calculate the solubility of all compounds that showed activity in the enzymatic assay (**1**, **2**, **9a**, **9e**, **10a-e**), 4-points calibration curves were made for each compound. Calibrations curves were obtained using four different concentrations of the corresponding compound (0.1 mg/mL, 0.01 mg/mL, 0.001 mg/mL and 0.0001 mg/mL). All samples were prepared in DMSO and measured in a LC-HRMS (Agilent 1260 Infinity II).



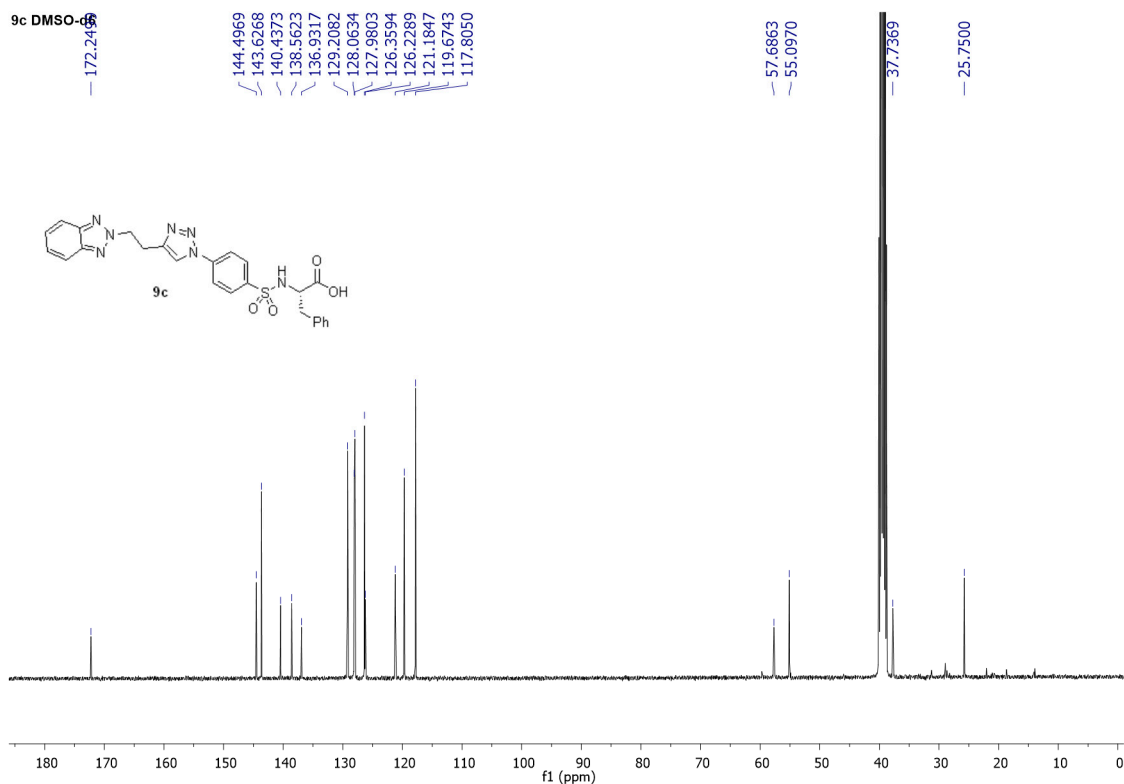
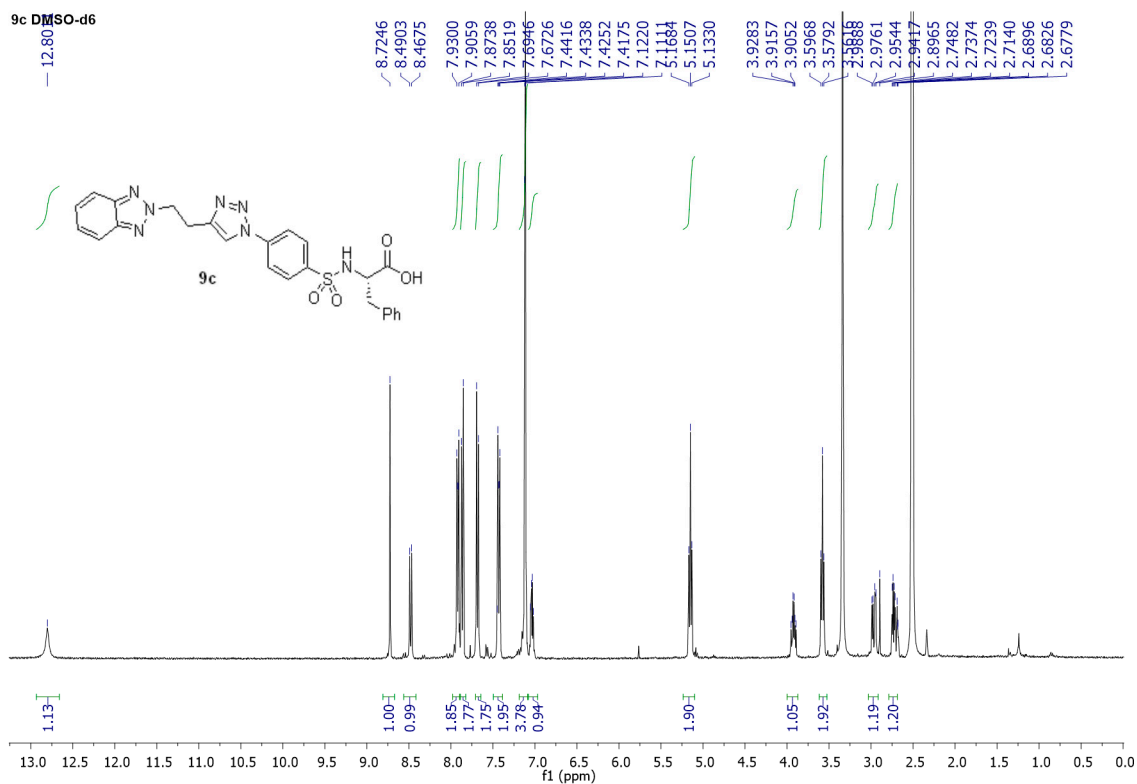
**Figure S1.** Calibration curves of all active compounds (solubility studies) (**1**, **2**, **9a**, **9e**, **10a-e**)

## PART B. $^1\text{H}$ and $^{13}\text{C}$ NMR spectra.

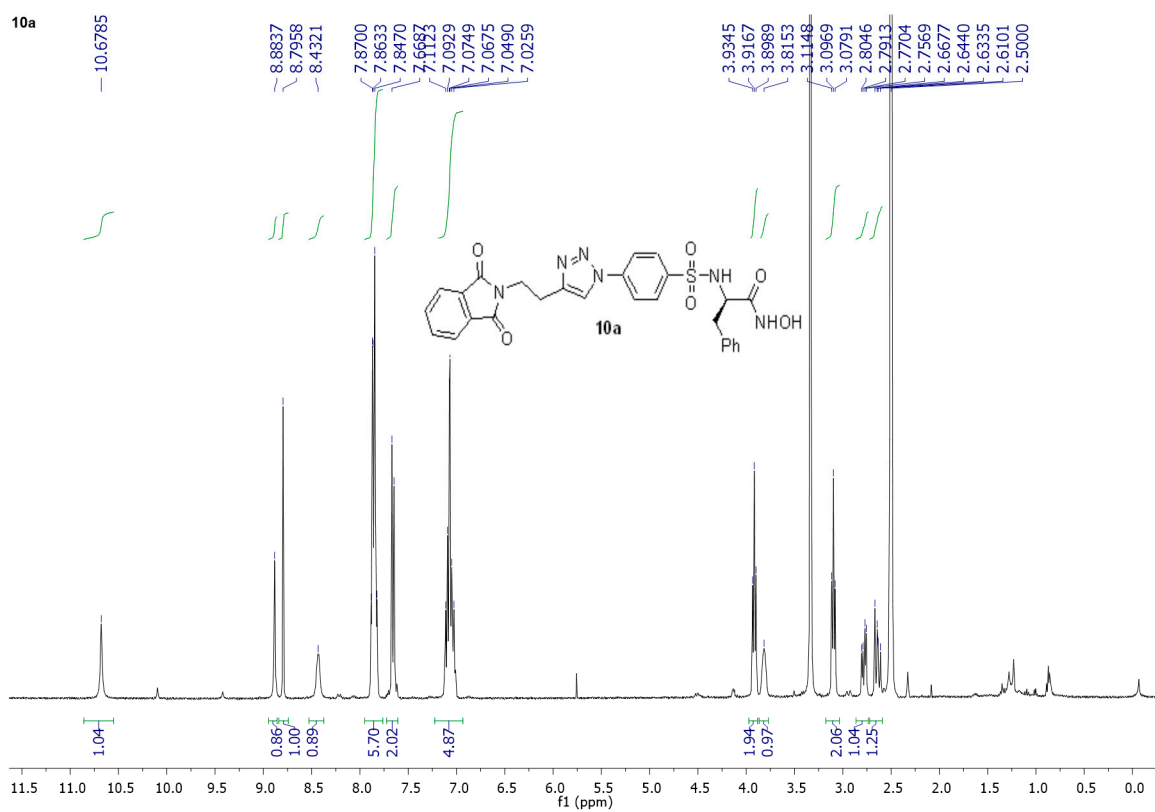
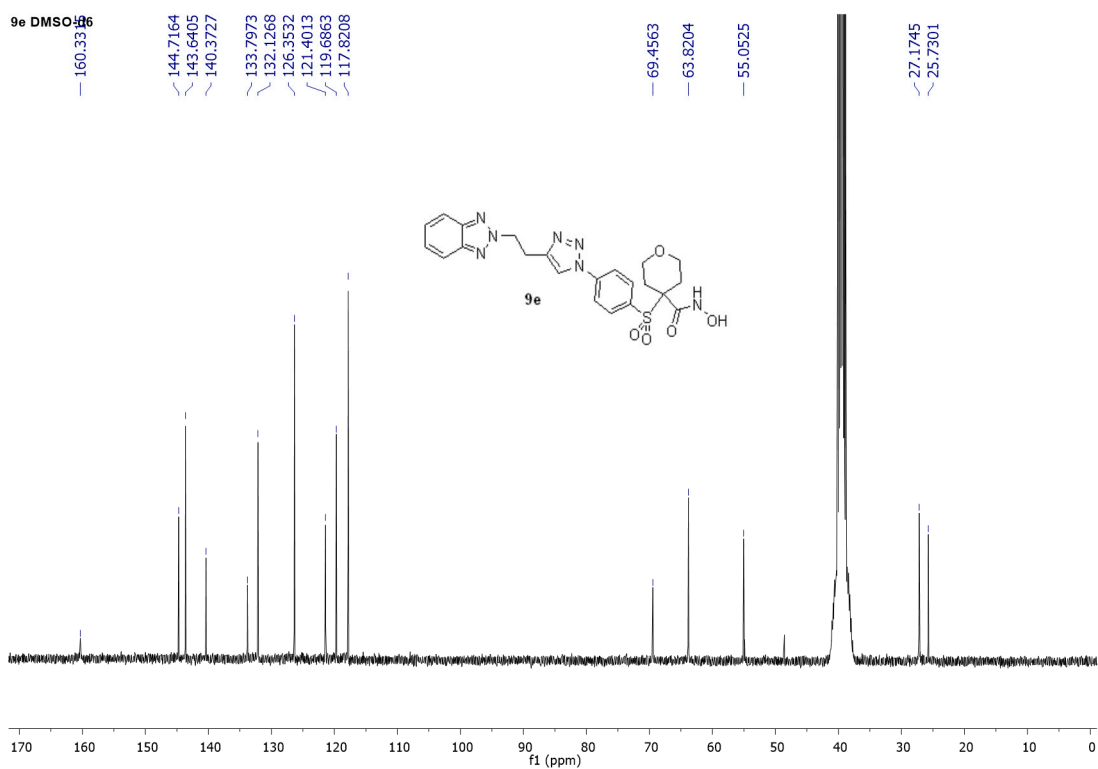


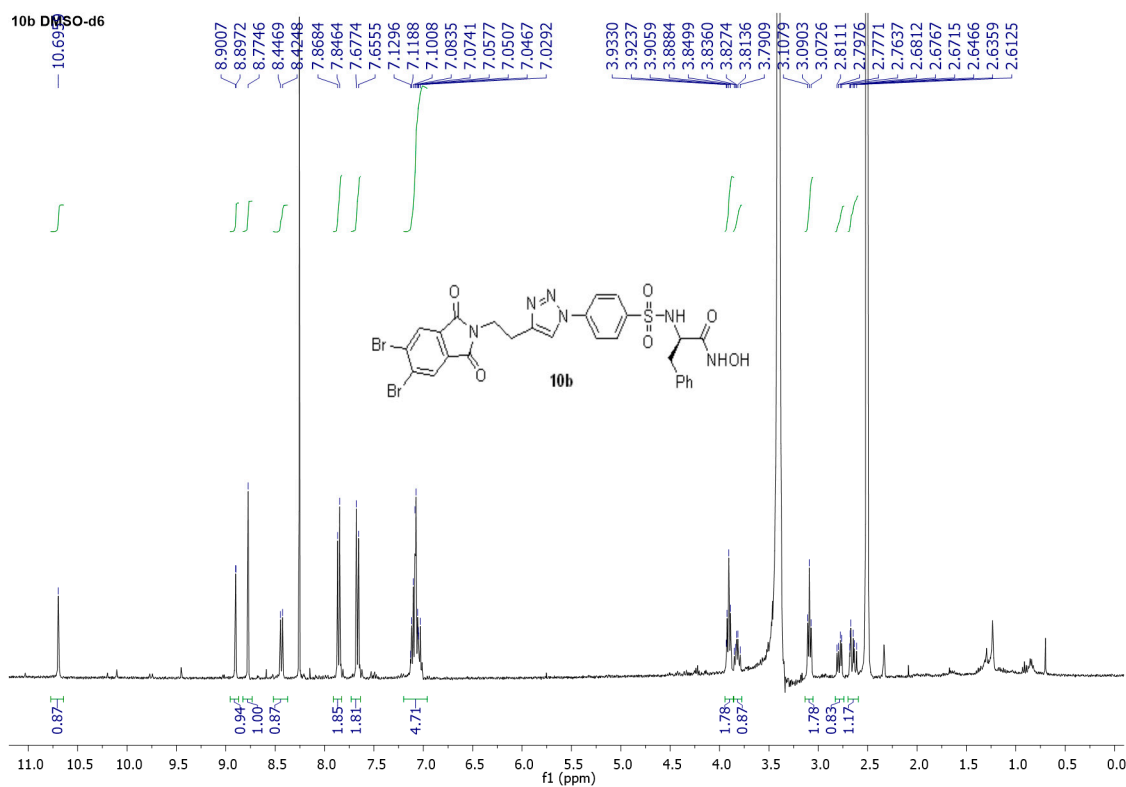
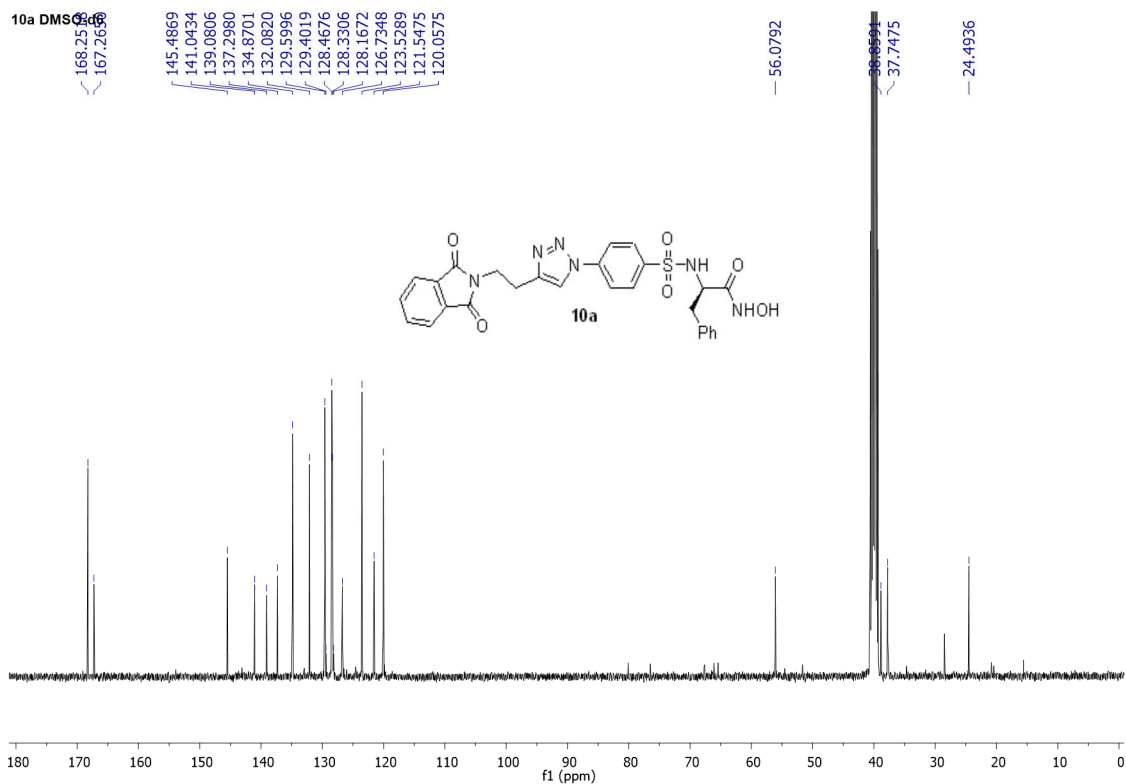


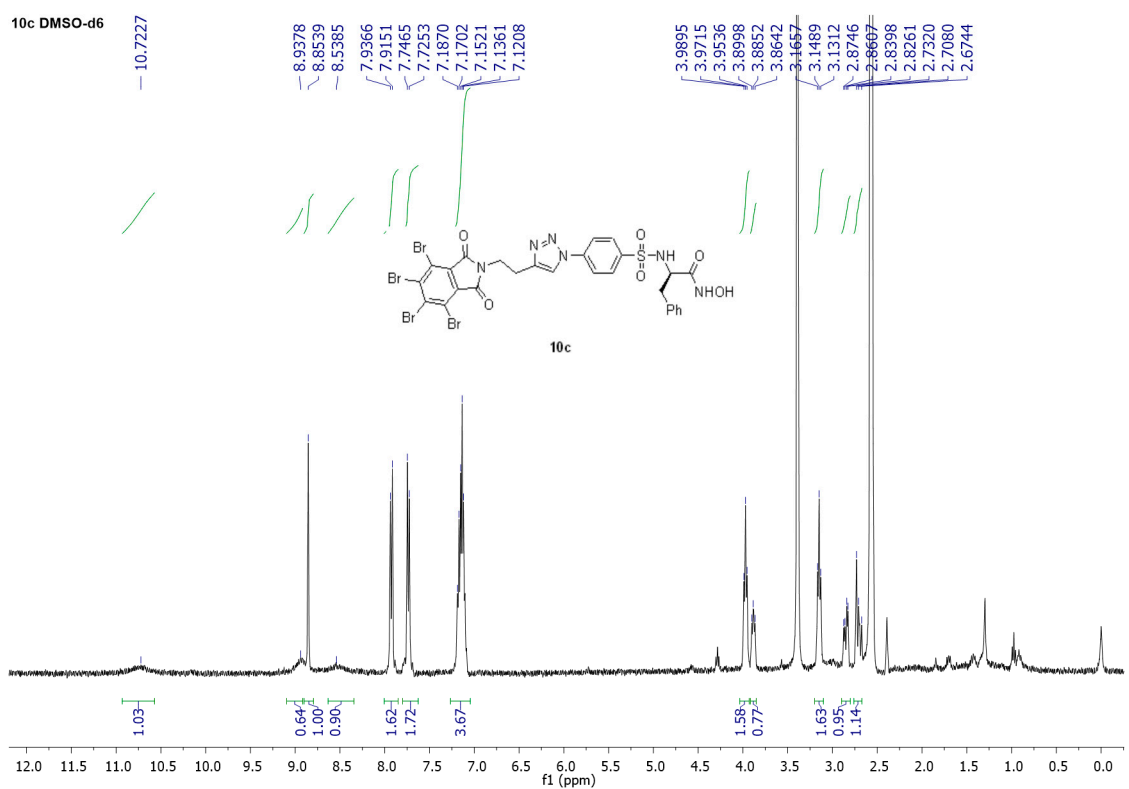
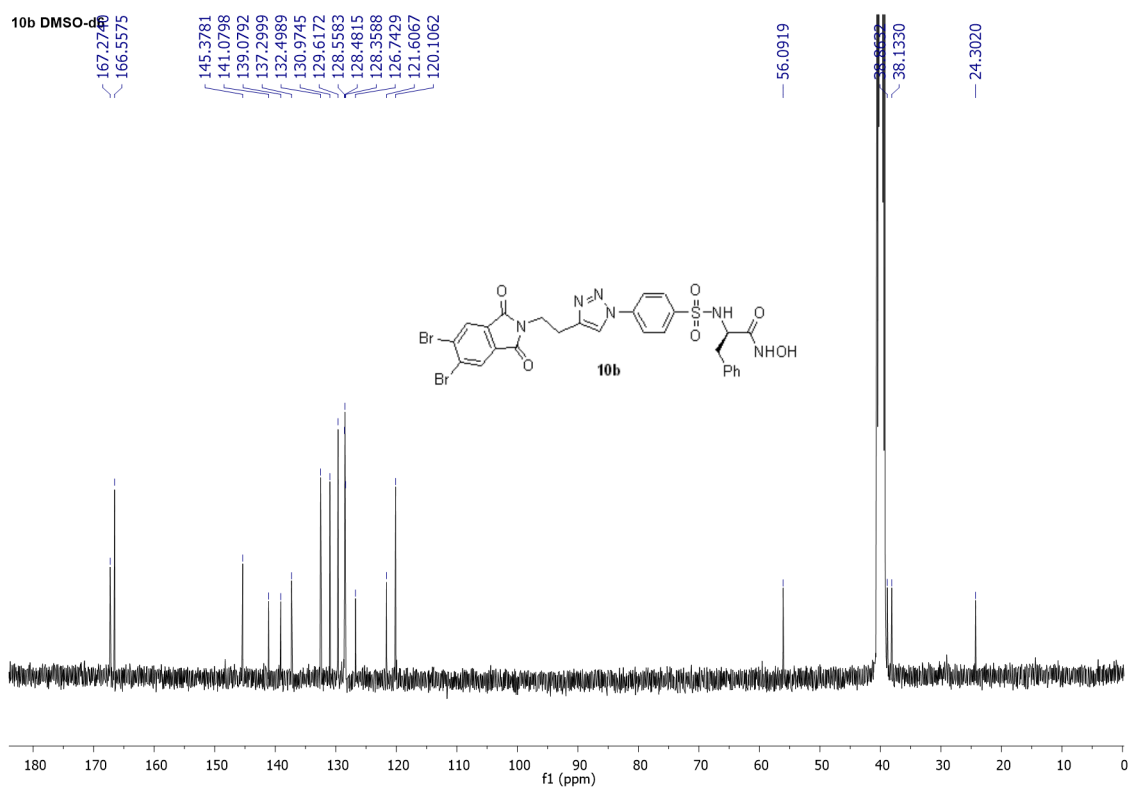


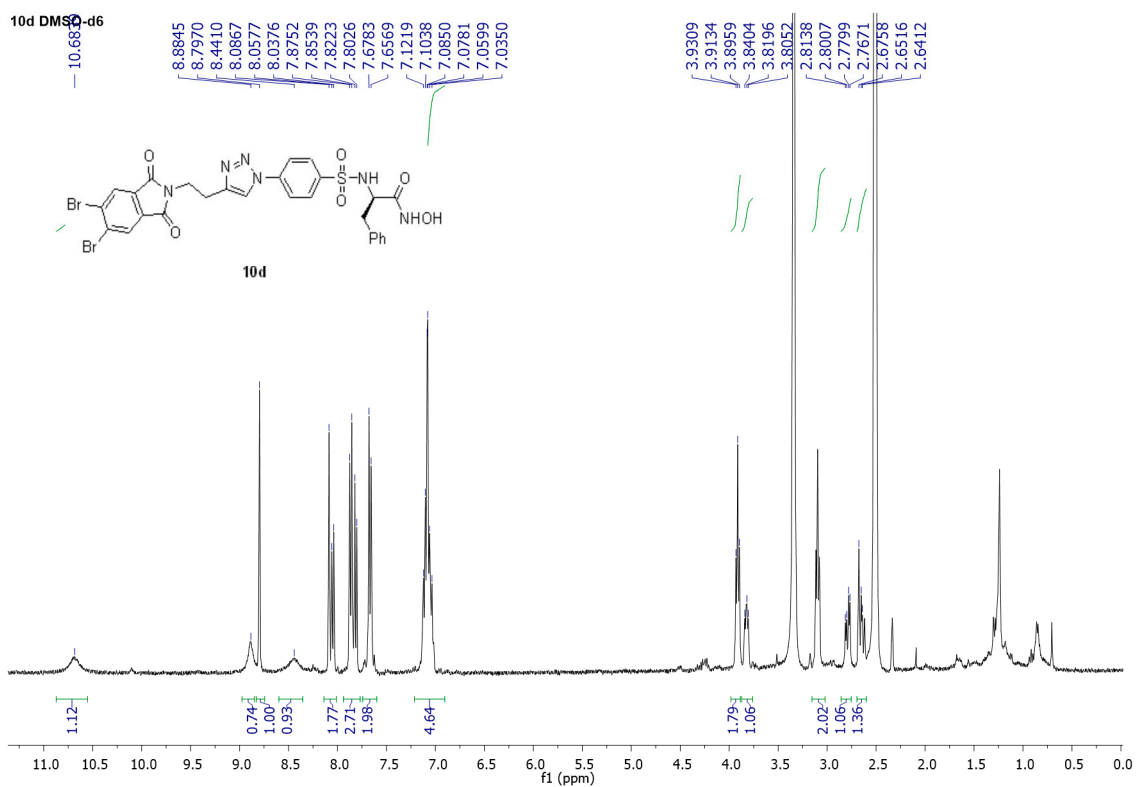
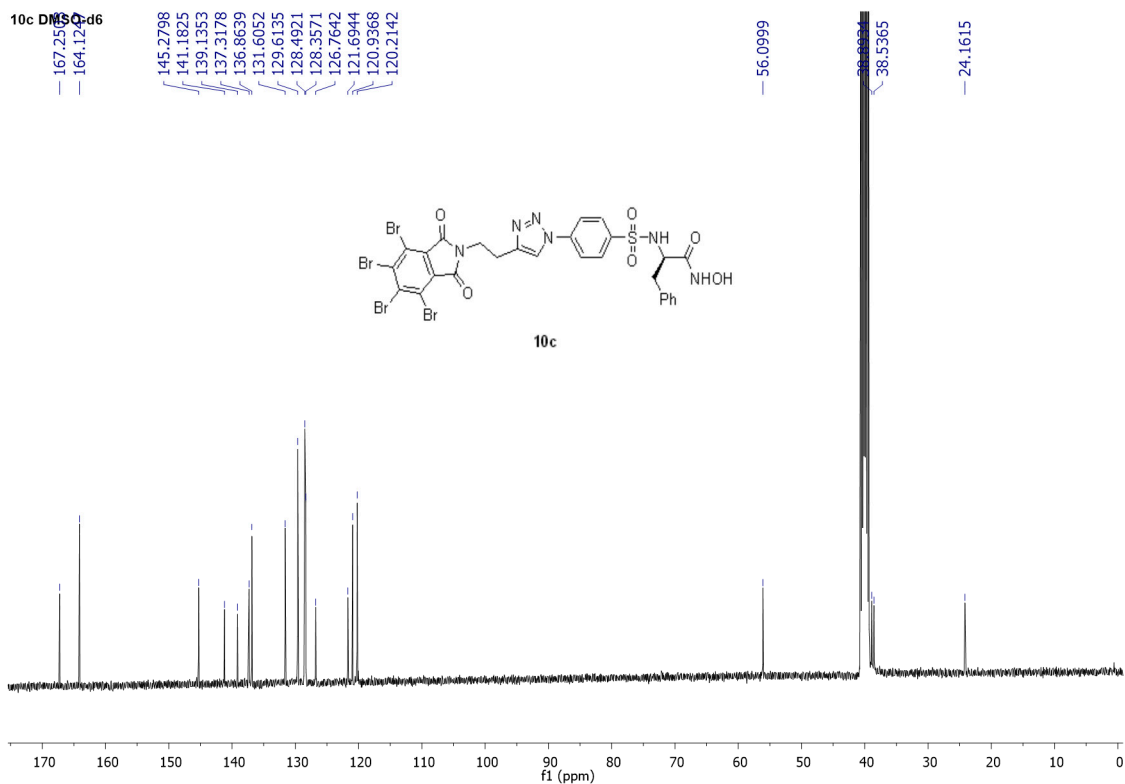


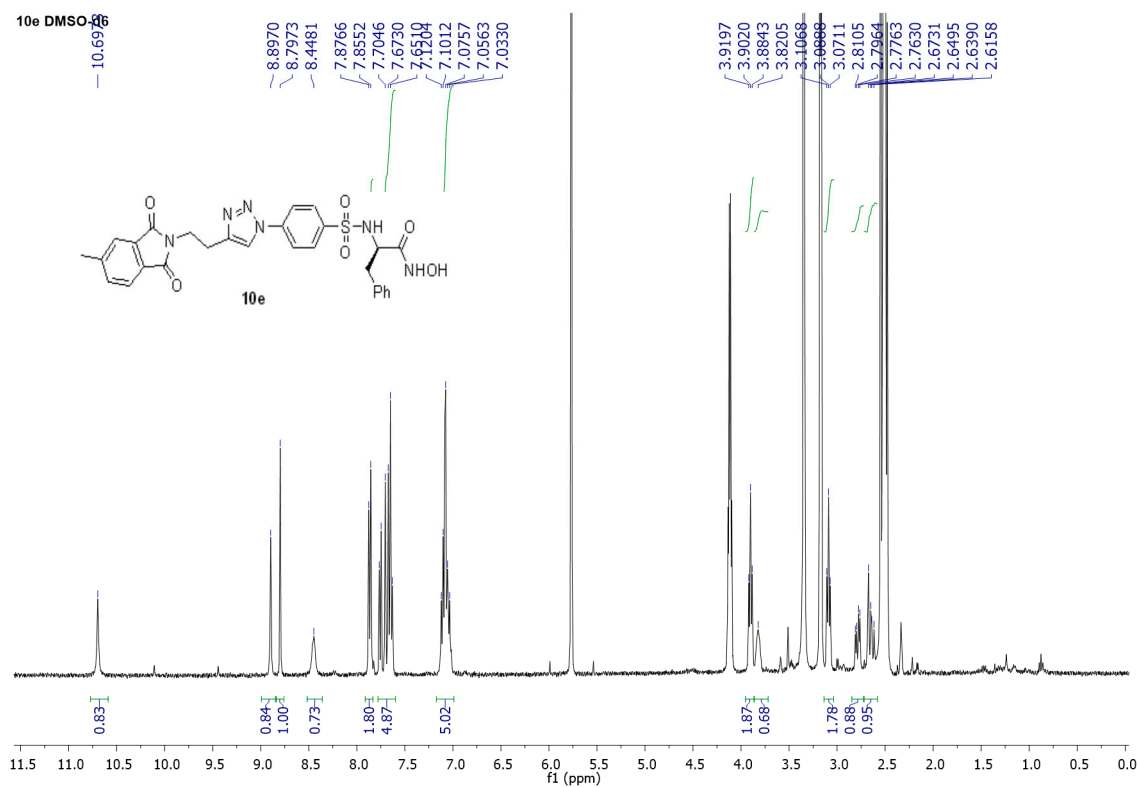
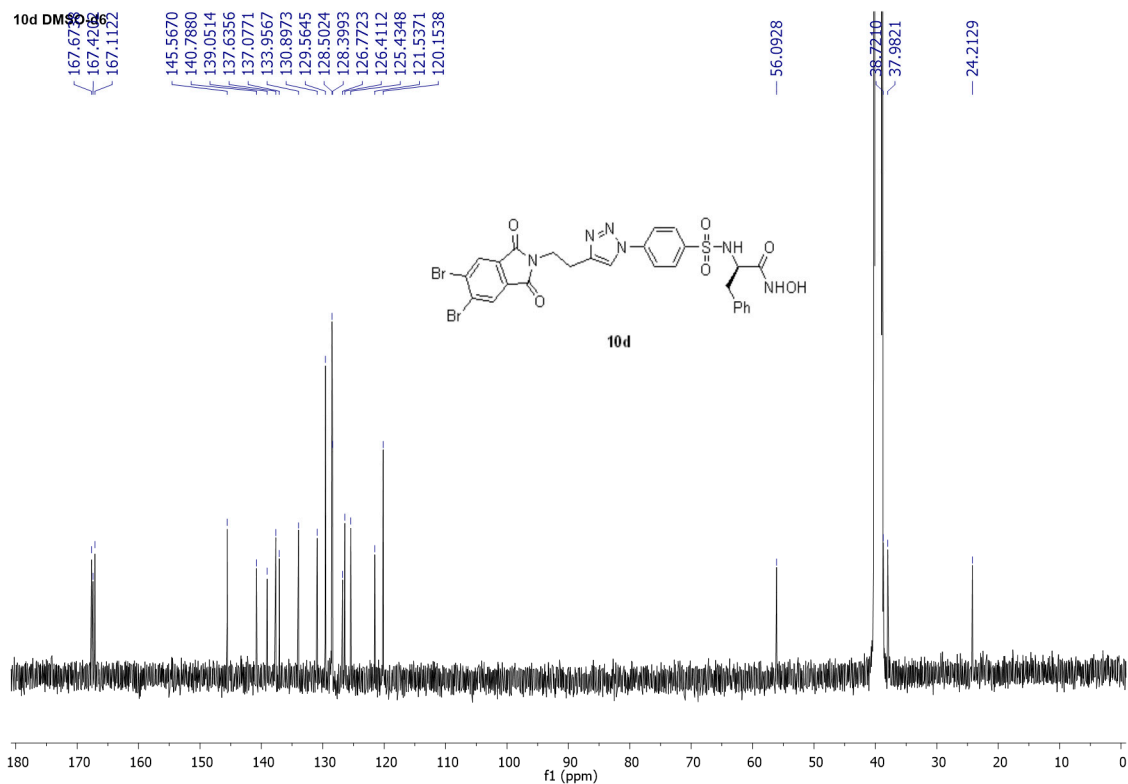


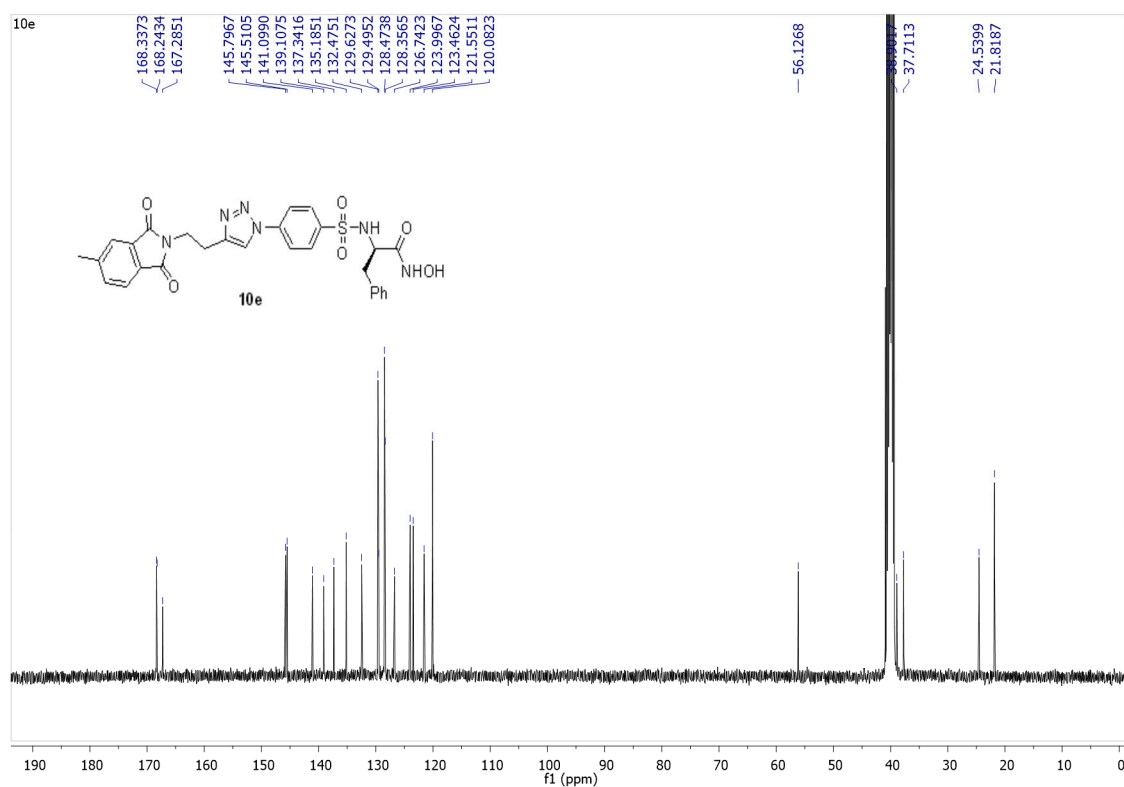








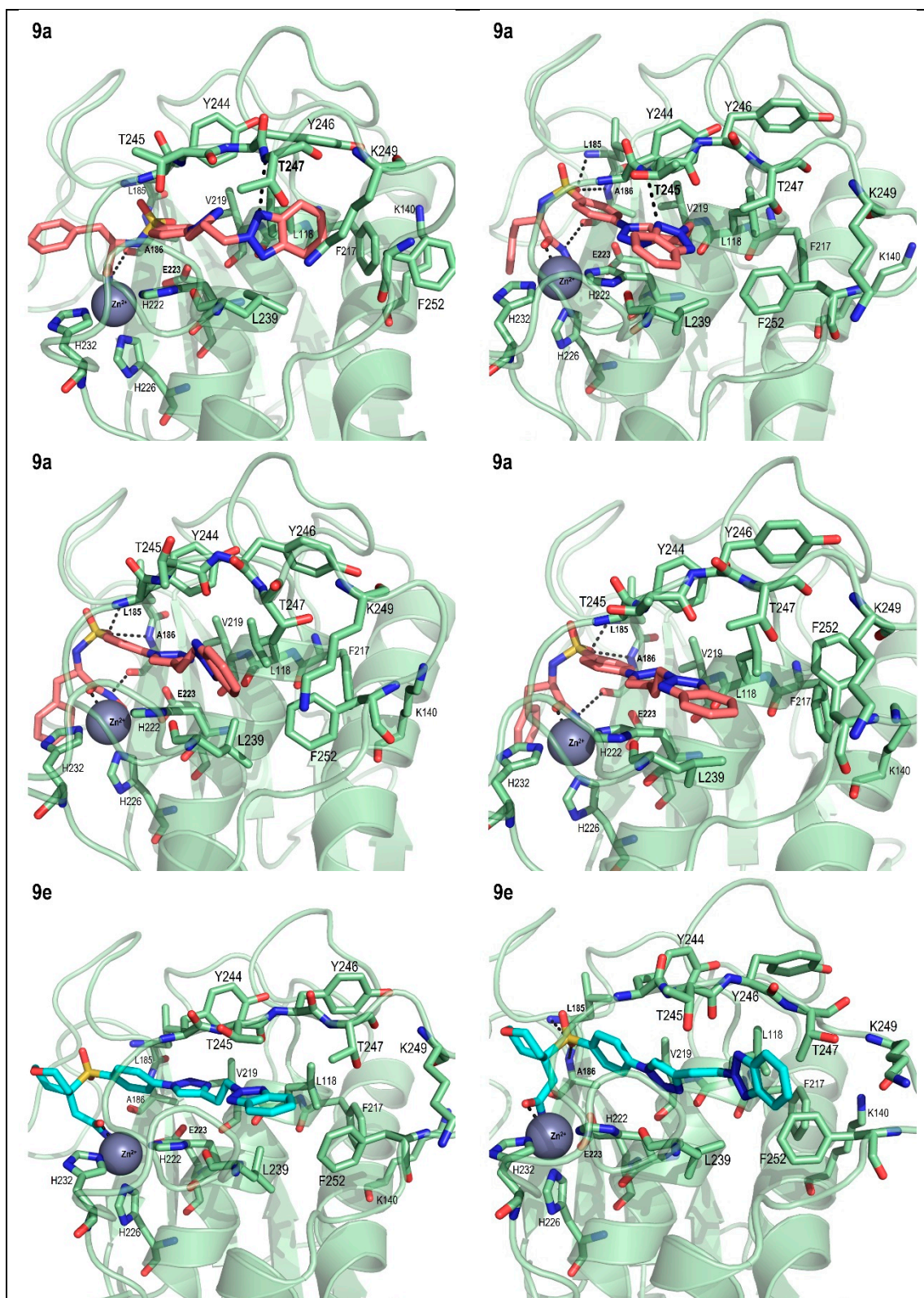


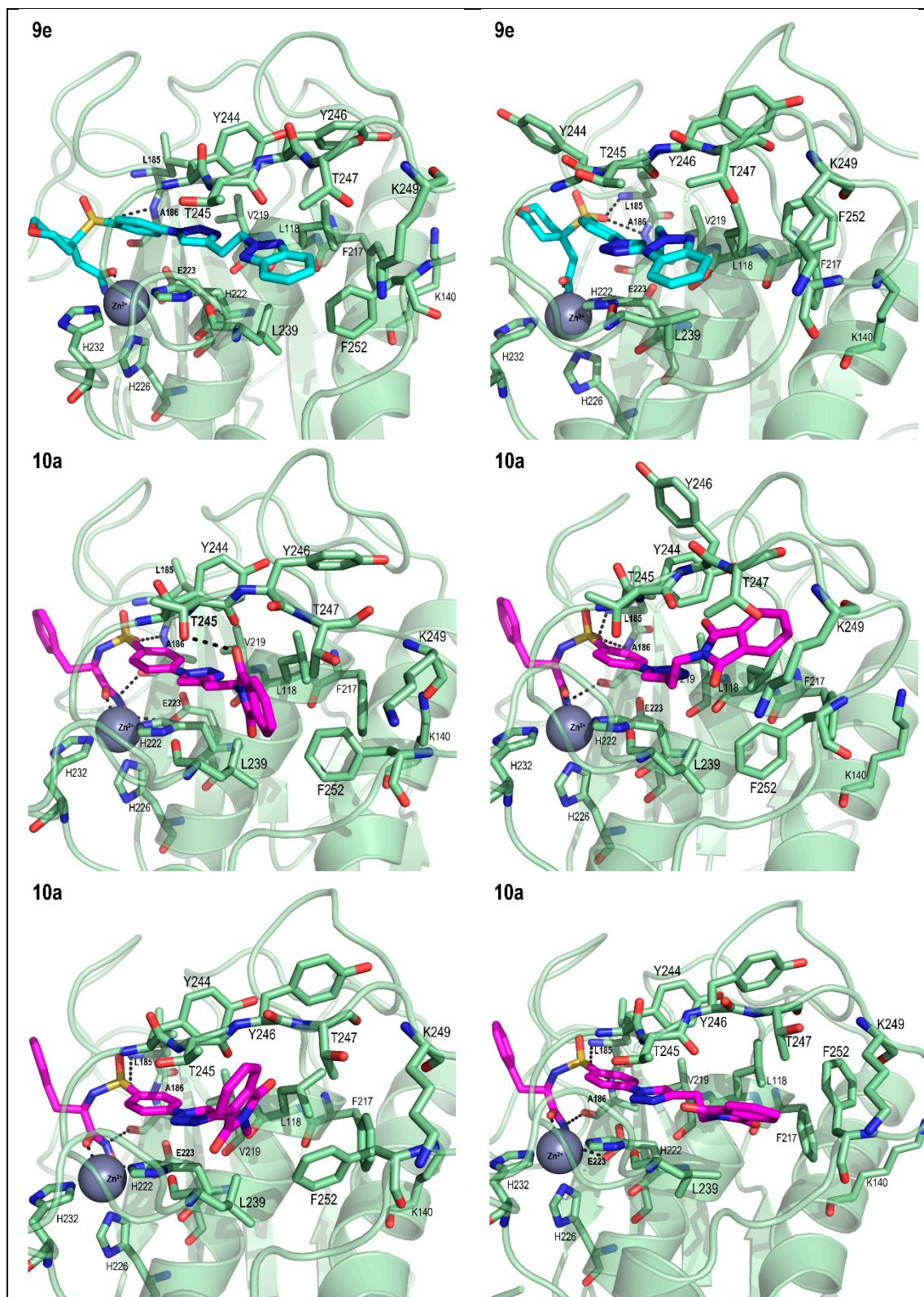


**Figure S2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for final compounds.

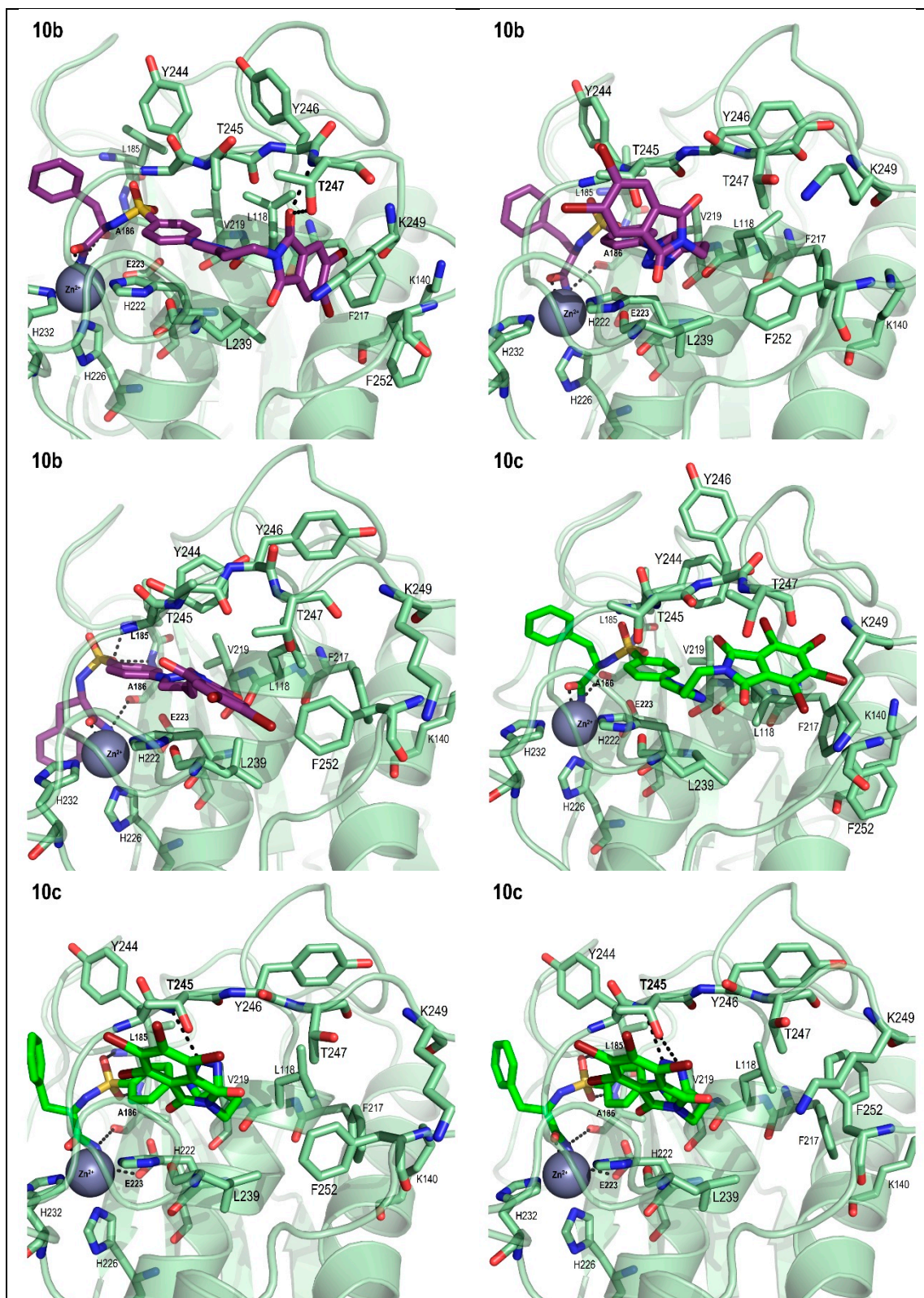


## PART C. Modeling

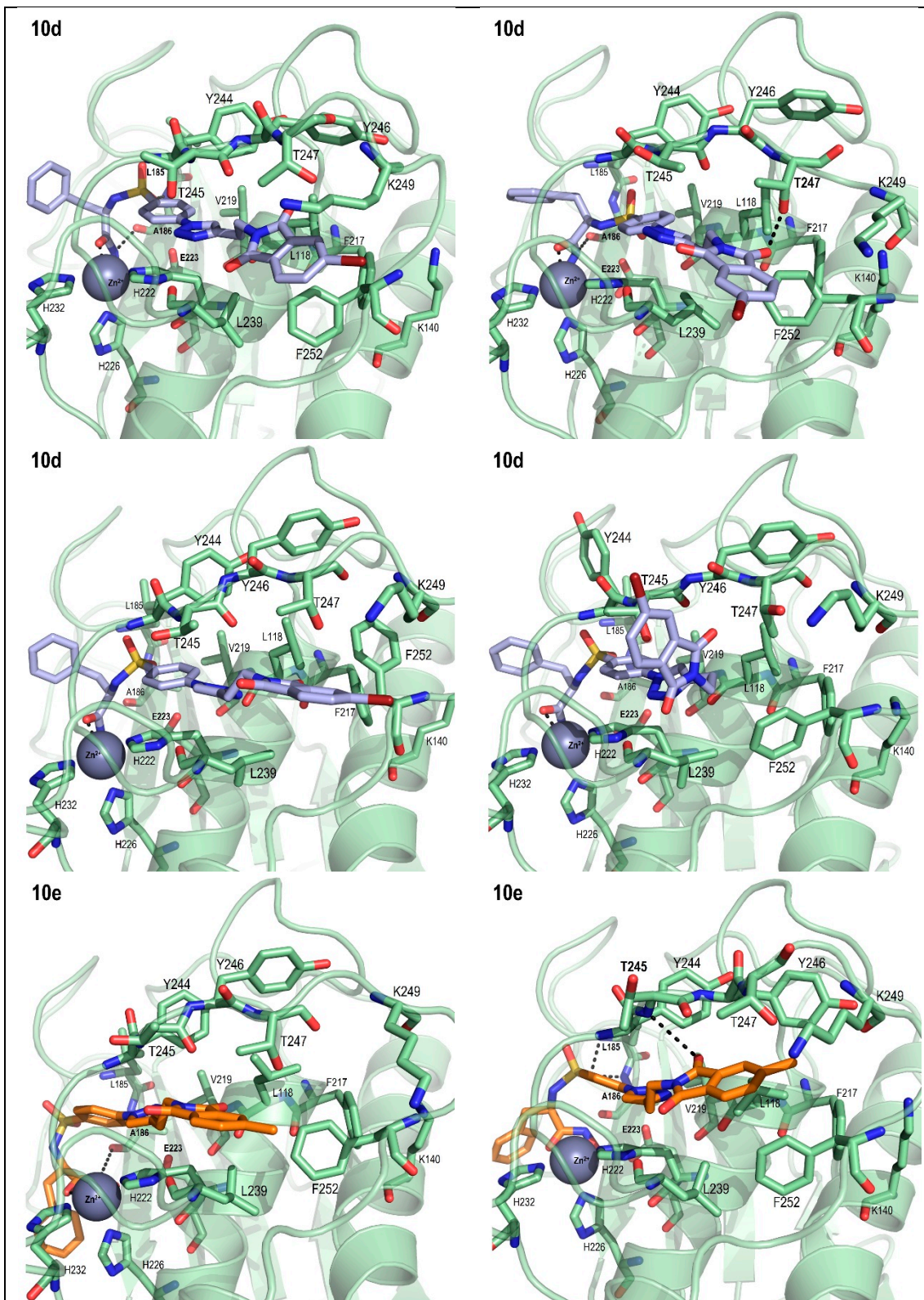




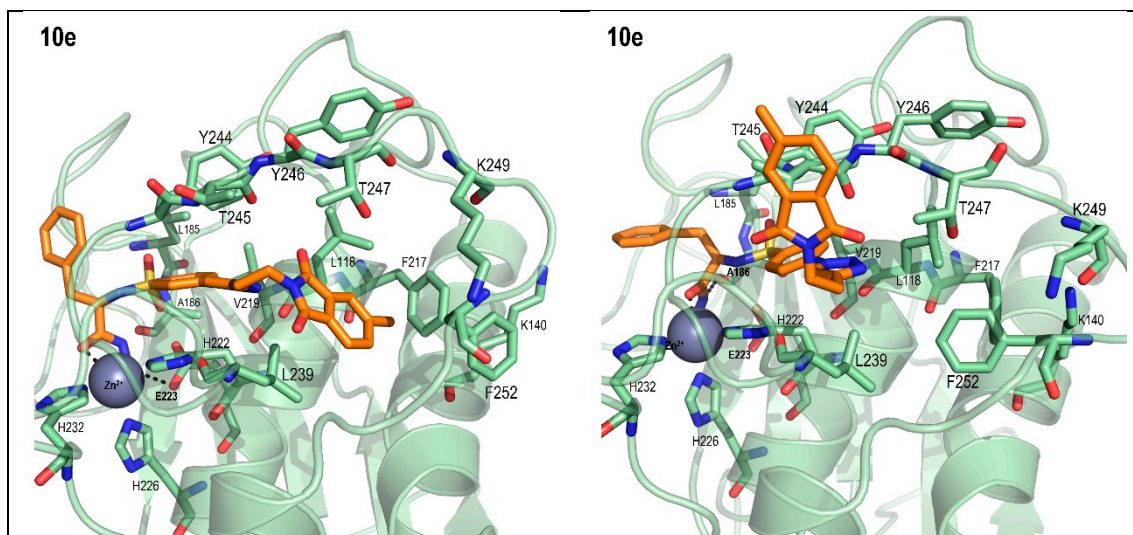




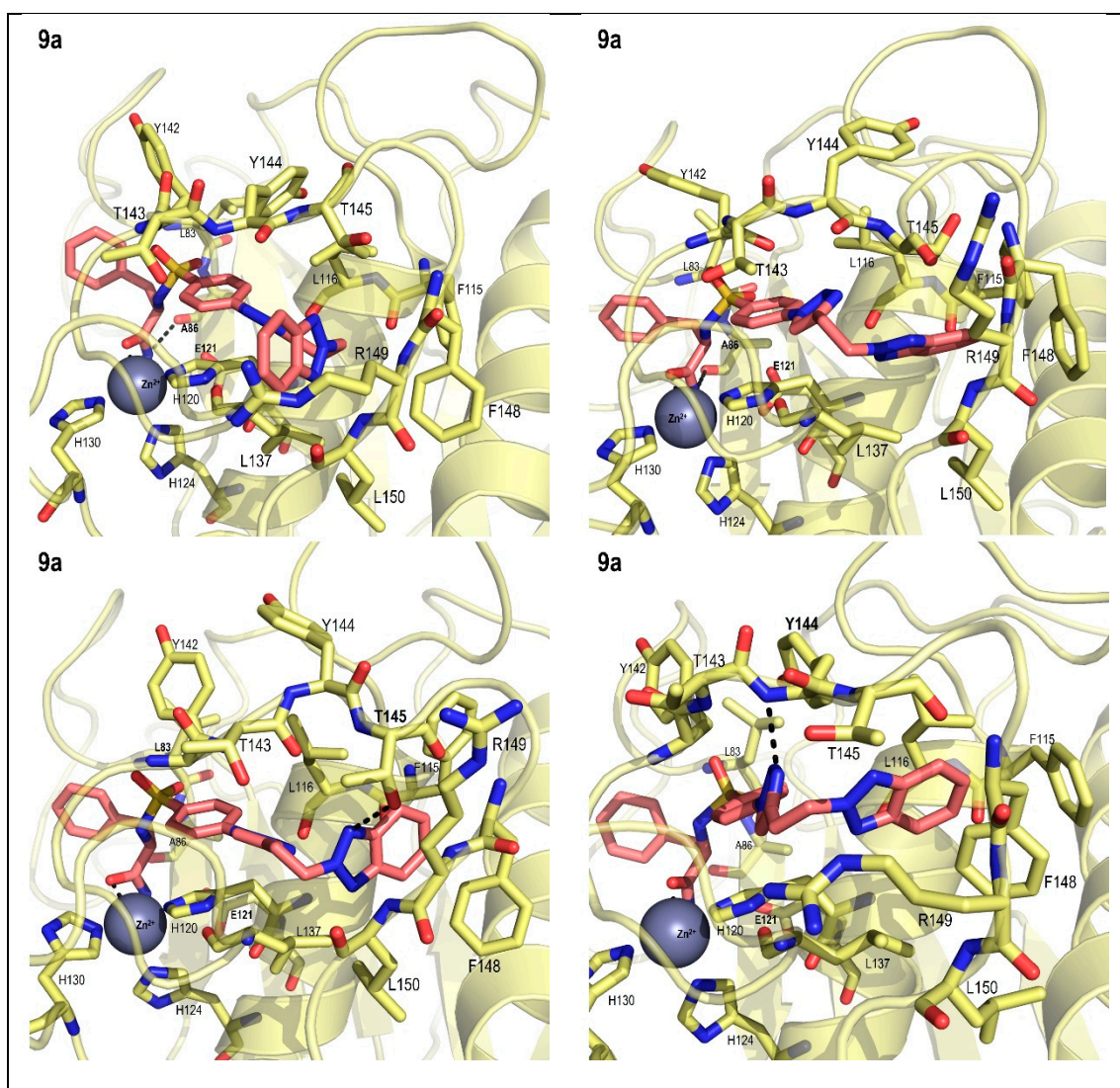




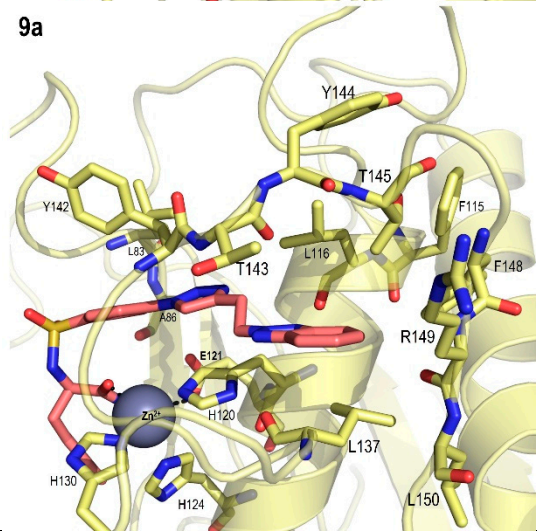
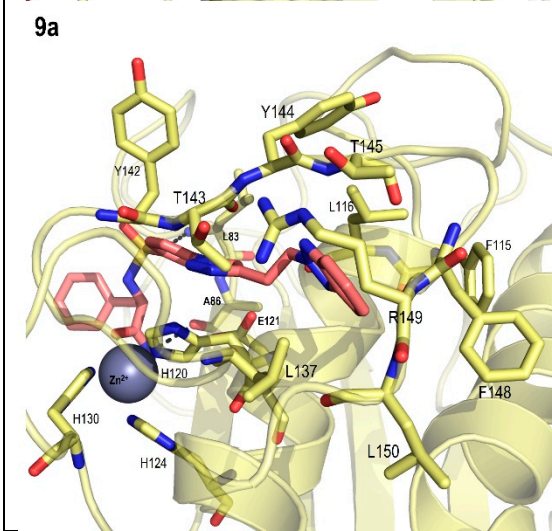
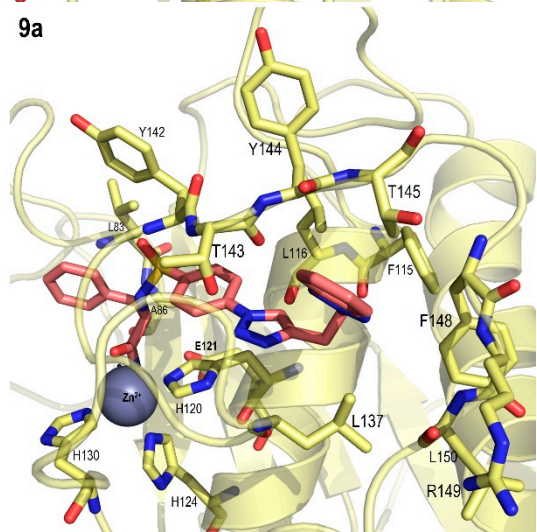
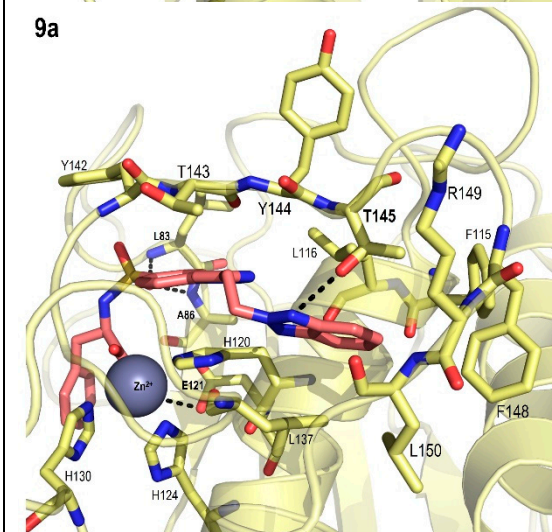
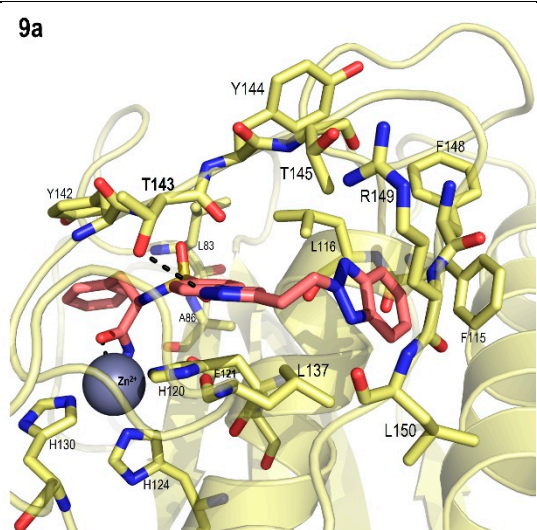
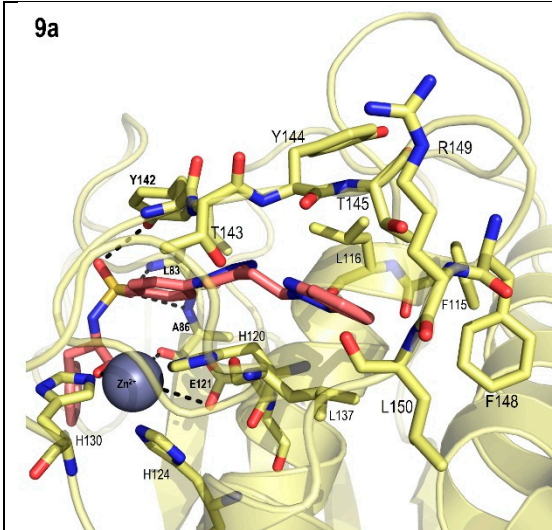




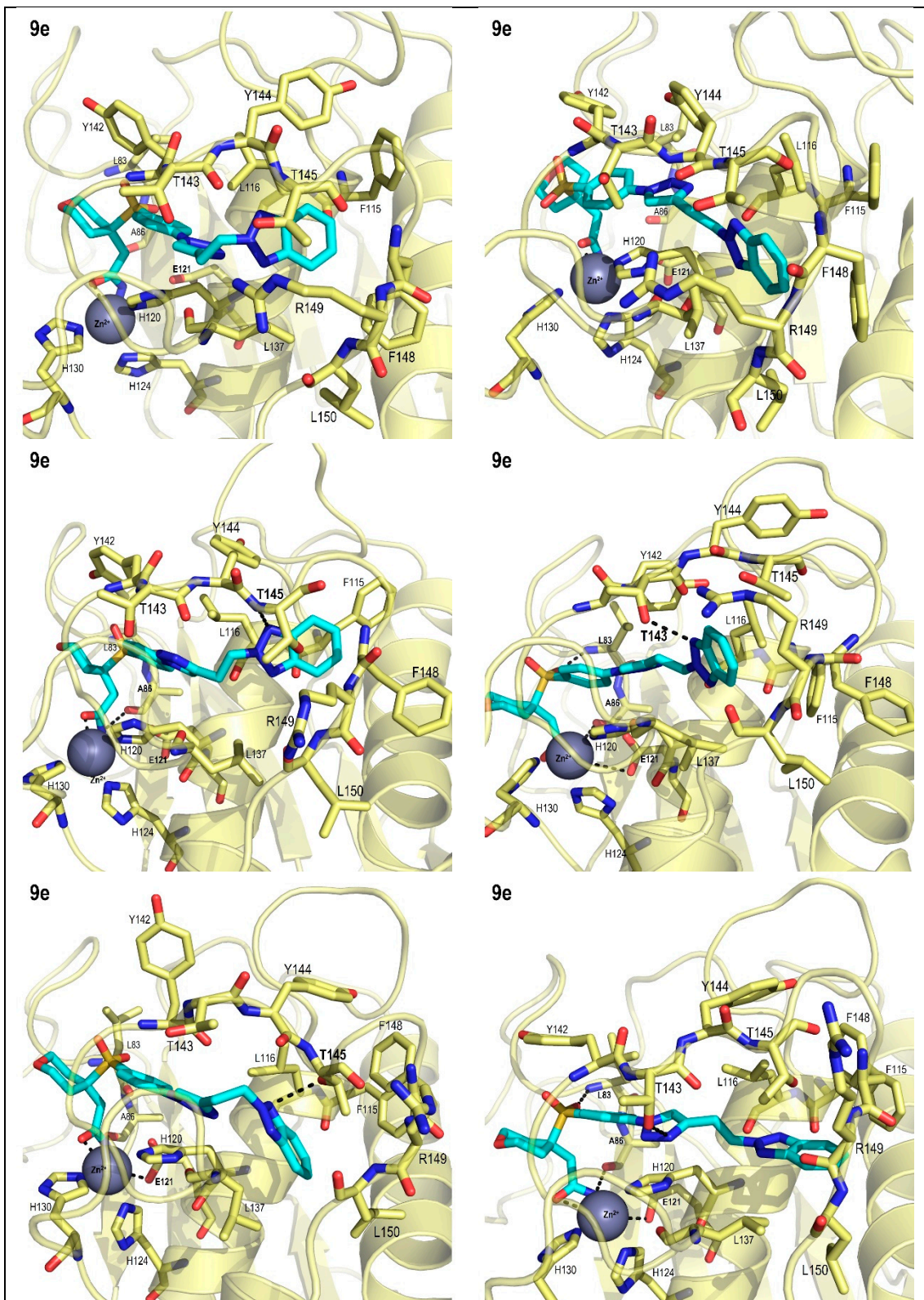
**Figure S3.** PyMOL stick and cartoon representation of the interactions established with the  $\Omega$ -loop and S1' obtained in the additional binding modes of the series of compounds when docked to the different crystal structures of MMP-13. Hydrogen bonds are shown in dashed lines, the catalytic zinc ion is shown as a grey sphere. For the sake of clarity, no hydrogens are shown.



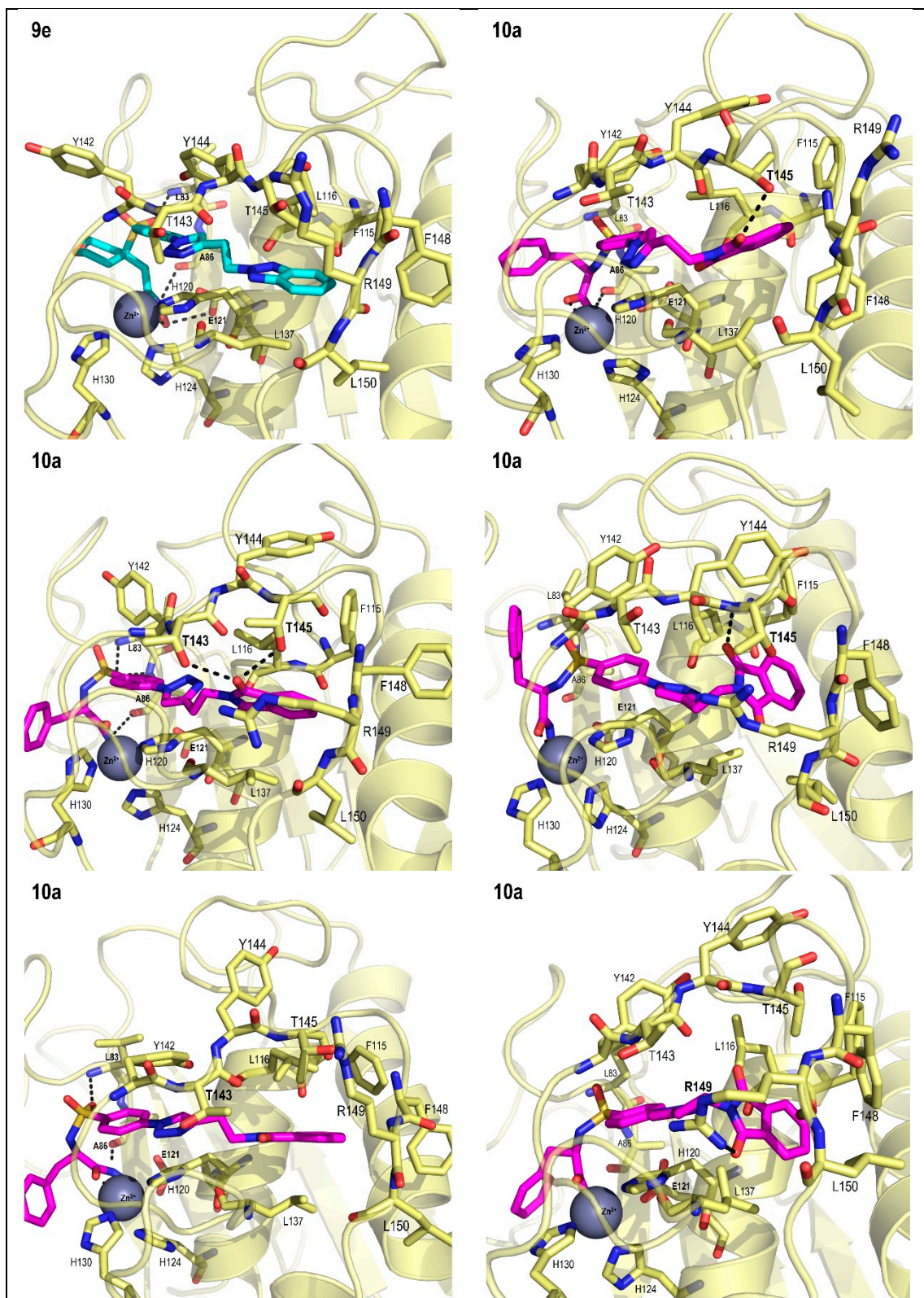




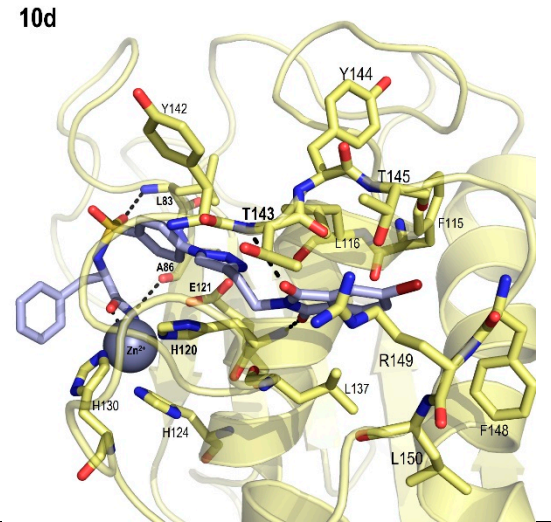
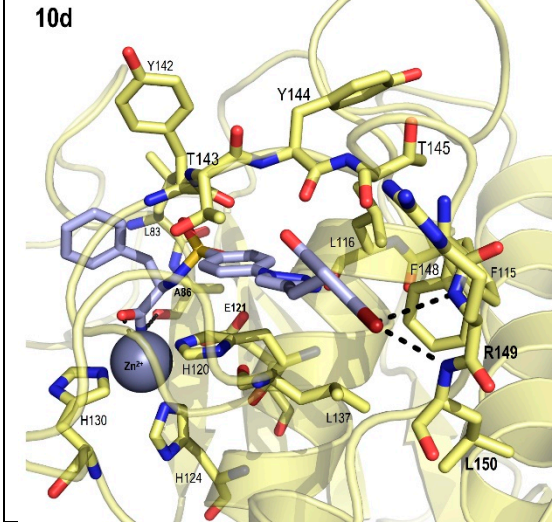
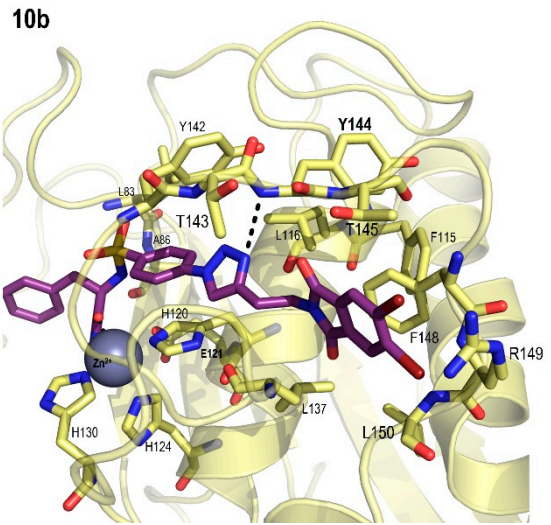
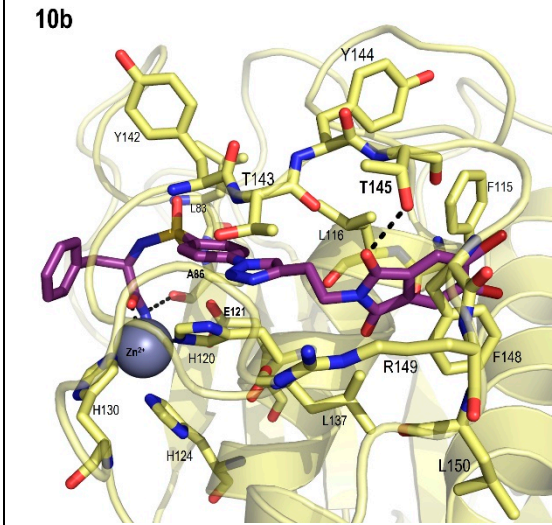
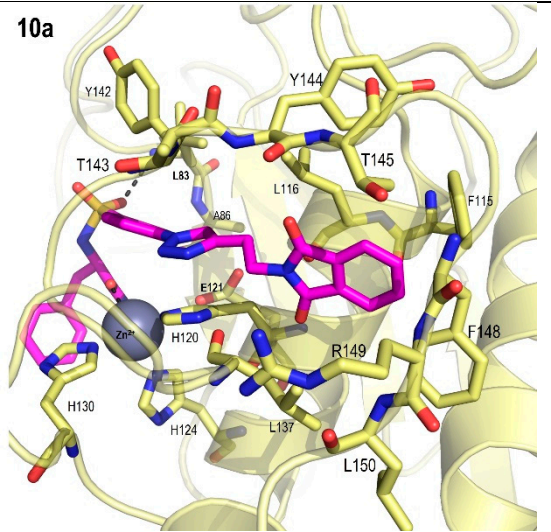
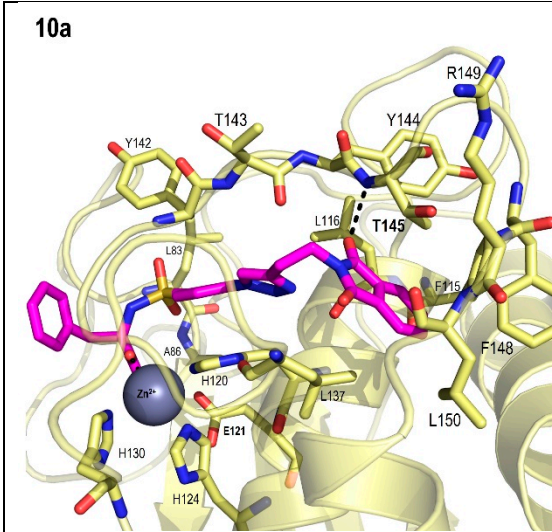




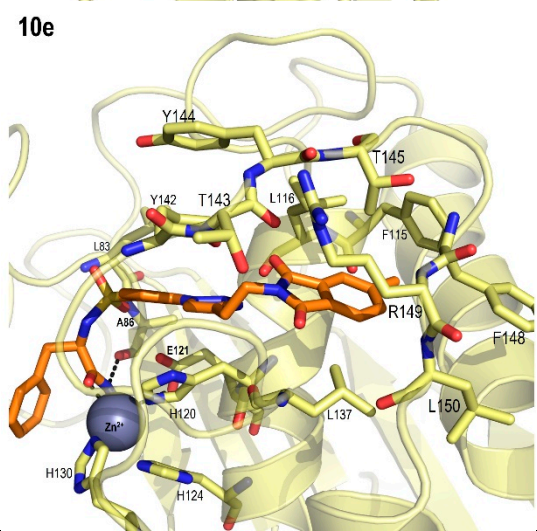
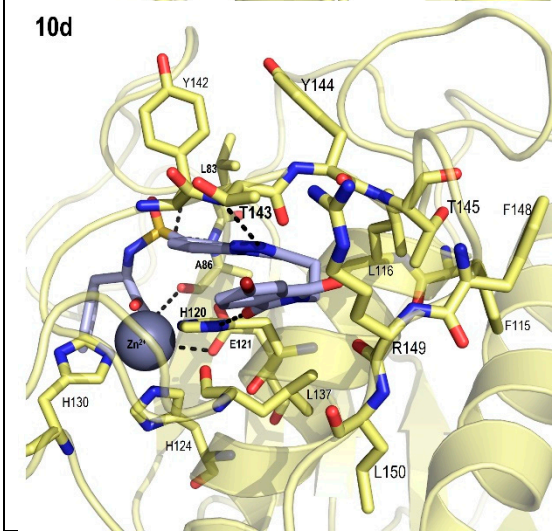
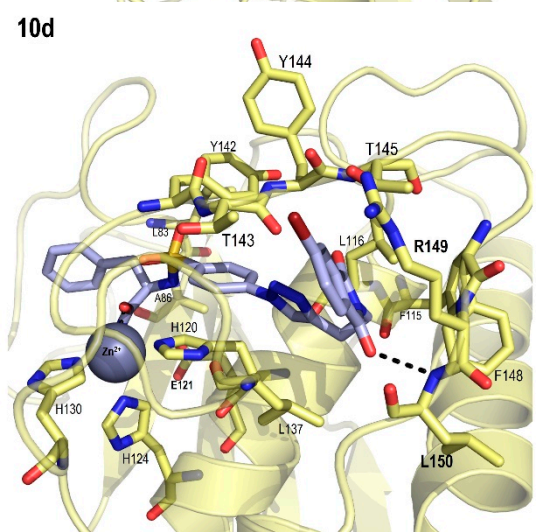
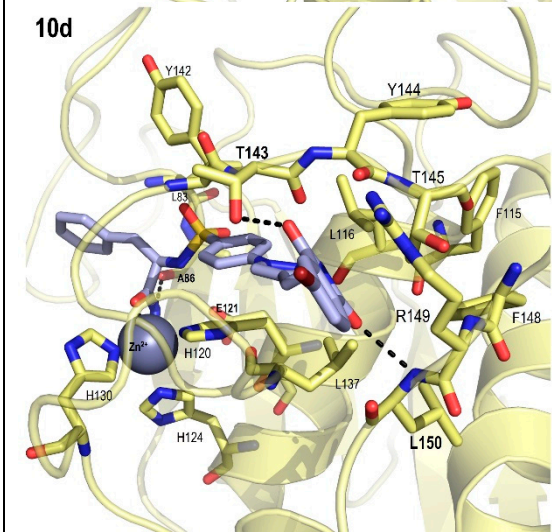
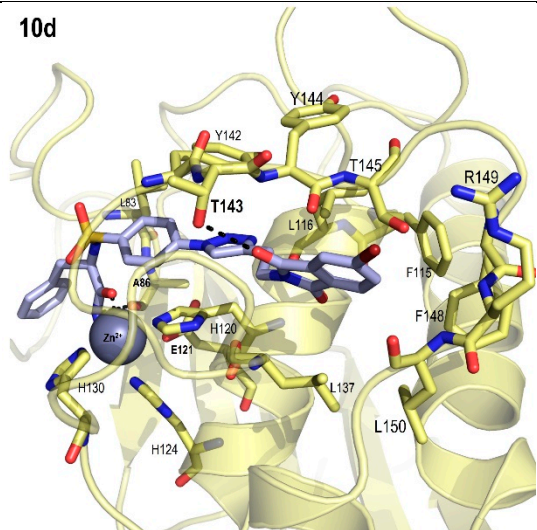
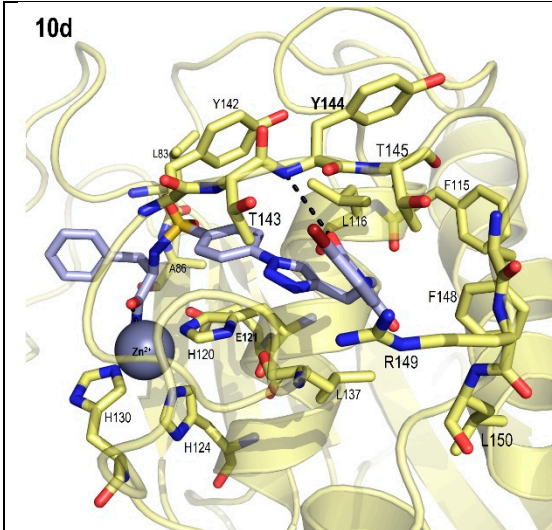


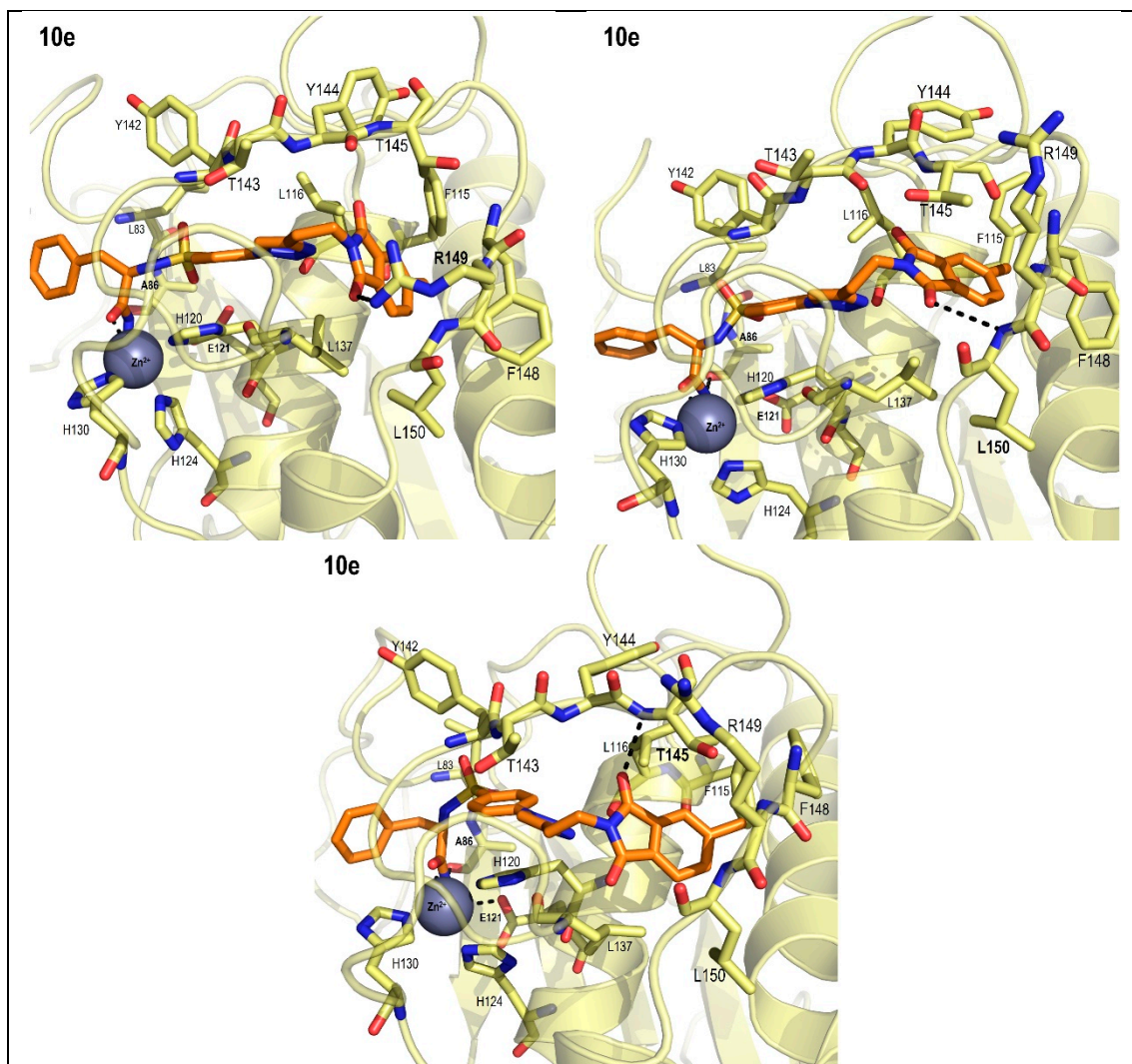




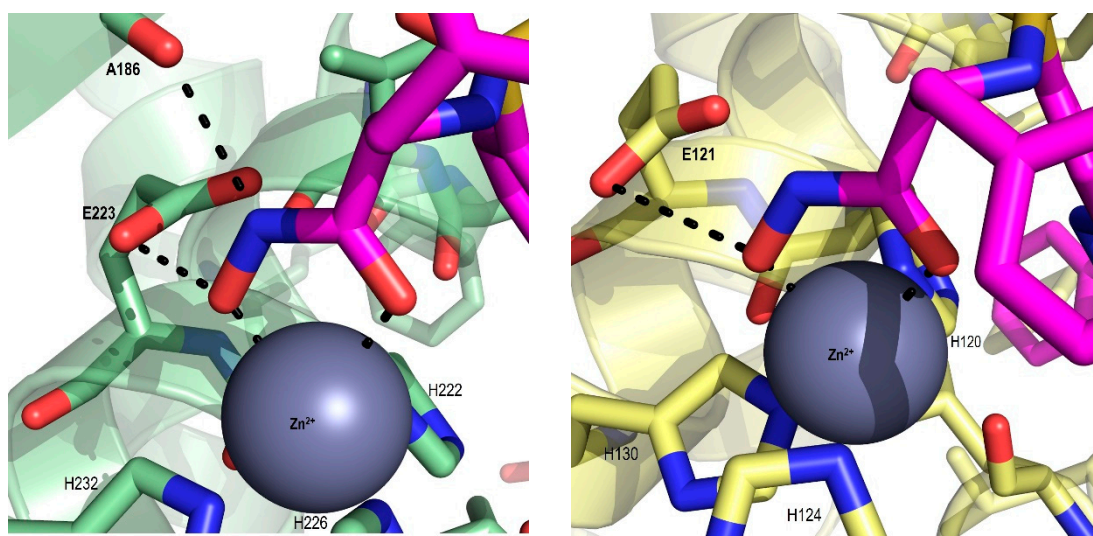








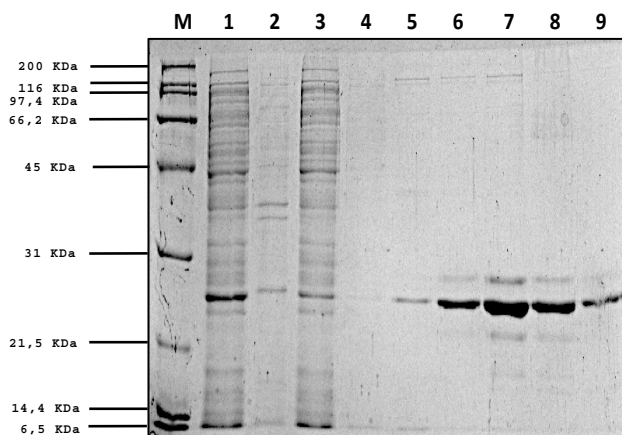
**Figure S4.** PyMOL stick and cartoon representation of the interactions established with the  $\Omega$ -loop and S1' pocket obtained in the additional binding modes of the series of compounds when docked to the different NMR models of MMP-2. Hydrogen bonds are shown in dashed lines, the catalytic zinc ion is shown as a grey sphere. For the sake of clarity, hydrogens are not shown.



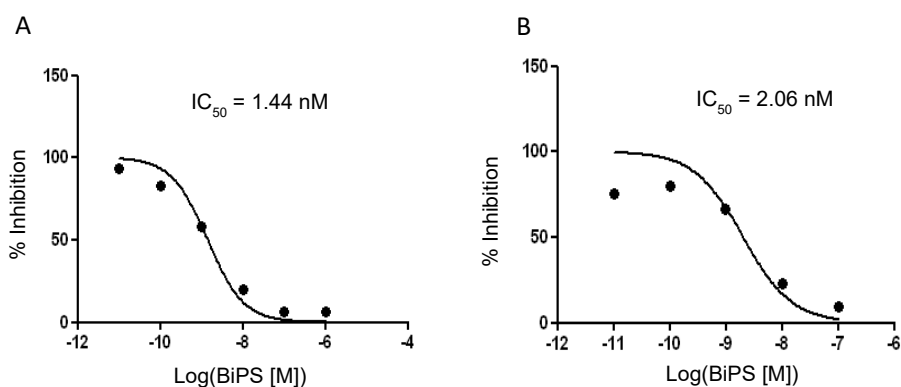


**Figure S5.** PyMOL stick and cartoon representation of the bidentate interaction established by the hydroxamate and the catalytic Zinc ion in both MMP-13 (left) and MMP-2 (right). Hydrogen bonds are shown in dashed lines, the catalytic zinc ion is shown as grey sphere. For the sake of clarity, no hydrogens are shown.

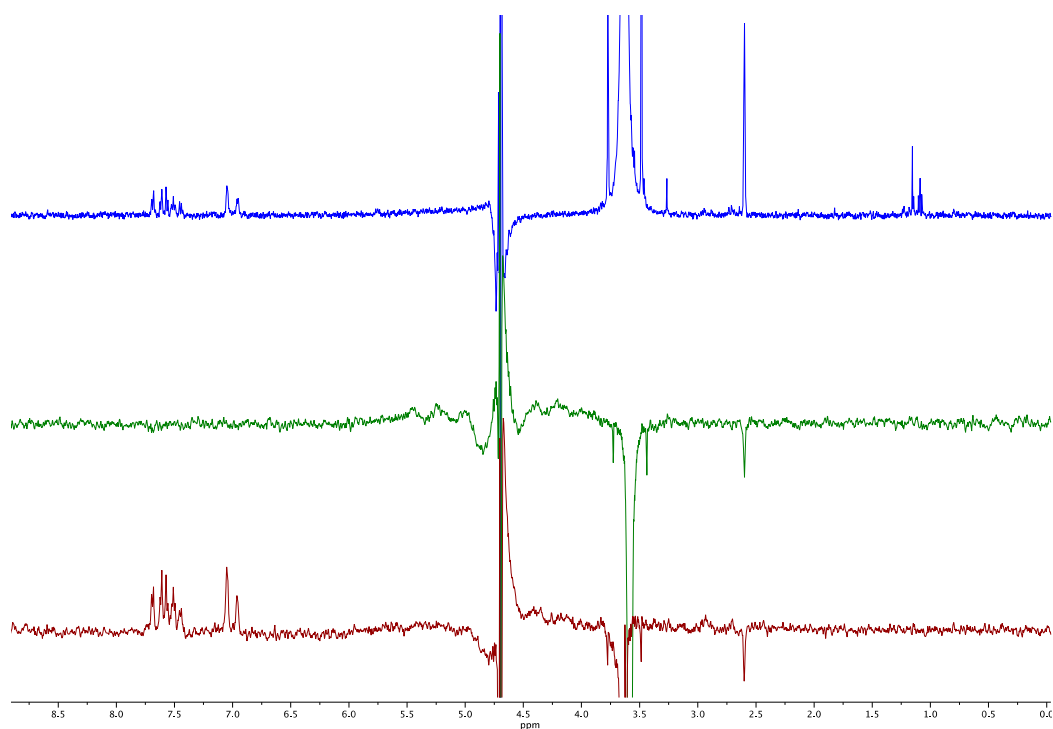
## PART D. NMR studies



**Figure S6.** SDS-PAGE analysis of the recombinantly expressed catalytic domain of MMP-13 purified by metal affinity chromatography. M corresponds to the molecular weight markers (kDa). Lane 1: supernatant of total bacterial protein; lane 2: precipitate of total bacterial protein; lane 3: flow-through; lane 4: wash with purification buffer; lane 5: wash with purification buffer containing 10 mM imidazole; lane 6: wash with purification buffer containing 30 mM imidazole; lane 7: elution with purification buffer containing 250 mM imidazole; lane 8: elution with purification buffer containing 500 mM imidazole; lane 9: elution with purification buffer containing 1 M imidazole.

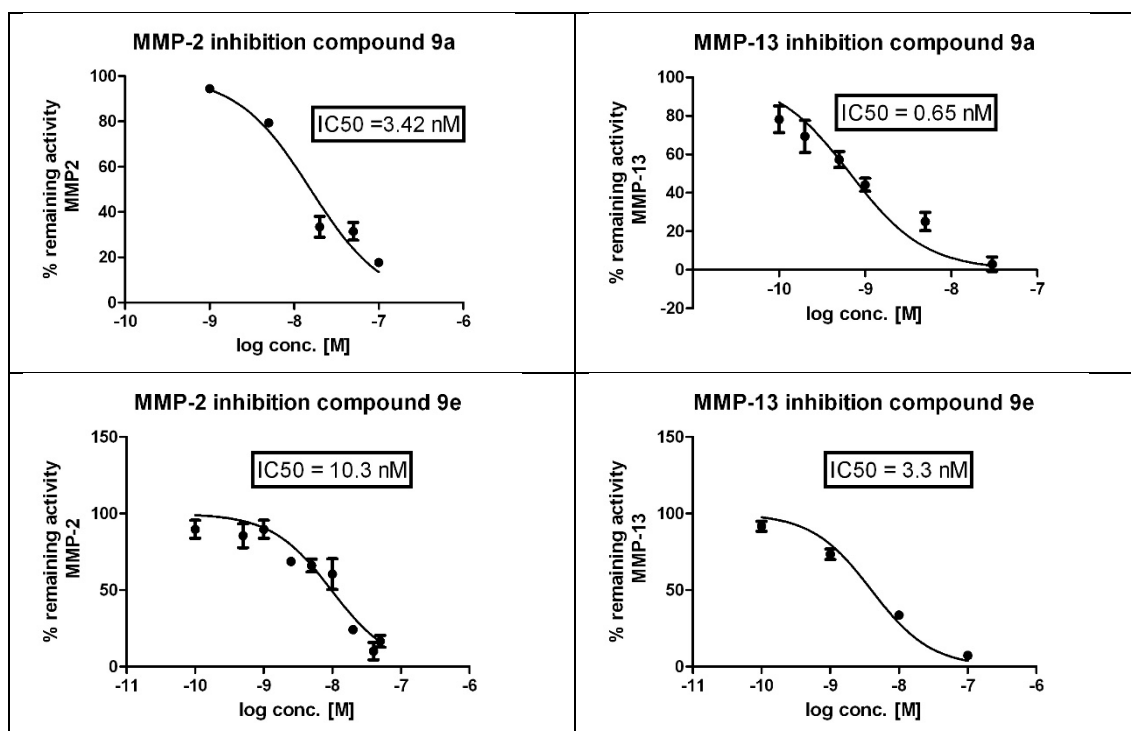


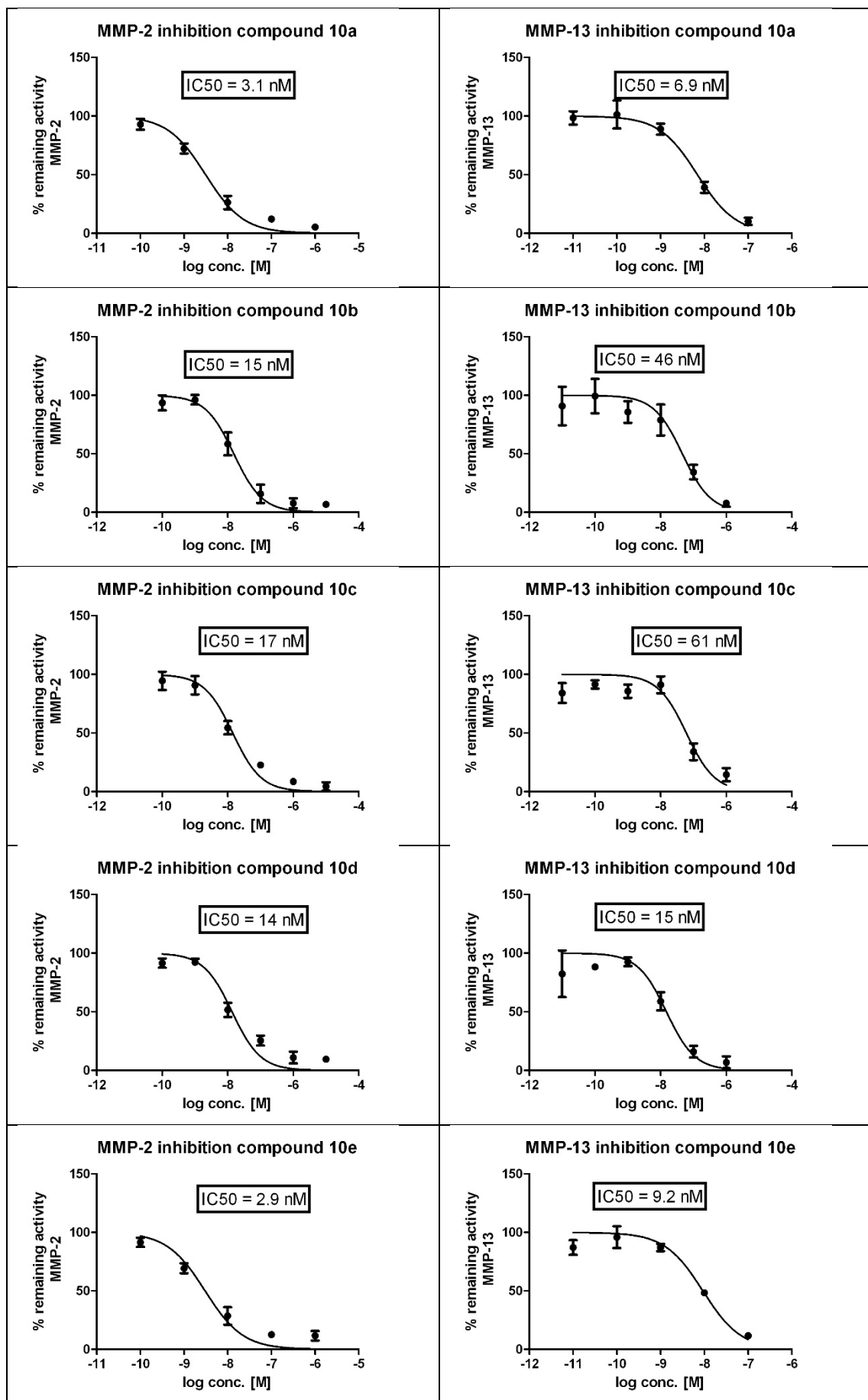
**Figure S7.** Dose response curve used to determine IC<sub>50</sub> of BiPS for A) recombinantly expressed catalytic domain of MMP-13, and B) commercially available MMP-13 active protein.



**Figure S8.** NMR studies for the interaction between the MMP-13 catalytic domain and BiPS. Blue: 1D  $^1\text{H}$  spectrum of BiPS and the MMP-13 catalytic domain; green: waterLOGSY experiment of BiPS; red: waterLOGSY experiment of BiPS in the presence of the MMP-13 catalytic domain.

## PART E. $\text{IC}_{50}$ calculations

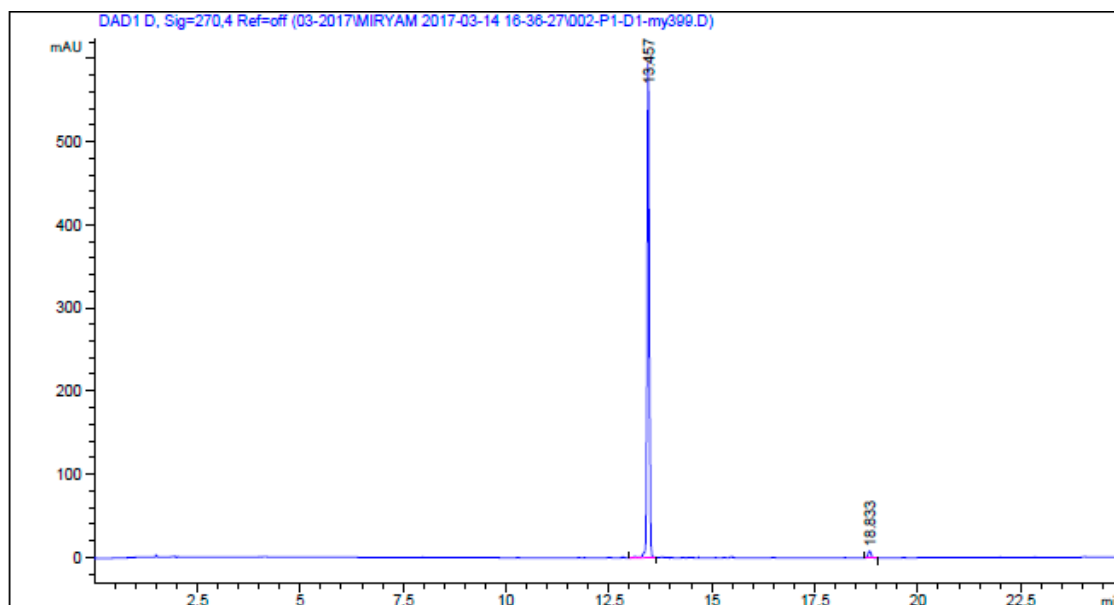




**Figure S9.** IC<sub>50</sub> calculations in MMP-13 and MMP-2 for active compounds.

## PART F. Purity by HPLC for the final compounds.

### HPLC for Compound 9a

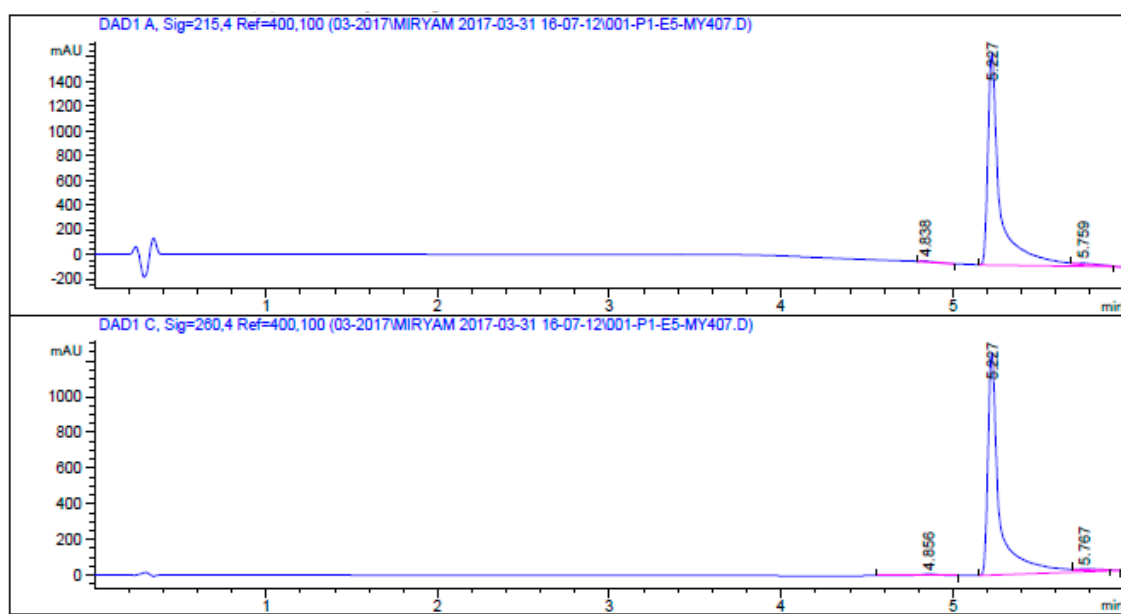


Signal 1: DAD1 D, Sig=270,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.457	VB R	0.0583	2259.47827	595.23047	98.2746
2	18.833	BB	0.0752	39.66923	8.38323	1.7254

Totals : 2299.14750 603.61370

### HPLC for Compound 9b



Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.838	BB	0.0878	36.47041	5.92225	0.4159
2	5.227	BV R	0.0707	8639.71191	1716.91211	98.5205

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	5.759	VBAE	0.1074	93.27305	11.65226	1.0636

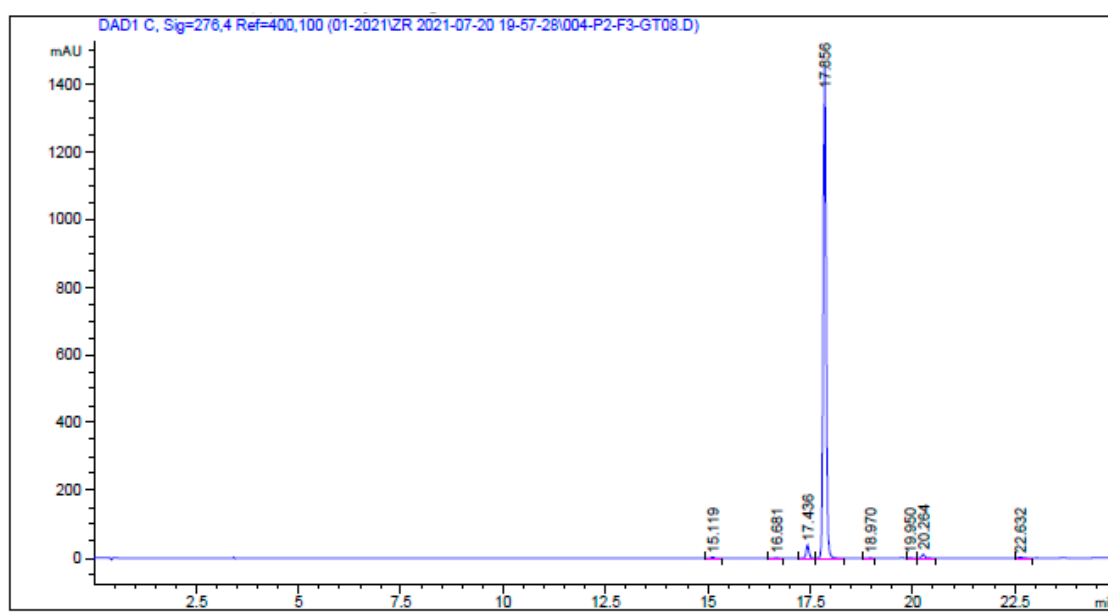
Totals : 8769.45538 1734.48662

Signal 2: DAD1 C, Sig=260,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.856	BB	0.1171	74.85628	8.46912	1.2333
2	5.227	BV R	0.0675	5943.94629	1249.23083	97.9313
3	5.767	VBAE	0.1091	50.70398	6.35159	0.8354

Totals : 6069.50654 1264.05155

## HPLC for Compound 9c

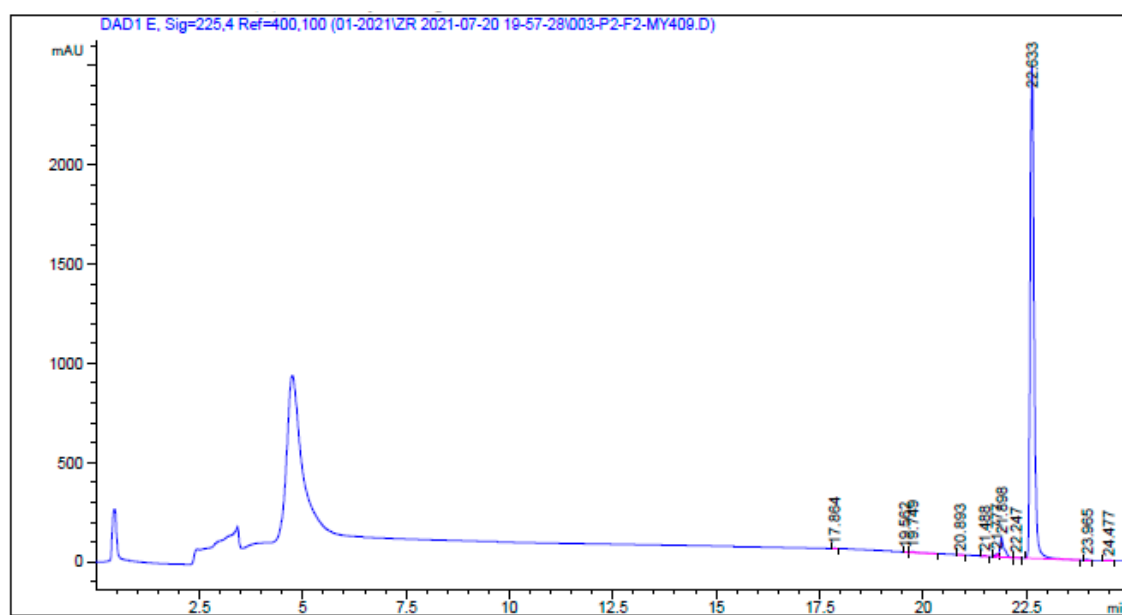




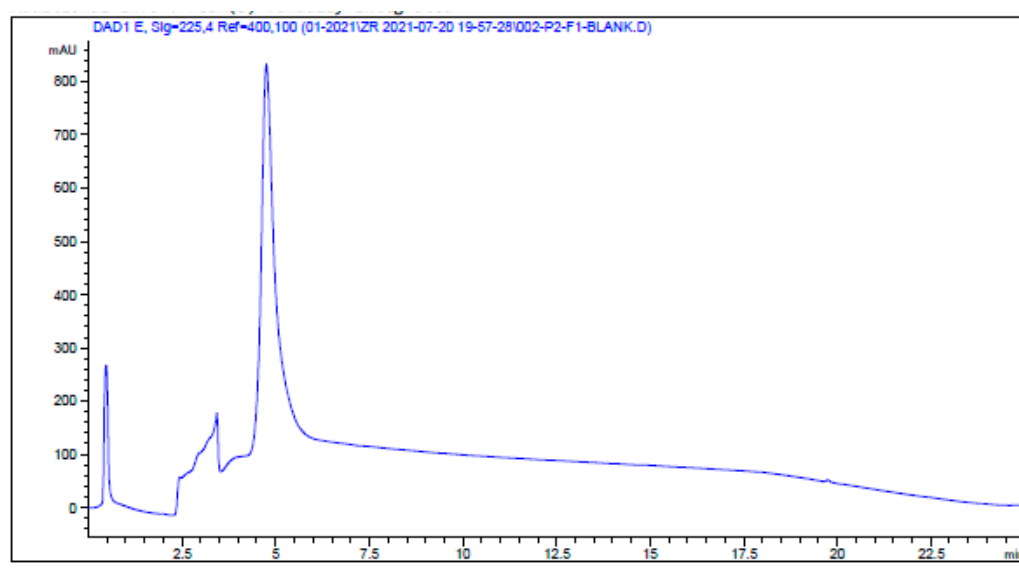
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.119	BB	0.0775	16.11996	3.16158	0.2147
2	16.681	BB	0.0930	12.50229	1.99875	0.1665
3	17.436	BB	0.0756	196.85049	39.85465	2.6220
4	17.856	BB	0.0757	7194.41211	1454.10107	95.8280
5	18.970	BV	0.0806	6.86097	1.27891	0.0914
6	19.950	BB	0.0798	6.80947	1.32931	0.0907
7	20.264	BV R	0.0800	56.38695	10.60598	0.7511
8	22.632	BB	0.0943	17.68854	2.85532	0.2356

Totals : 7507.63078 1515.18557

### HPLC for Compound 9d



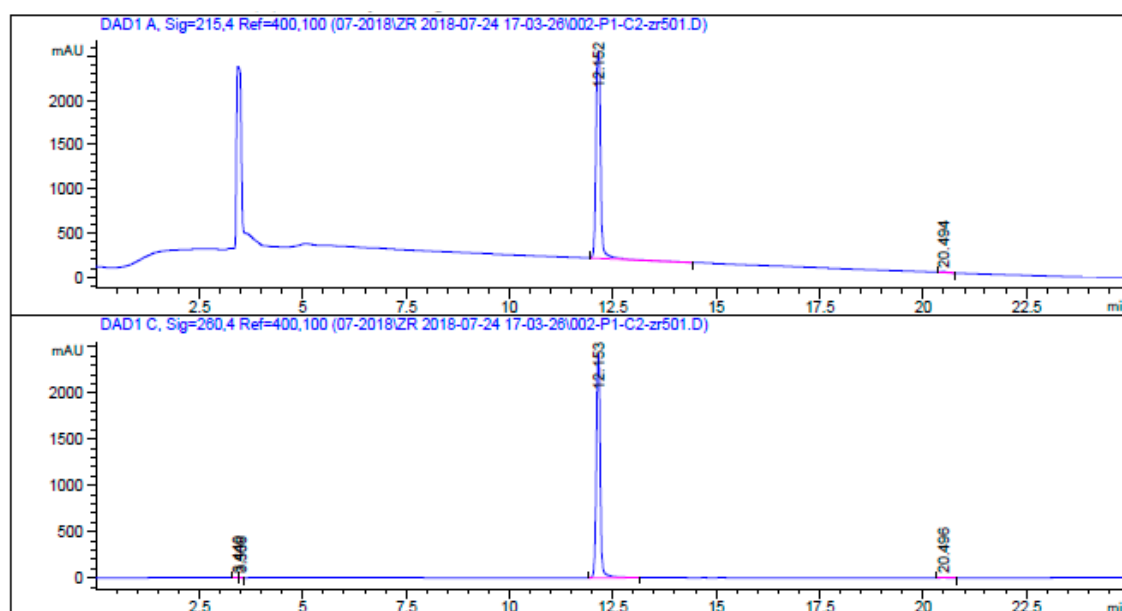
(Blank with DMSO)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.864	BV	0.0895	8.26097	1.34708	0.0477
2	19.562	VV	0.0946	11.94984	1.81893	0.0690
3	19.749	VB	0.1401	47.16131	4.65719	0.2722
4	20.893	BB	0.0779	6.38866	1.28624	0.0369
5	21.488	BB	0.0820	10.55691	1.98744	0.0609
6	21.777	BV E	0.0660	48.15607	11.23072	0.2780
7	21.898	VB R	0.1009	682.95239	96.13583	3.9425
8	22.247	BB	0.0951	7.37572	1.14606	0.0426
9	22.633	BB	0.1033	1.64512e4	2482.61694	94.9675
10	23.965	BB	0.0835	20.65994	3.79639	0.1193
11	24.477	BB	0.1354	28.32399	3.46351	0.1635

Totals : 1.73230e4 2609.48632

### HPLC for Compound 9e



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.152	BB	0.1277	1.87126e4	2329.23779	99.6838
2	20.494	BB	0.0871	59.35725	10.31442	0.3162

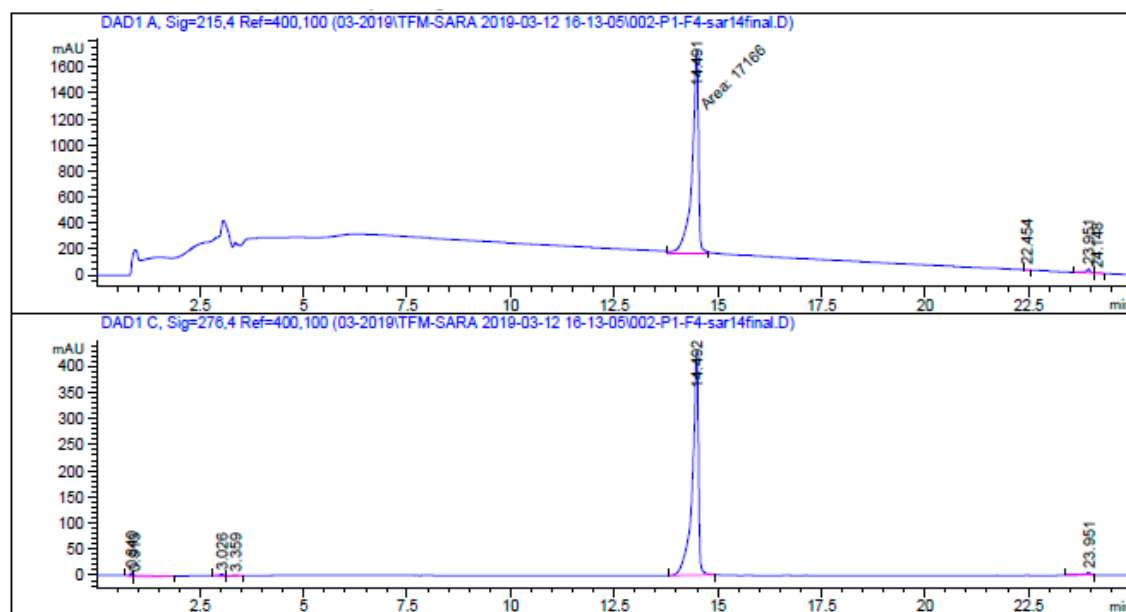
Totals : 1.87719e4 2339.55221

Signal 2: DAD1 C, Sig=260,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.440	BV	0.0616	6.71400	1.57931	0.0415
2	3.508	VB	0.0700	7.67466	1.78961	0.0475
3	12.153	BB	0.1034	1.61354e4	2430.89648	99.7939
4	20.496	BB	0.0902	18.93077	3.14770	0.1171

Totals : 1.61687e4 2437.41310

### HPLC for Compound 10a



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.491	MM	0.1828	1.71660e4	1565.27454	98.6214
2	22.454	BB	0.0959	12.01511	2.00604	0.0690
3	23.951	BB	0.1030	195.08801	26.77514	1.1208
4	24.148	BBA	0.1125	32.85143	4.14433	0.1887

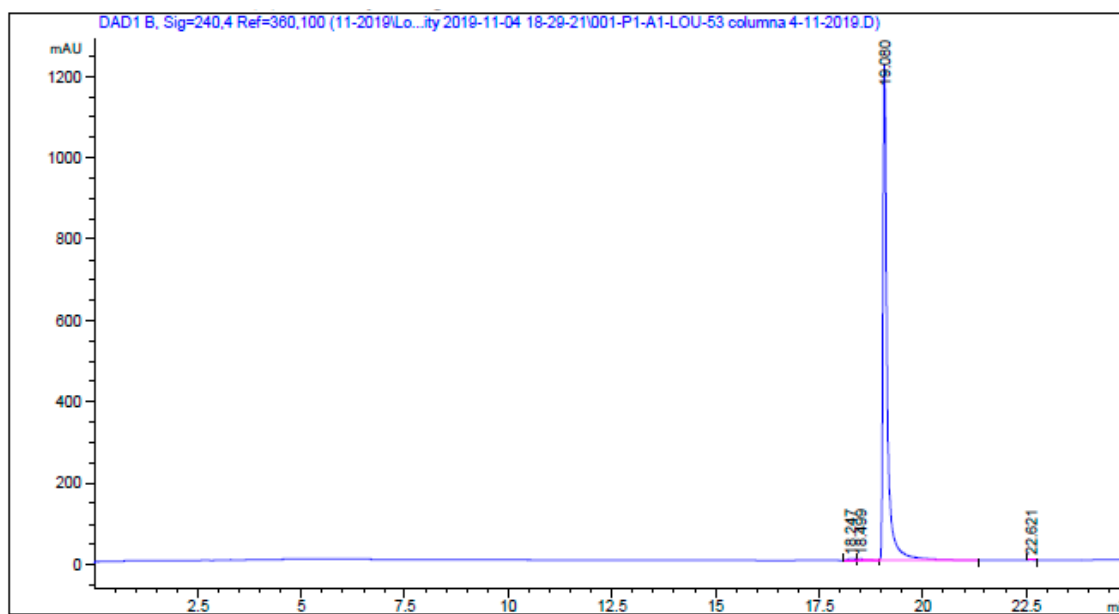
Totals : 1.74059e4 1598.20005

Signal 2: DAD1 C, Sig=276,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.840	BB	0.0490	17.75322	5.31471	0.3945
2	0.913	BB	0.2234	44.63164	2.46326	0.9917
3	3.026	BB	0.0876	20.10593	3.18593	0.4467
4	3.359	BB	0.1560	13.92740	1.35393	0.3094
5	14.492	BB	0.1387	4352.80371	427.95197	96.7139
6	23.951	BB	0.1139	51.48116	6.26349	1.1438

Totals : 4500.70307 446.53328

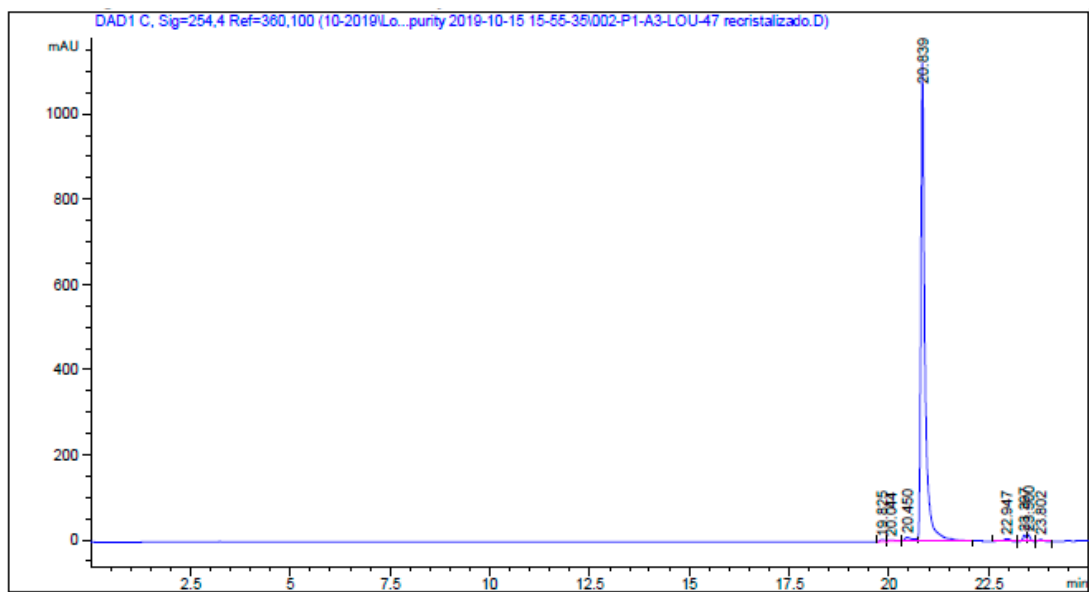
#### HPLC for Compound 10b



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.247	BV E	0.2020	41.00013	2.99033	0.4693
2	18.499	VV E	0.2619	51.83469	2.50011	0.5933
3	19.080	VB R	0.1026	8635.04395	1219.69983	98.8448
4	22.621	BB	0.0820	8.08163	1.52106	0.0925

Totals : 8735.96039 1226.71132

## HPLC for Compound 10c

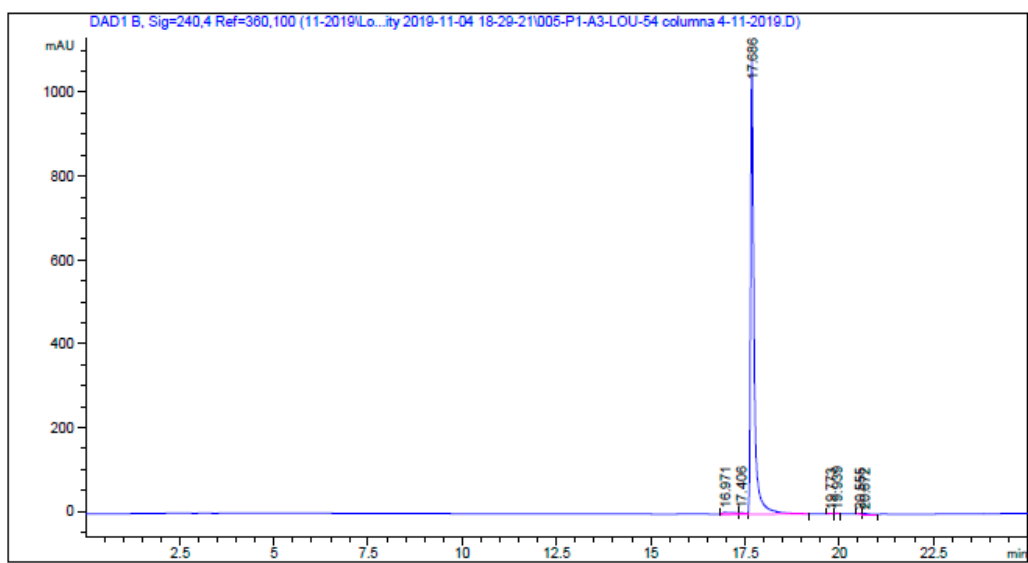


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.825	BV	0.1249	33.94621	4.00282	0.3982
2	20.044	VB	0.1706	27.32478	2.14544	0.3206

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	20.450	BV E	0.2018	118.82546	8.56540	1.3940
4	20.839	VB R	0.1041	8101.08252	1124.42224	95.0401
5	22.947	VB R	0.1428	61.60908	5.67054	0.7228
6	23.397	BV	0.0793	72.70608	13.84328	0.8530
7	23.500	VV	0.0816	79.34451	14.56338	0.9309
8	23.802	VB	0.0961	29.01904	4.56726	0.3404

Totals : 8523.85768 1177.78035

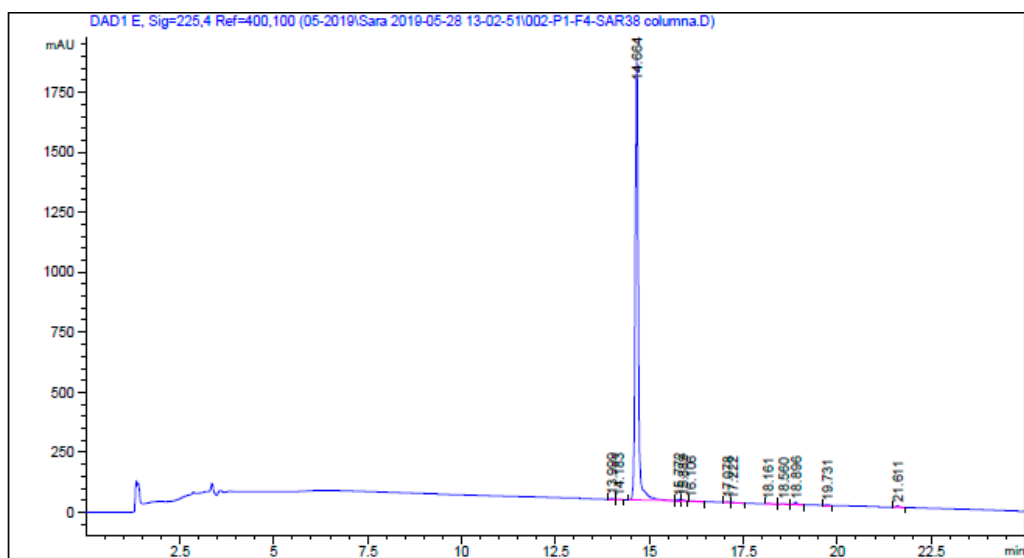
## HPLC for Compound 10d



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.971	BV E	0.2757	92.51758	4.42853	1.2917
2	17.406	VV E	0.1366	31.11412	3.11244	0.4344
3	17.686	VB R	0.0934	7000.71289	1083.49072	97.7423

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	19.773	BV	0.0801	5.61738	1.08983	0.0784
5	19.939	VB	0.0852	12.26579	2.26520	0.1713
6	20.555	BV	0.0863	9.44953	1.66328	0.1319
7	20.672	VB	0.0956	10.74185	1.61454	0.1500

### HPLC for Compound 10e



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.999	BV	0.0754	17.90581	3.64128	0.1674
2	14.183	VB	0.0914	6.80771	1.14525	0.0636

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	14.664	BV R	0.0864	1.04494e4	1836.38232	97.6760
4	15.772	BV	0.0795	32.74617	6.21522	0.3061
5	15.889	VB	0.0746	24.74622	4.92883	0.2313
6	16.106	BB	0.1176	12.60213	1.47627	0.1178
7	17.078	BV	0.0856	11.97303	2.12864	0.1119
8	17.222	VB	0.1257	10.50355	1.15931	0.0982
9	18.161	BB	0.0884	9.88424	1.63735	0.0924
10	18.560	BV	0.0952	20.95824	3.34188	0.1959
11	18.896	VB	0.0857	51.57423	8.88674	0.4821
12	19.731	BB	0.0817	14.04190	2.65661	0.1313
13	21.611	BB	0.0853	34.88150	6.23371	0.3261

Totals : 1.06980e4 1879.83341

Figure S10. HPLC results for final compounds.