Supplementary information for

Vanillin enones as selective inhibitors of the cancer associated carbonic anhydrase isoforms IX and XII. The out of the active site pocket for the design of selective inhibitors ?

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1. Chemistry

1.1. Molecular Docking

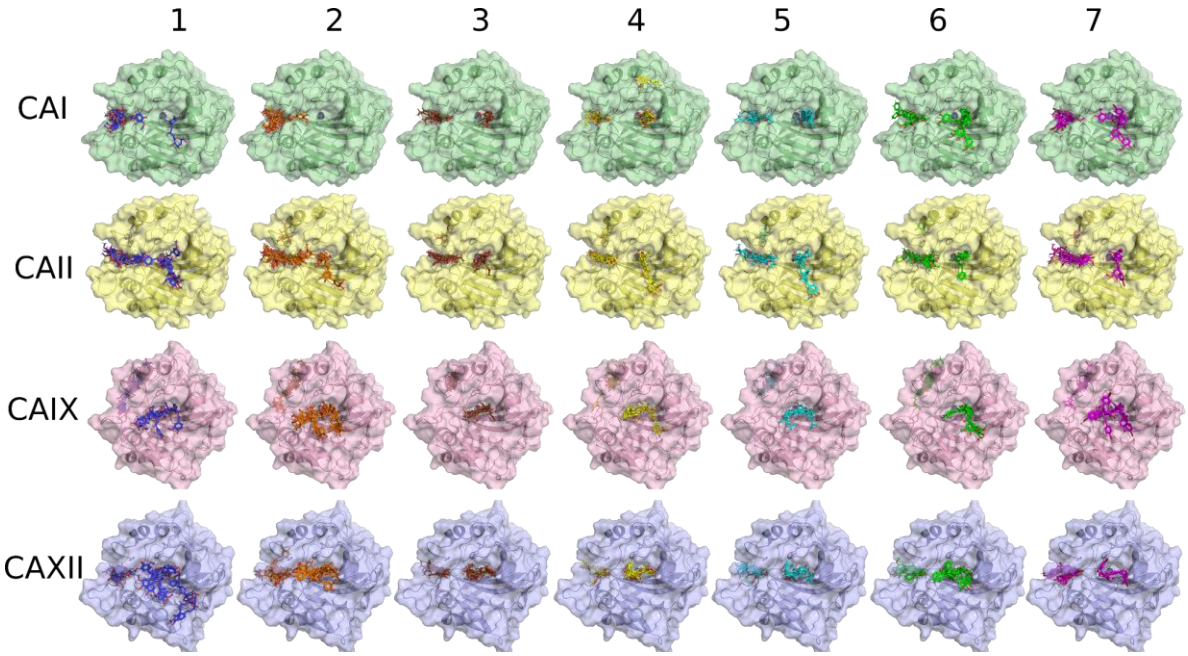
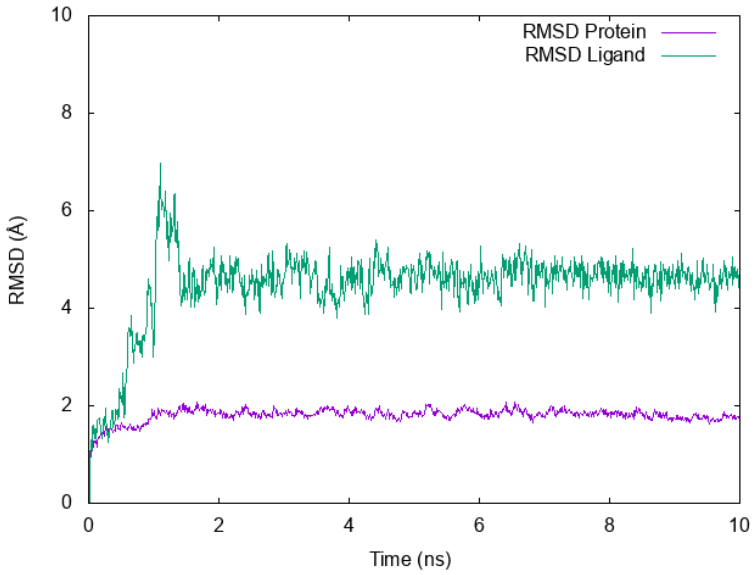
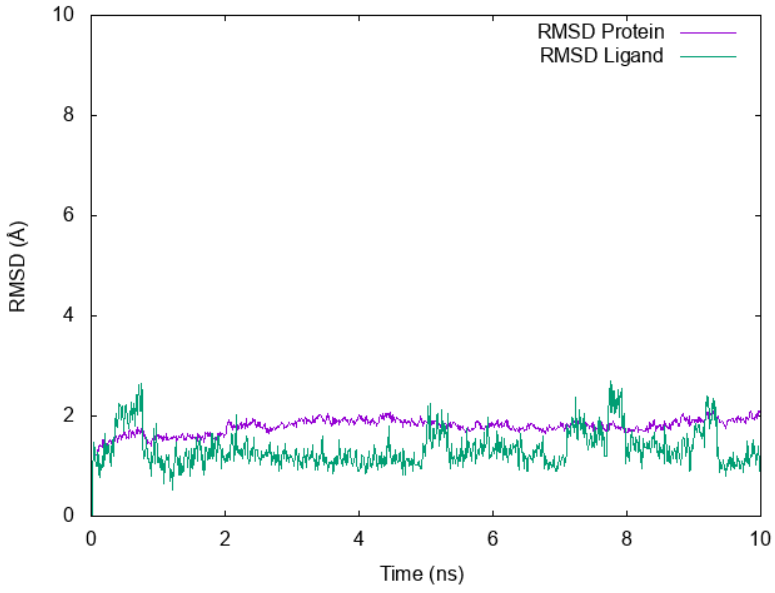


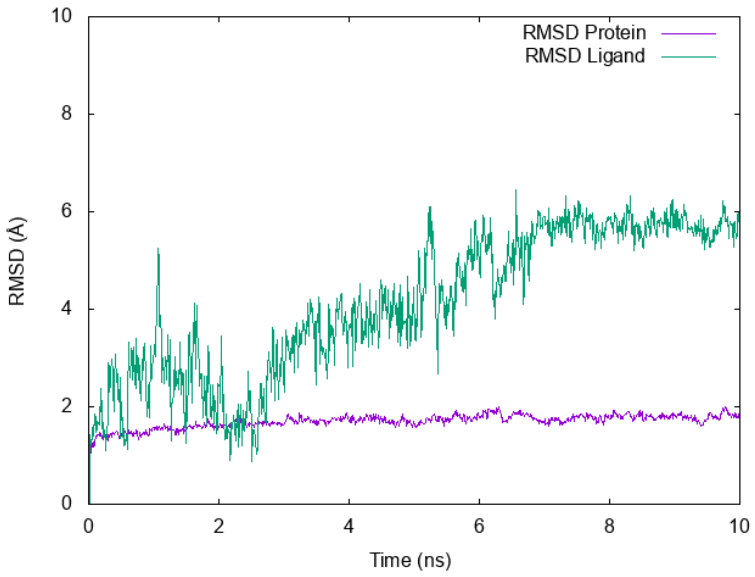
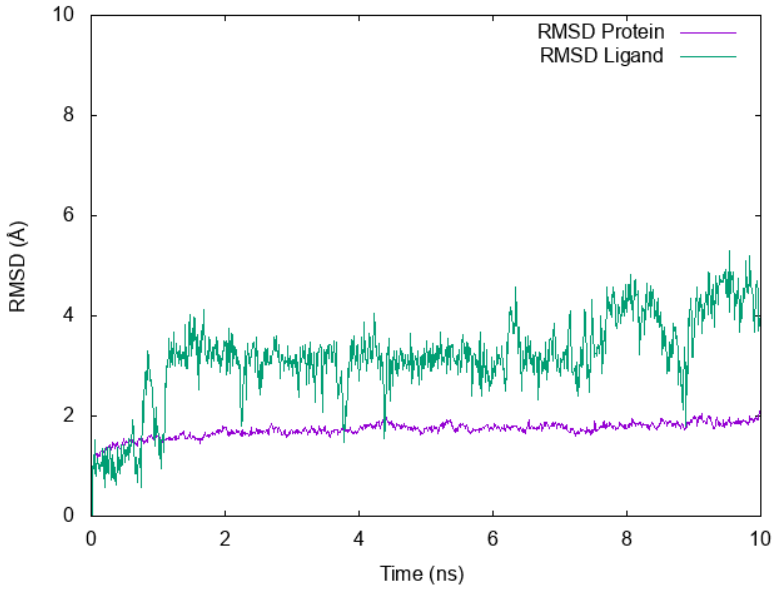
Figure S1. Ligand poses obtained with docking on the carbonic anhydrase isozymes.

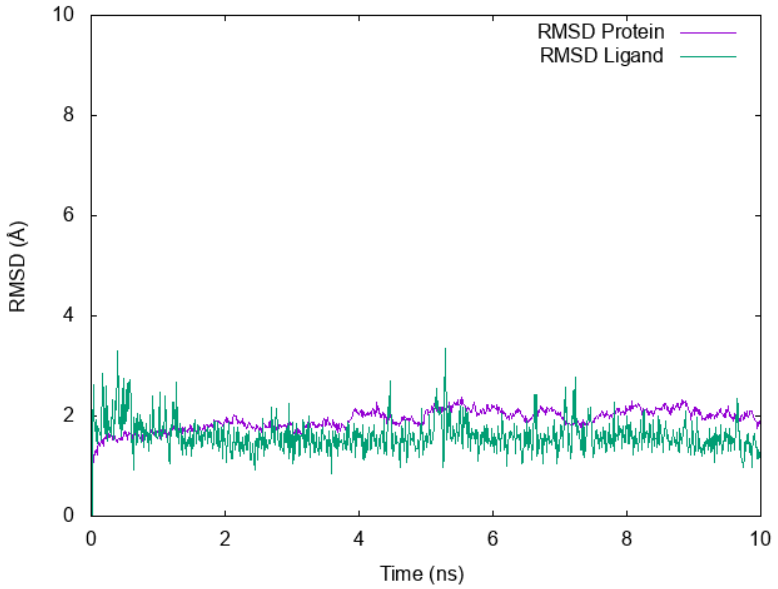
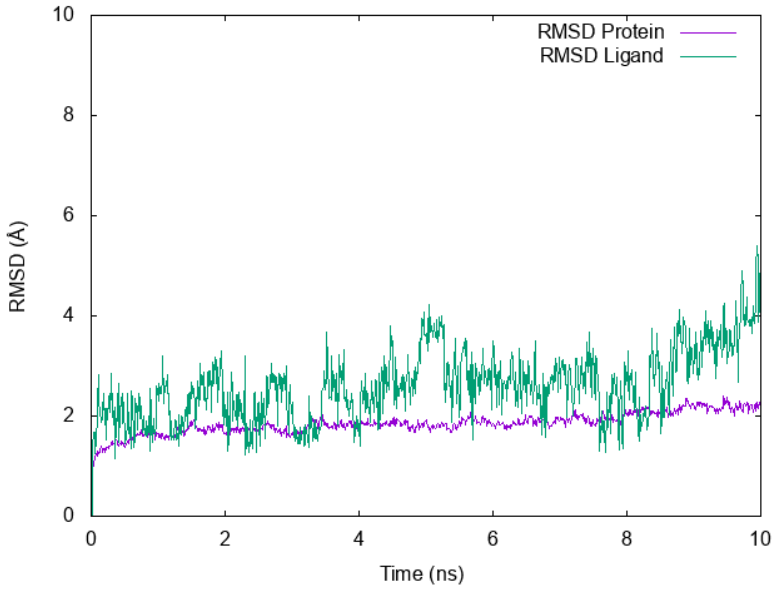
Table S1. Binding enthalpies (kcal/mol) calculated with PM6 and MOZYME approach using MOPAC. Location classification of ligand poses: *down*, poses placed inside the catalytic cavity with the methoxyphenol close the Zn^{2+} cation (distance <3Å); *up*, poses localized inside the catalytic cavity and close de Zn^{2+} cation (distance <3Å) with outward-facing methoxyphenol; *top down* and *top up*, poses that also block the catalytic site at a distance between 3Å and 7Å from the Zn^{2+} cation; *pocket*, poses located in the adjacent alternative pocket (in case of CAIX, poses at a distance < 3.5Å from pocket residues); *unclassified*, poses that were not located at the catalytic site or in the neighboring pocket.

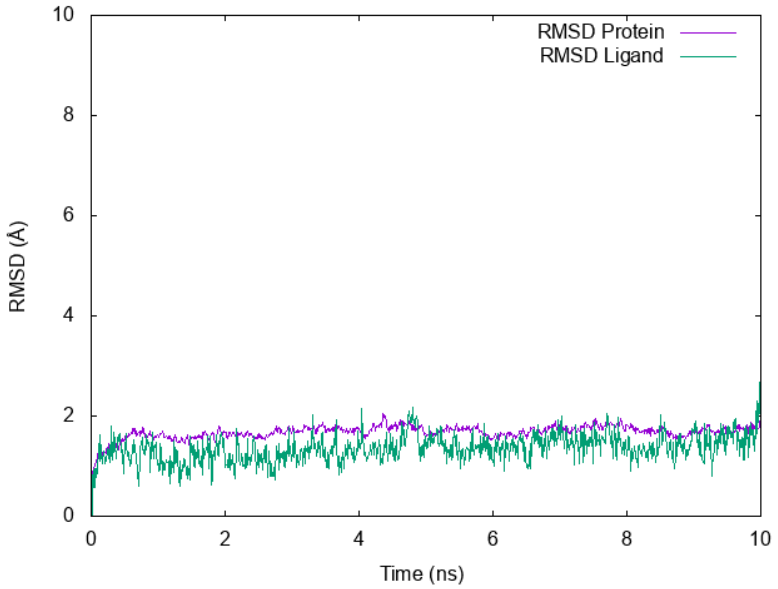
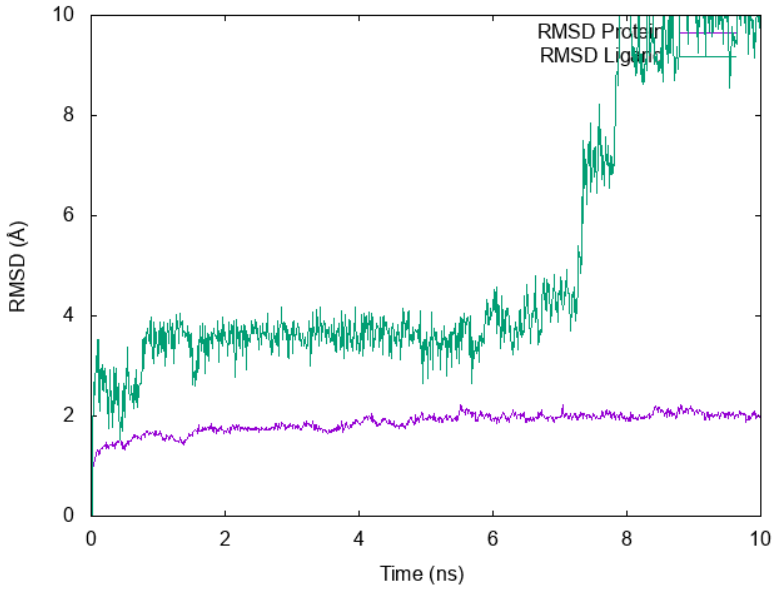
Compound	Location	CAI (2FW4)	CAII (3KS3)	CAIX (6FE2)	CAXII (1JCZ)
1	down	-	-	-	-
	up	-	-	-	-
	top down	-17	-12	-33	-28
	top up	-	-24	-30	-22
	pocket	-34	-28	-26	-33
	unclassified	-1	-27	-24	-32
2	down	-	-	-22	-
	up	-	-	-	-
	top down	-	-	-20	-26
	top up	-	-25	-34	-18
	pocket	-43	-28	-28	-42
	unclassified	-	-18	-33	-32
3	down	-	-	-	-
	up	-	-	9	-
	top down	-6	0	-17	-7
	top up	5	-17	-6	-1
	pocket	-17	-15	-	-24
	unclassified	-1	-11	-18	-
4	down	-	-	-	-
	up	10	-	2	-
	top down	-9	-7	-17	-8
	top up	3	-	-9	-7
	pocket	-16	-19	-	-23
	unclassified	-15	-18	-20	-
5	down	-	-	-9	-
	up	2	-	-	-
	top down	-10	-9	-18	-10
	top up	6	-	-13	-20
	pocket	-20	-17	-	-28
	unclassified	-	-13	-21	-
6	down	-	-	-	-
	up	-	-	-10	-
	top down	-15	-5	-21	-21
	top up	-22	-5	-20	-4
	pocket	-20	-21	-18	-37
	unclassified	-	-16	-26	-
7	down	-	-	-	-
	up	-	-	-	-
	top down	-18	-15	-26	-14
	top up	0	-10	-	-3
	pocket	-18	-22	-24	-27
	unclassified	-24	-16	-26	-

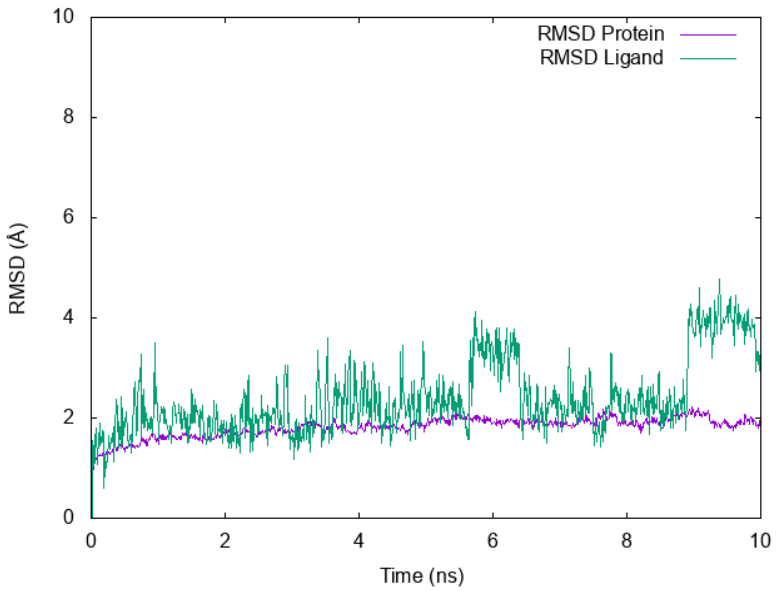
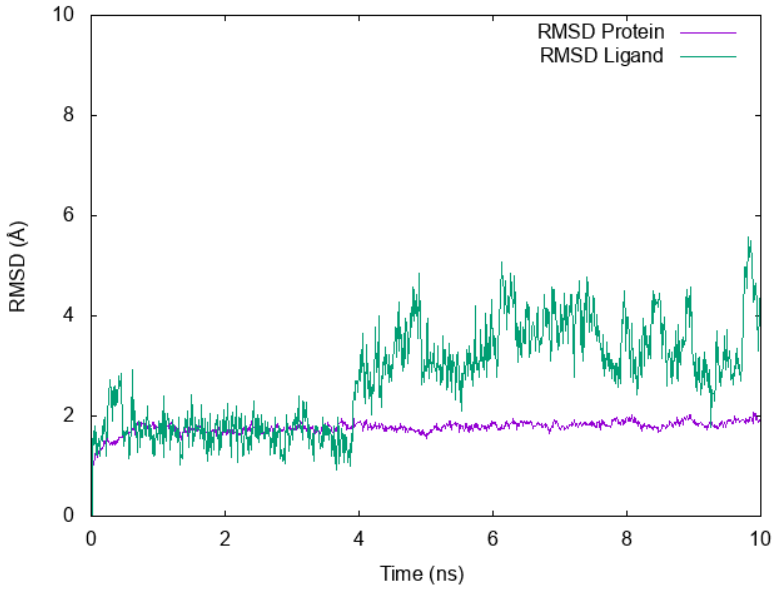
Figure S2. RMSD profiles of the equilibration/production stage are shown for CA I with ligands. “RMSD Protein” was measured considering all residues in the complex aligned using the protein backbone. The plots were generated with CPPTRAJ and GNUPLOT.

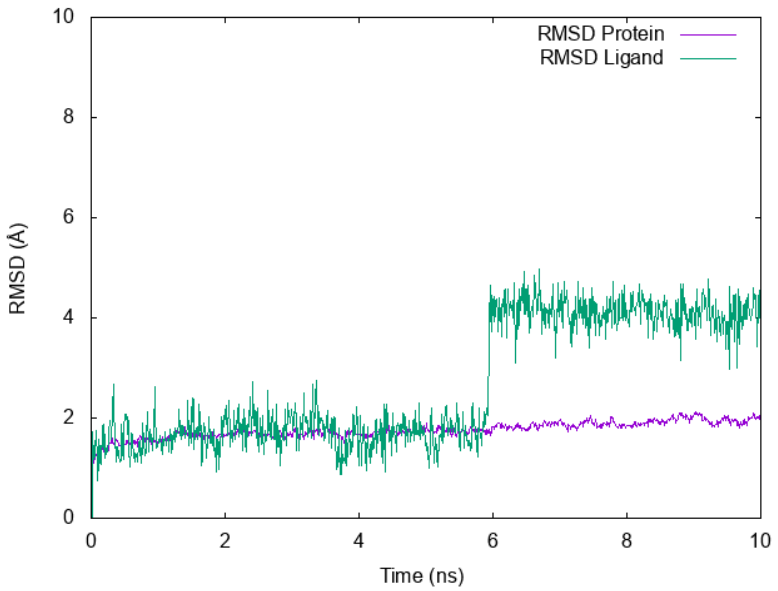
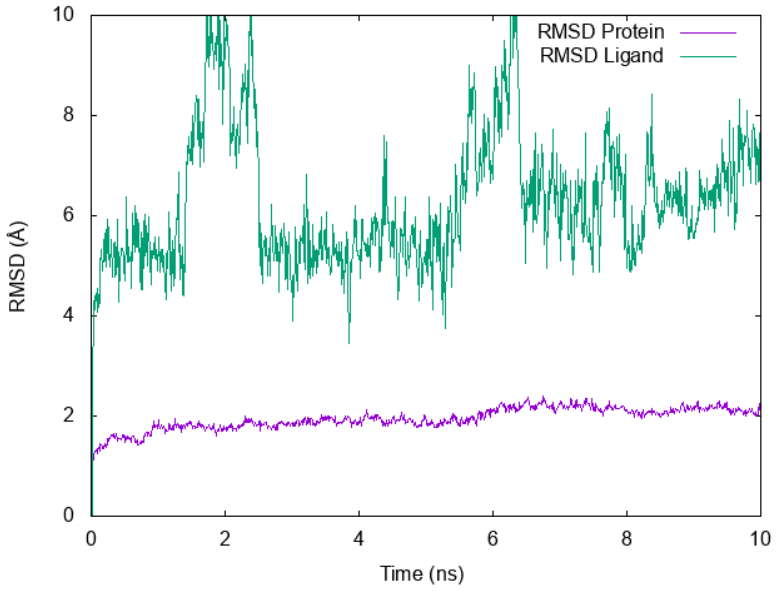
Ligand	Location	RMSD
1	catalytic down	
	pocket	

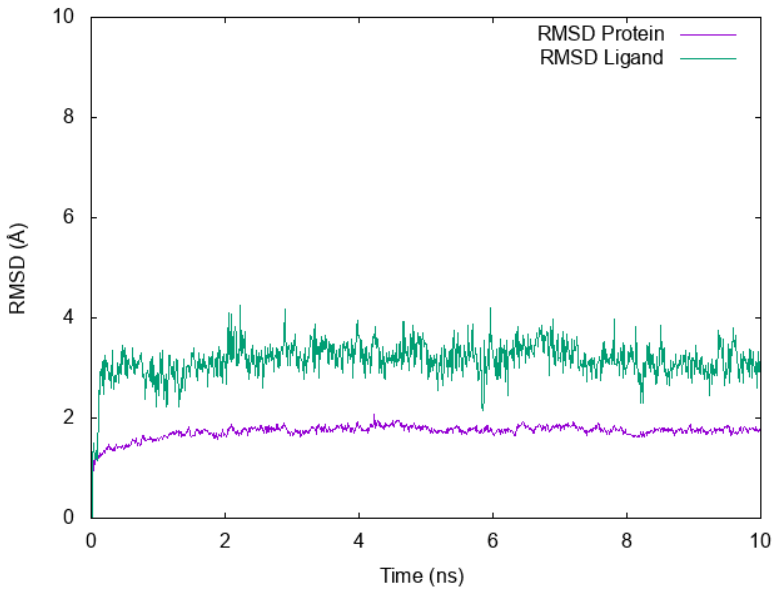
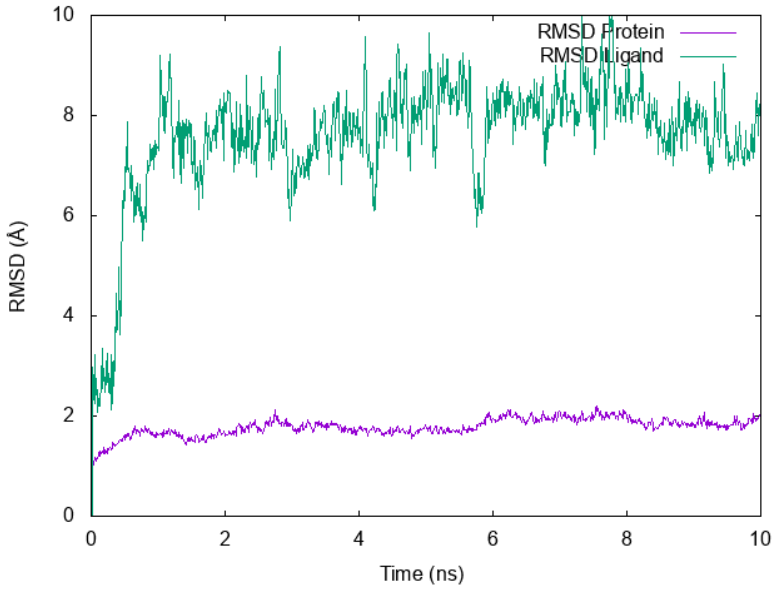
2	catalytic down	
	pocket	

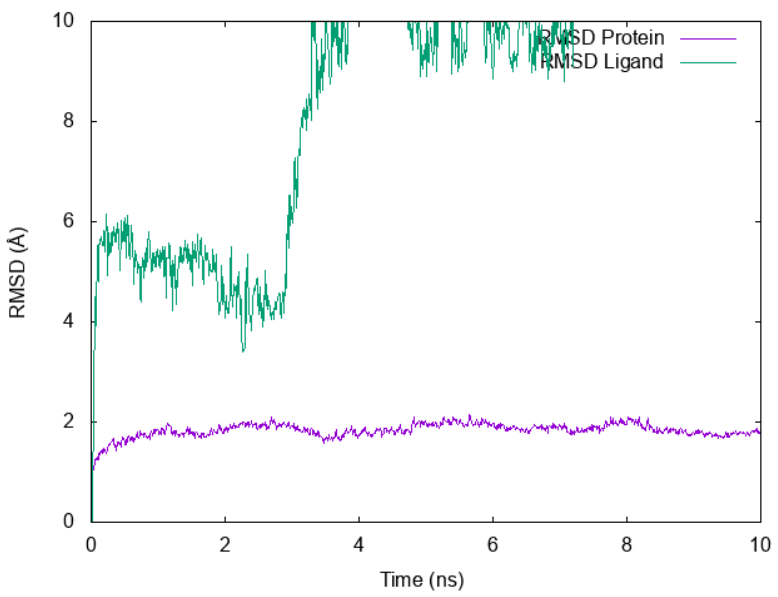
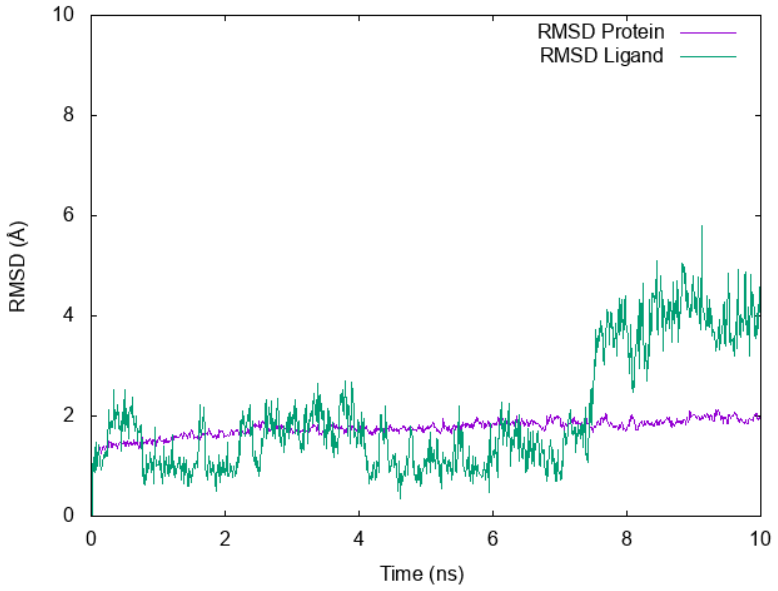
3	catalytic down	
	catalytic up	

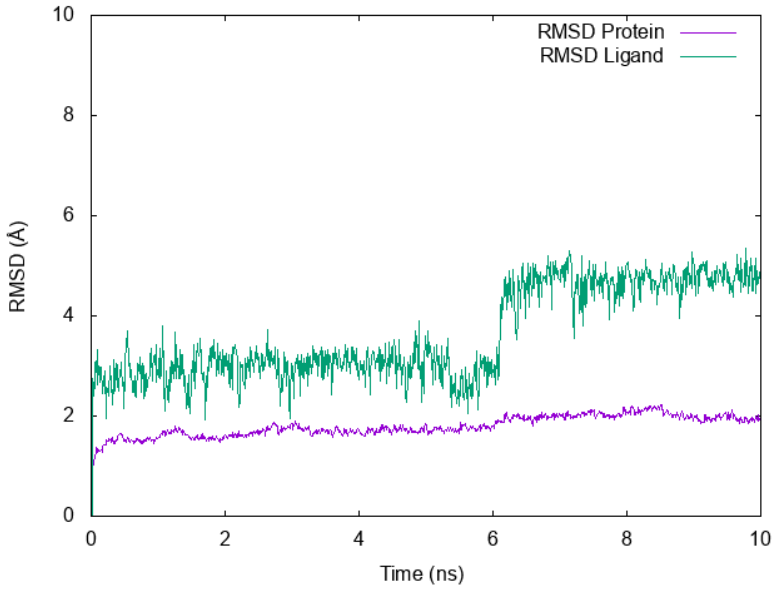
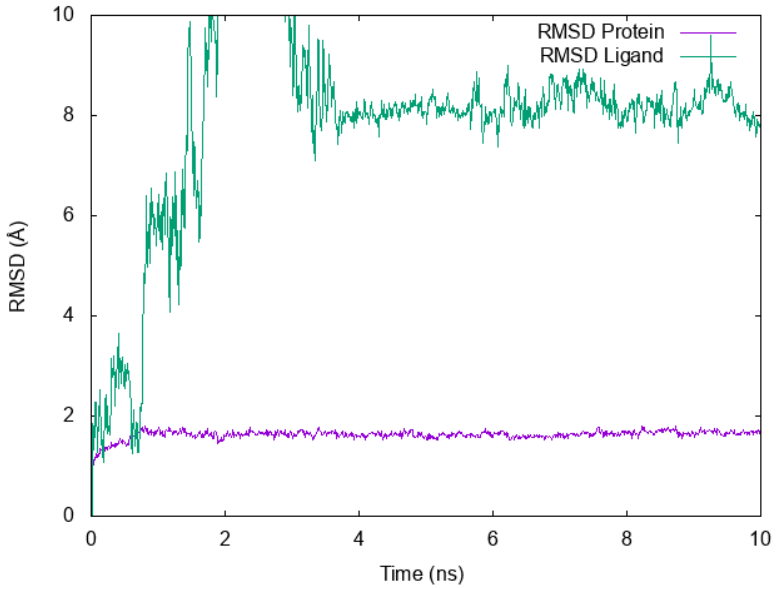
	pocket	
4	catalytic down	

	catalytic up	
	pocket	

5	catalytic down	
	catalytic up	

	pocket	
6	catalytic down	

	catalytic up	
	pocket	

7	catalytic down	
	catalytic up	

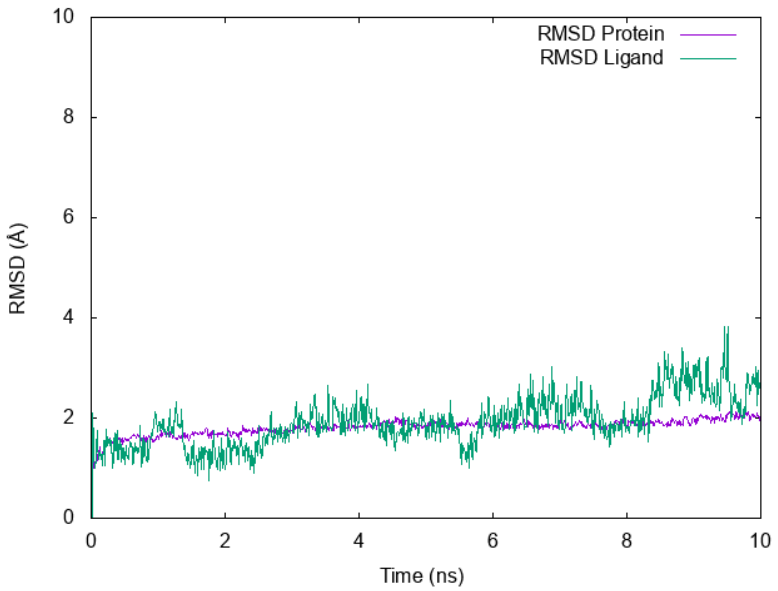
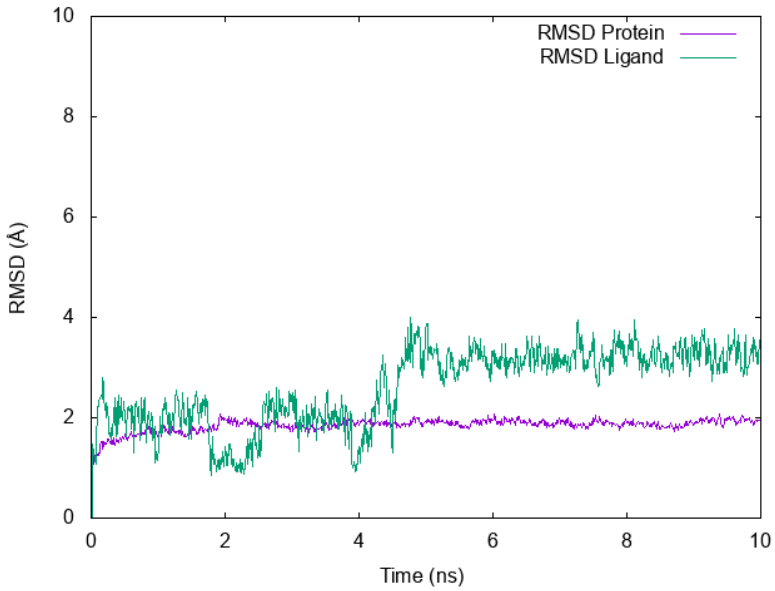
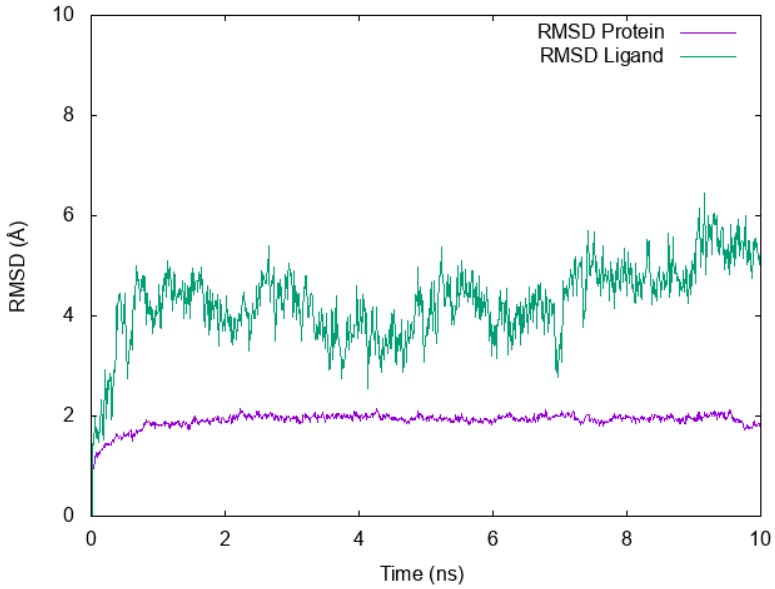
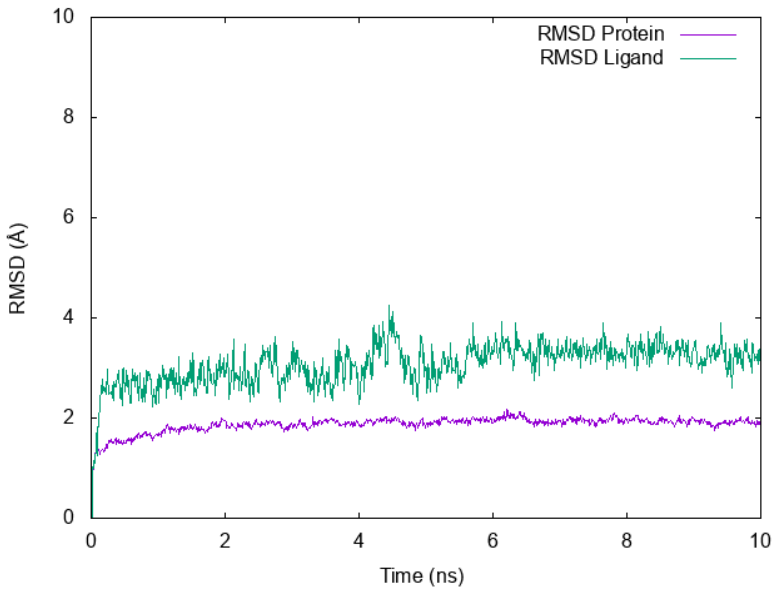
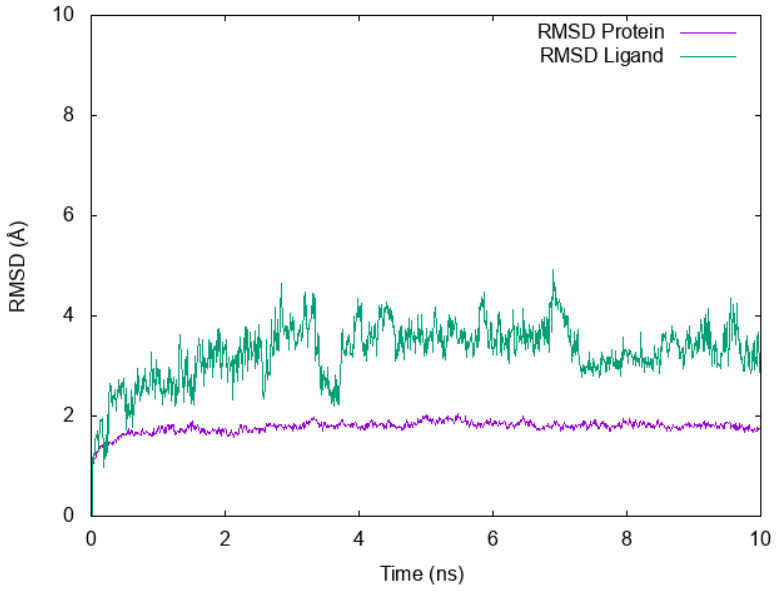
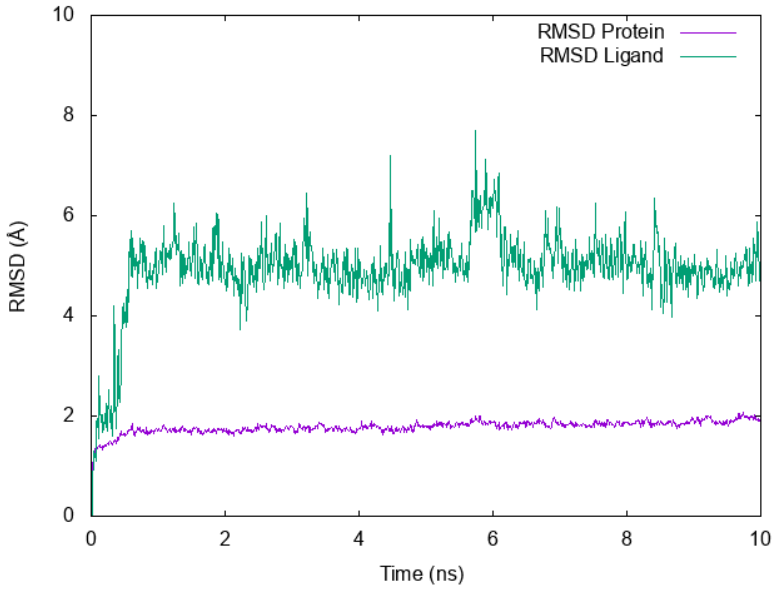
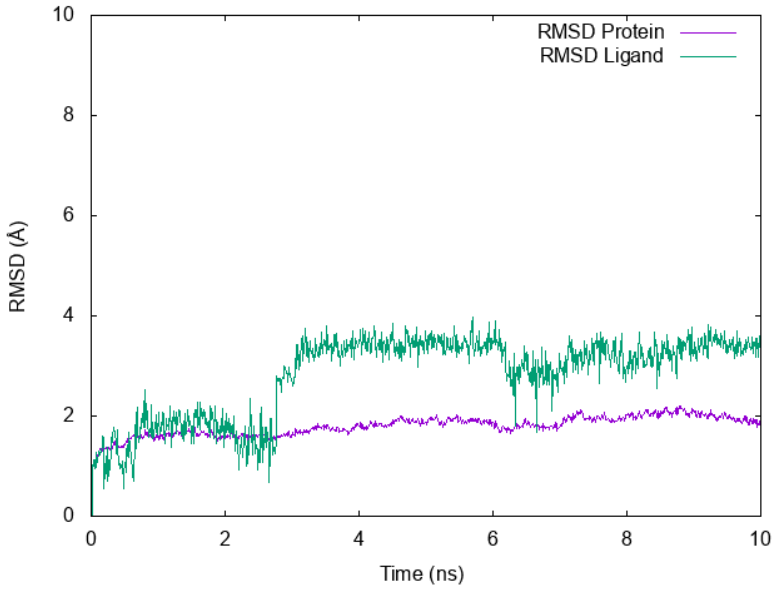
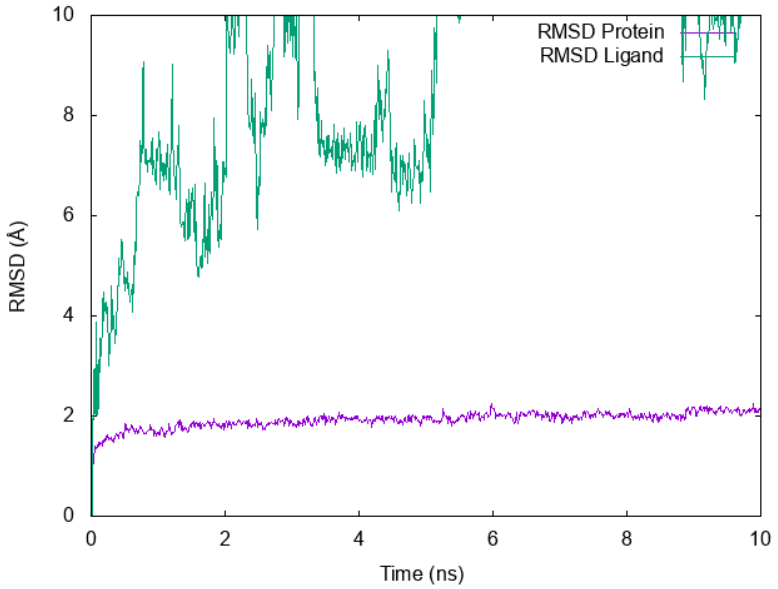
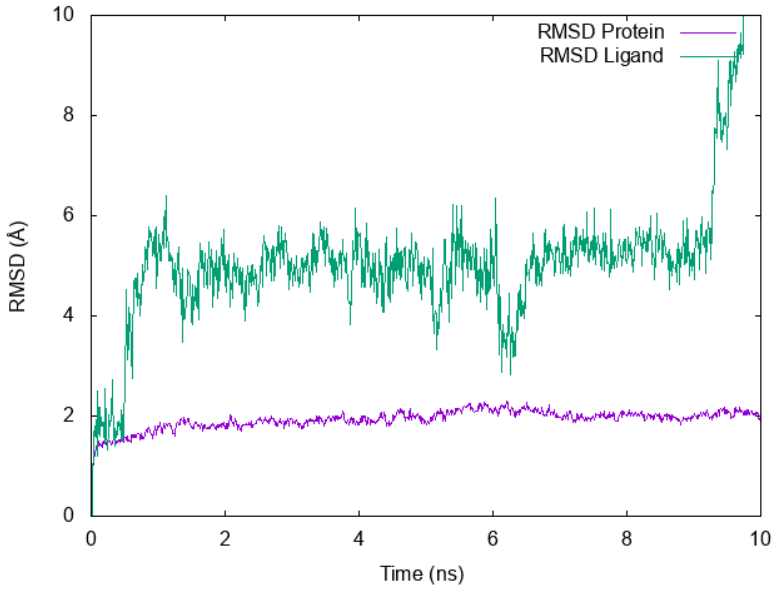
	pocket	 RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10

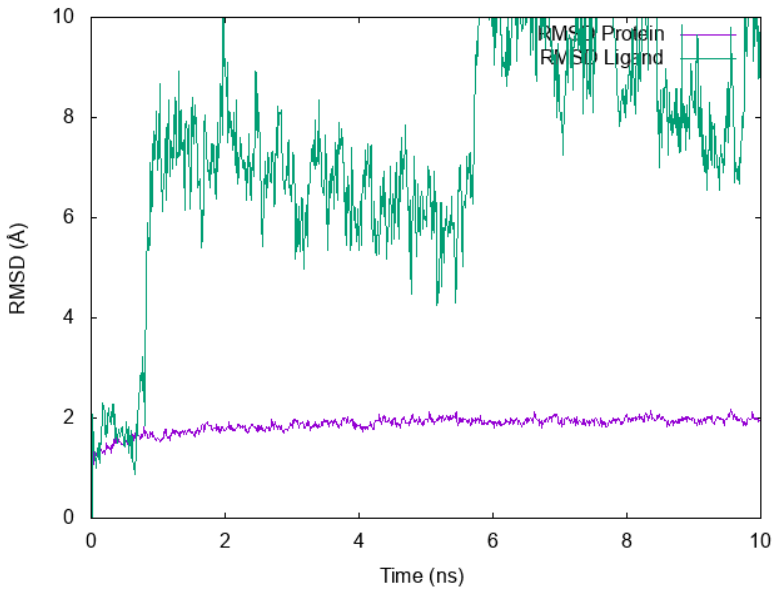
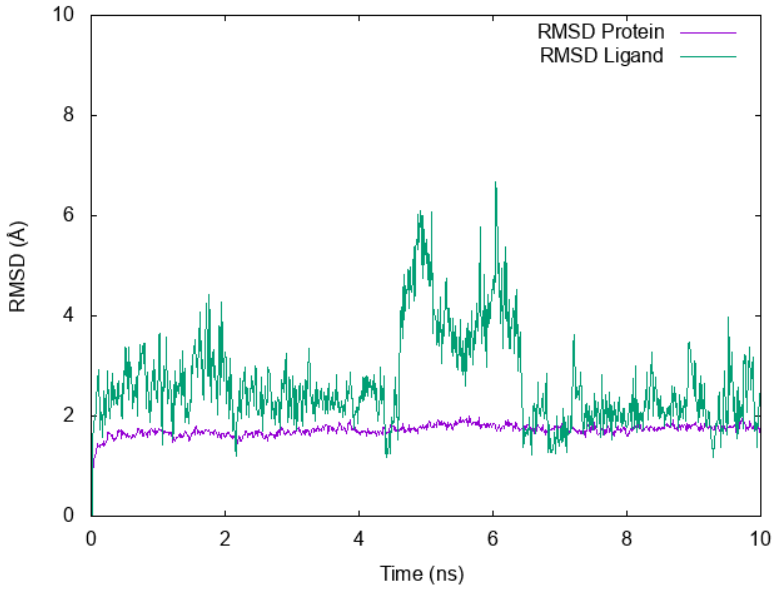
Figure S3. RMSD profiles of the equilibration/production stage are shown for CA II with ligands. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. The plots were generated with CPPTRAJ and GNUPLOT.

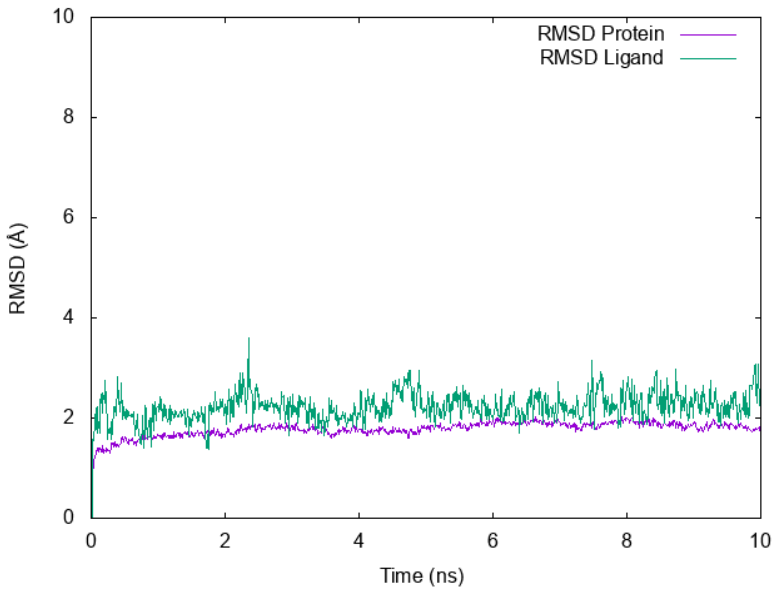
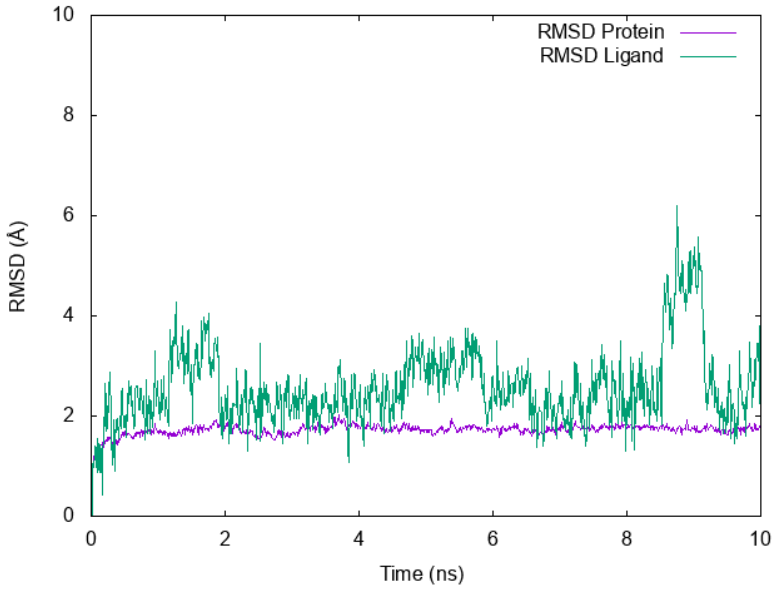
Ligand	Location	RMSD
1	catalytic down	
	pocket	

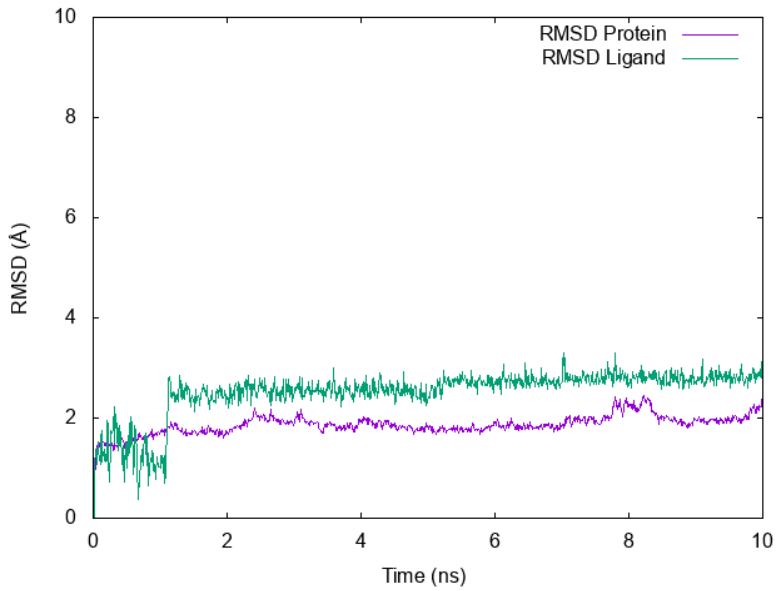
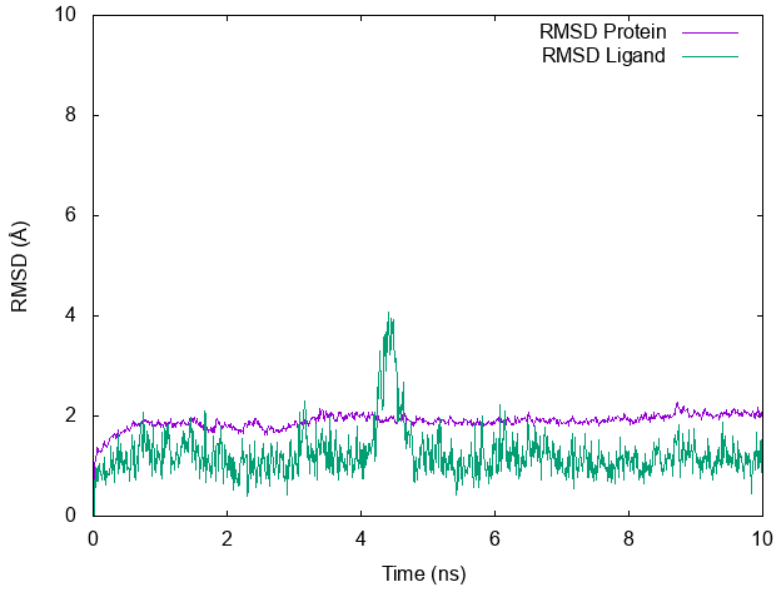
2	catalytic down	
	pocket	

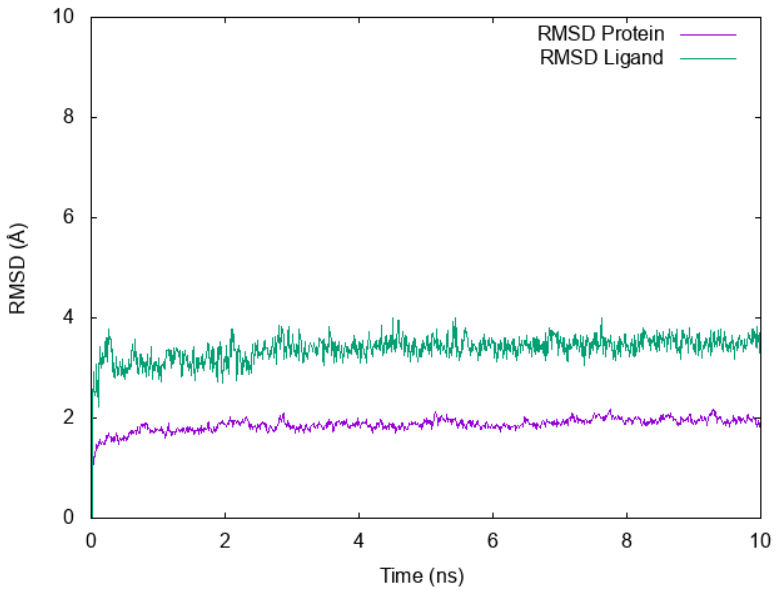
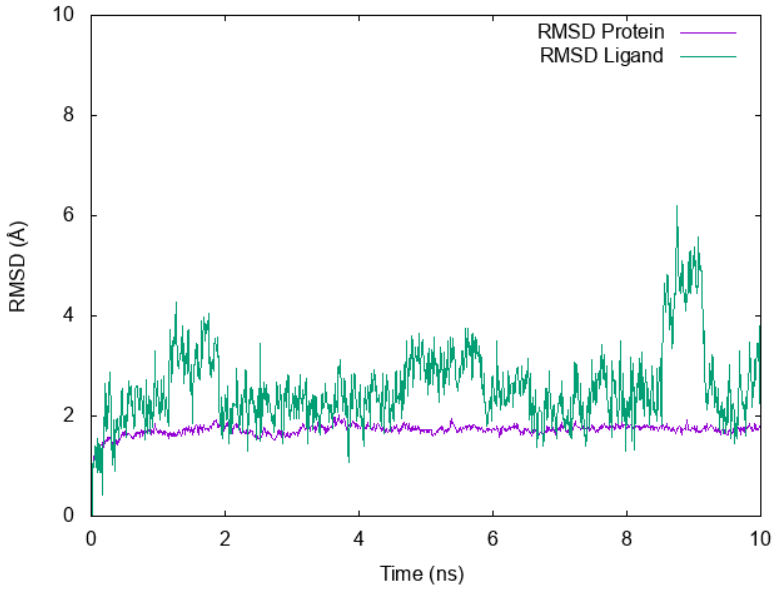
3	catalytic down	
	catalytic up	

	pocket	
4	catalytic down	

	catalytic up	
	pocket	

5	catalytic down	
	pocket	

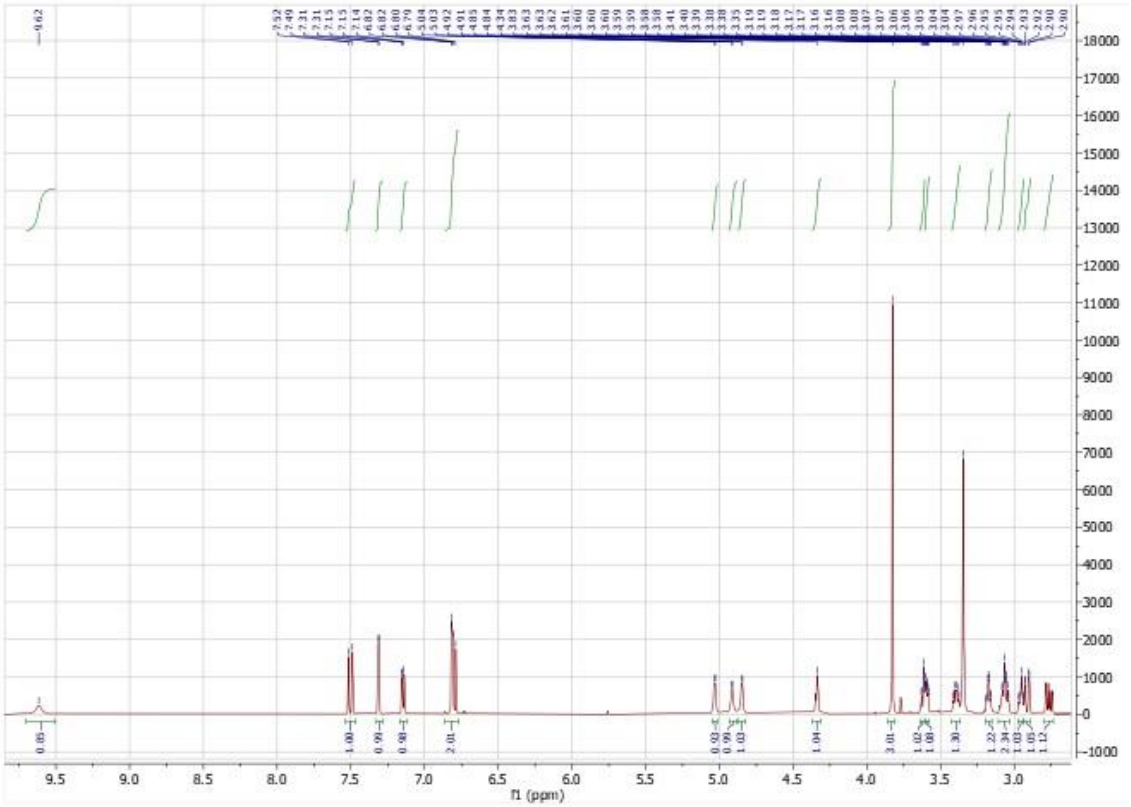
6	catalytic down	
	pocket	

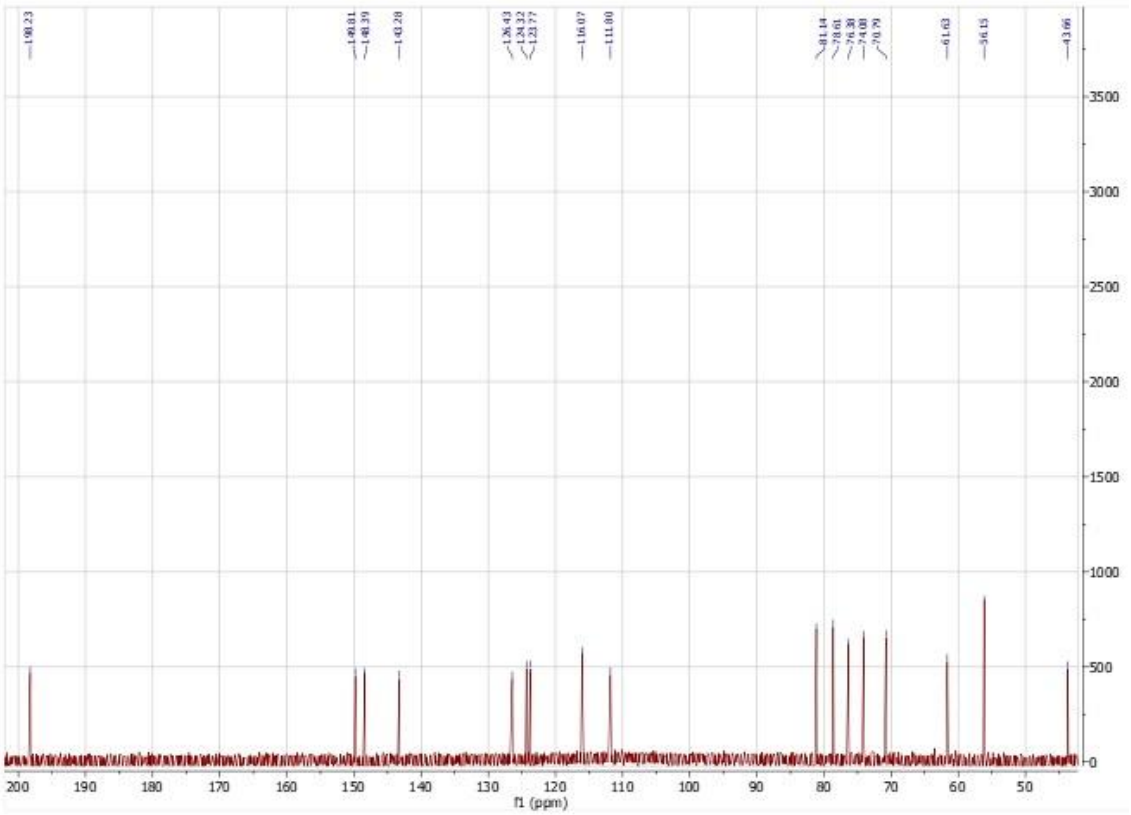
7	catalytic down	
	pocket	

2. Experimental

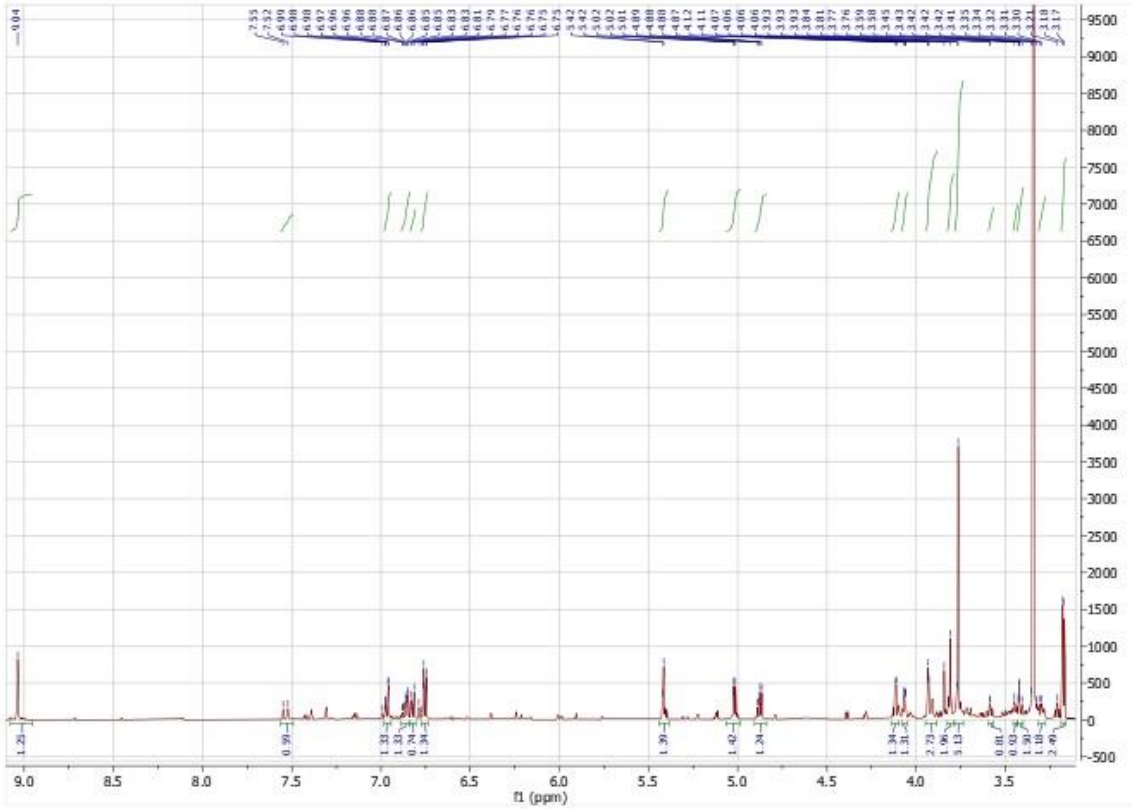
2.1 NMR snapshots

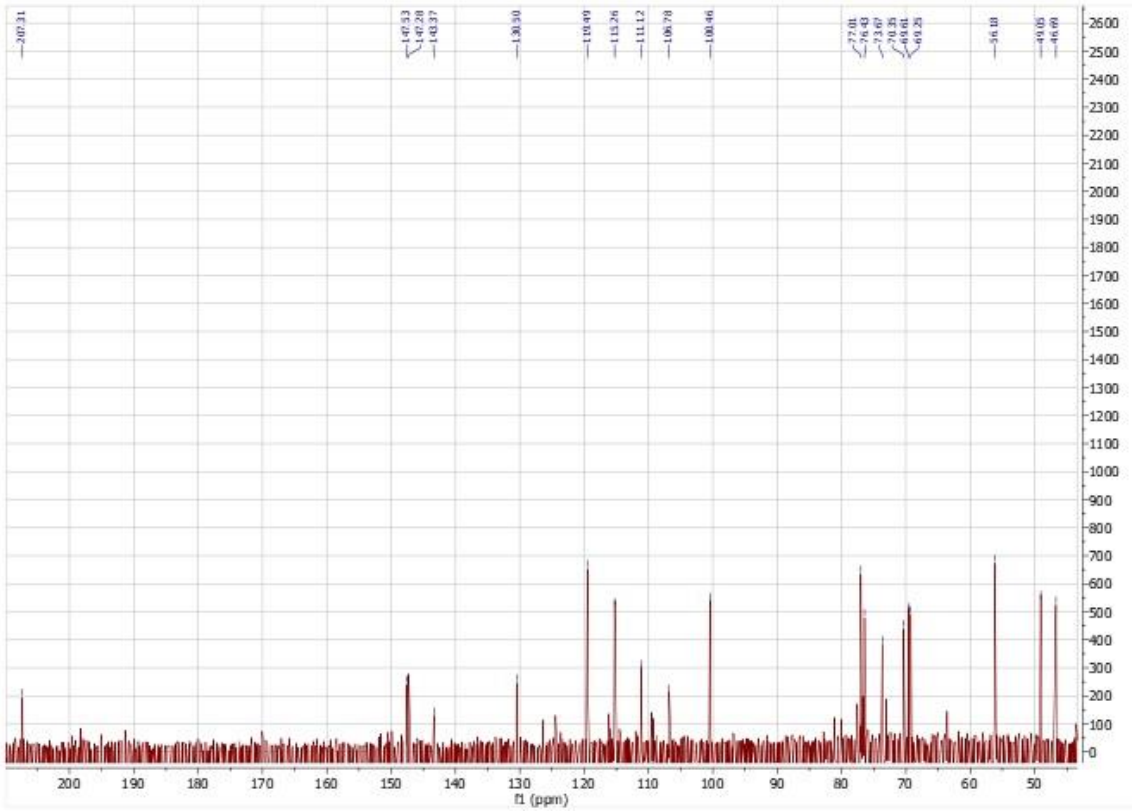
1-(β-D-glucopiranosyl)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (1)



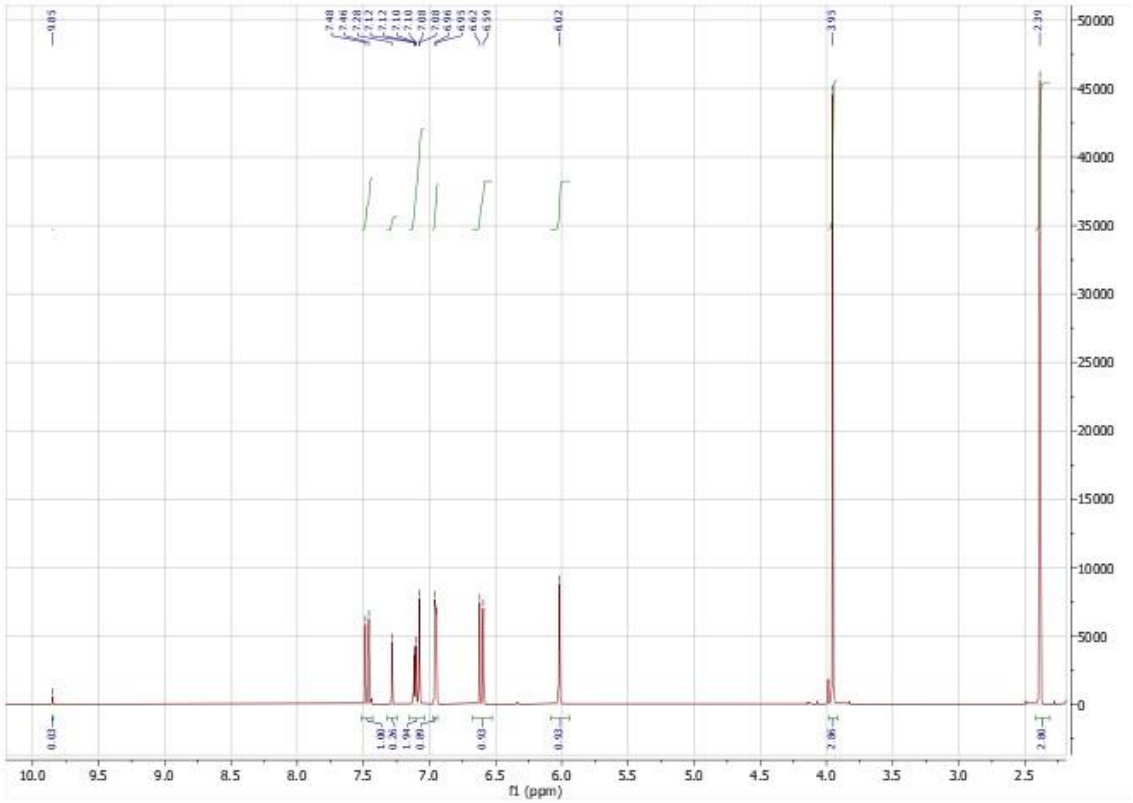


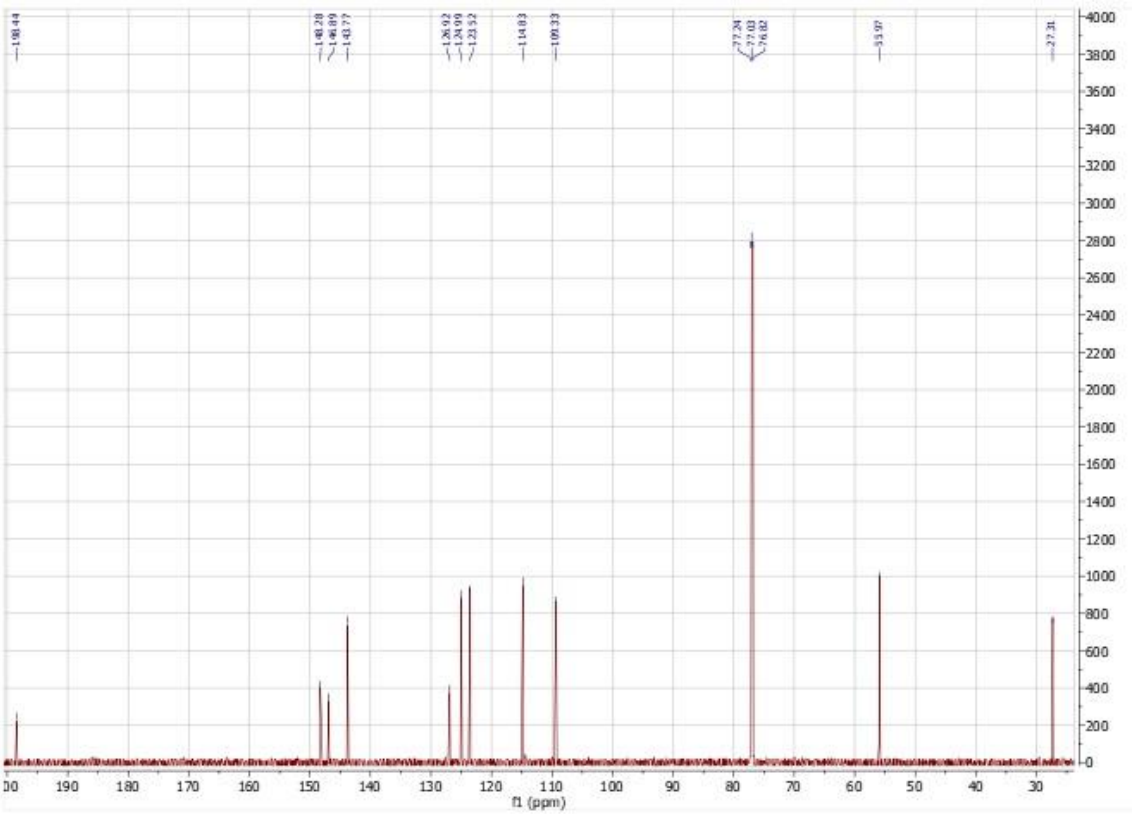
1-(β-D-galactopiranosyl)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (2)



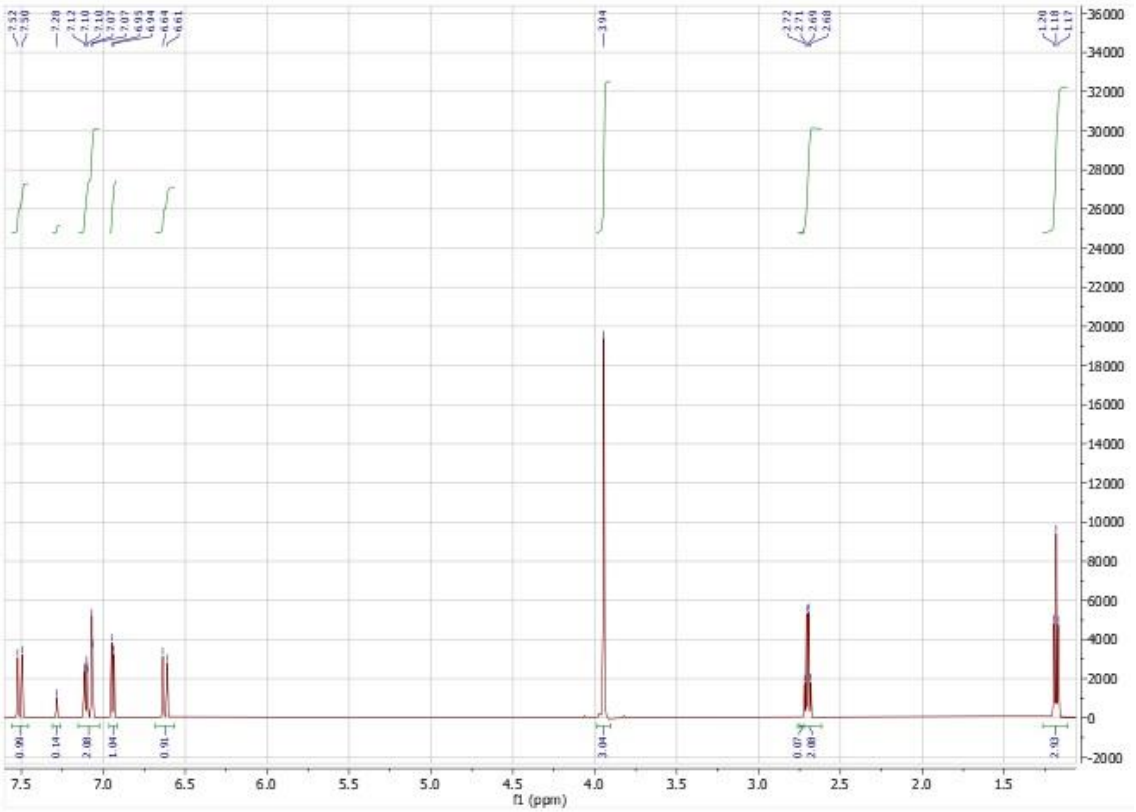


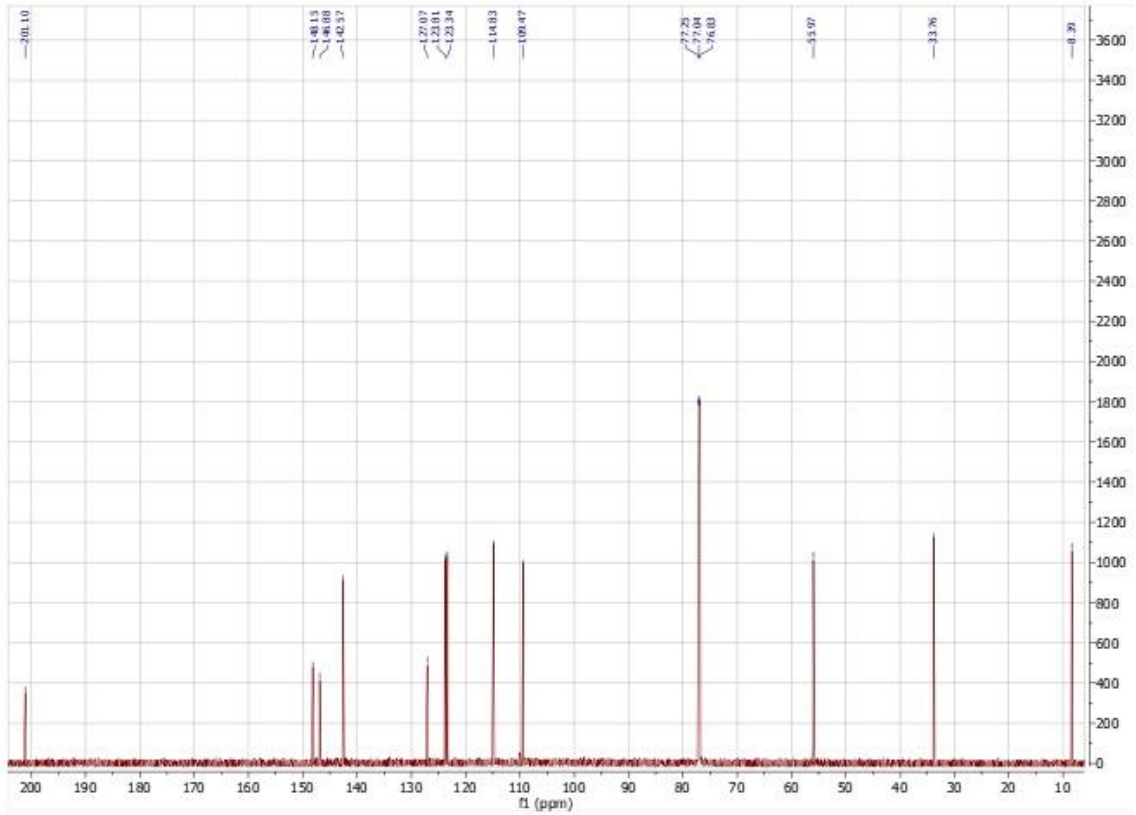
(*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (3)



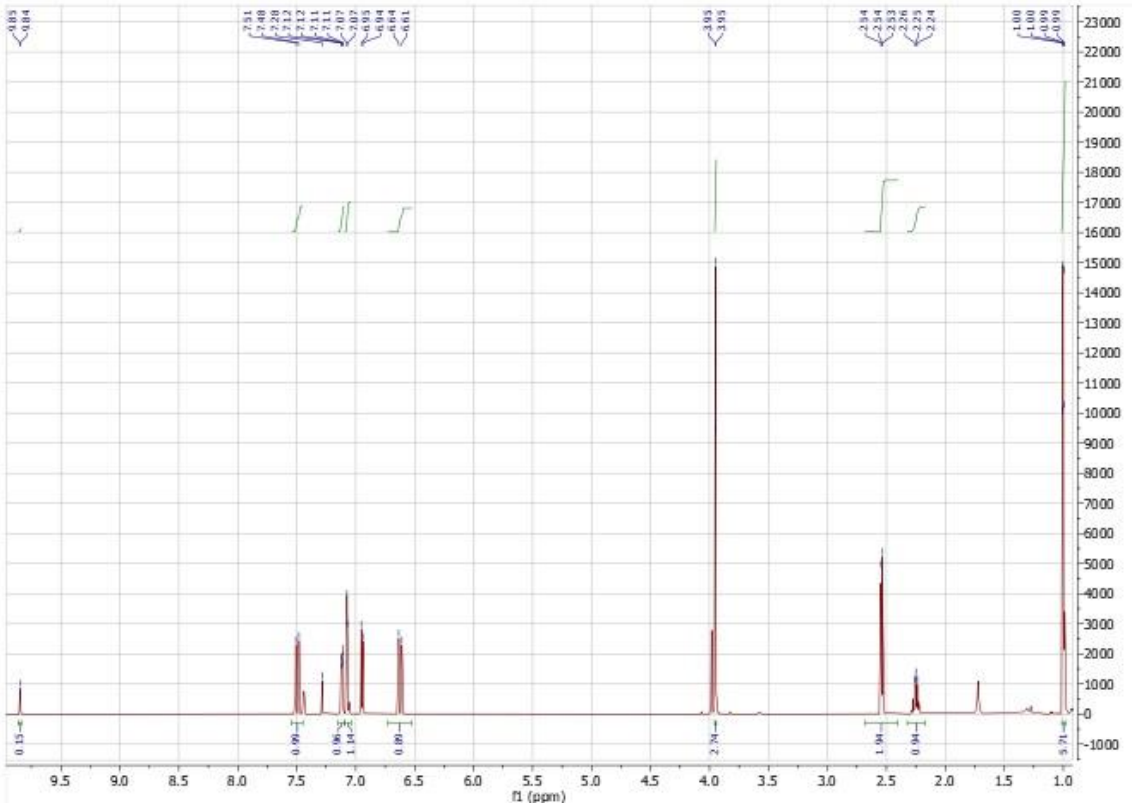


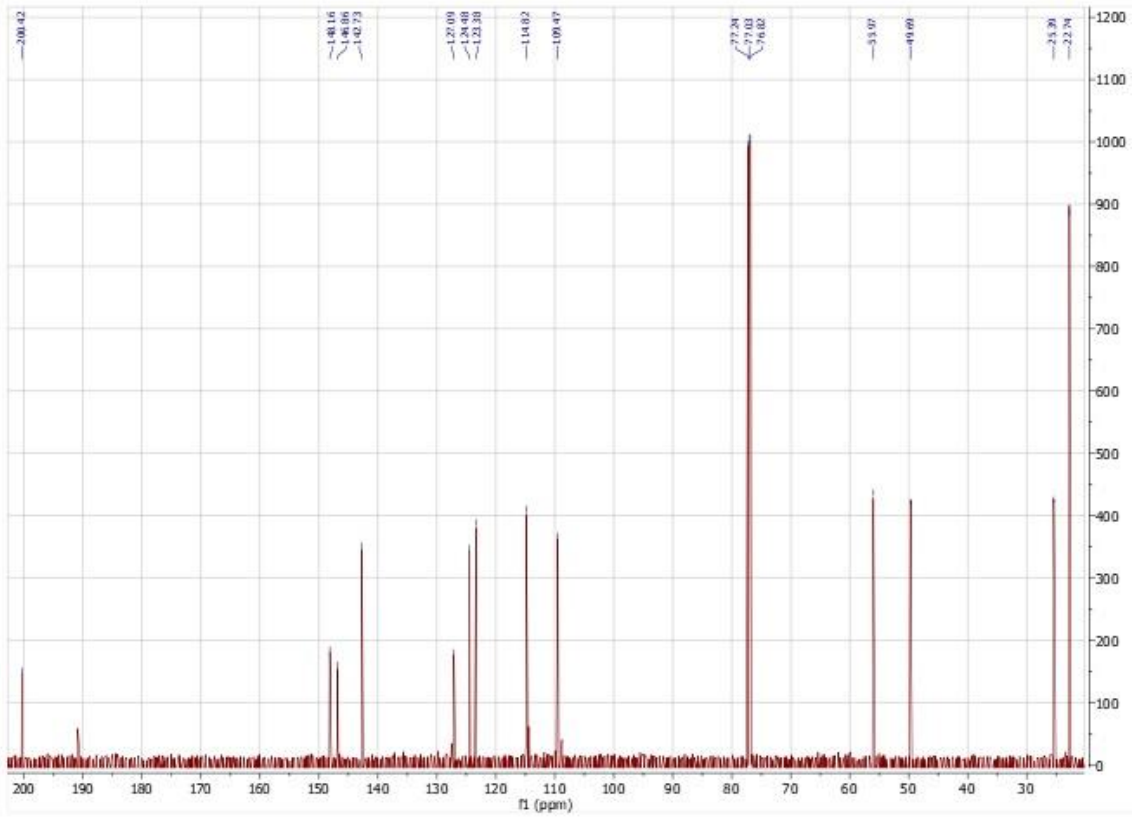
(*E*)-1-(4-hydroxy-3-methoxyphenyl)pent-1-en-3-one (4)





(*E*)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one (5)





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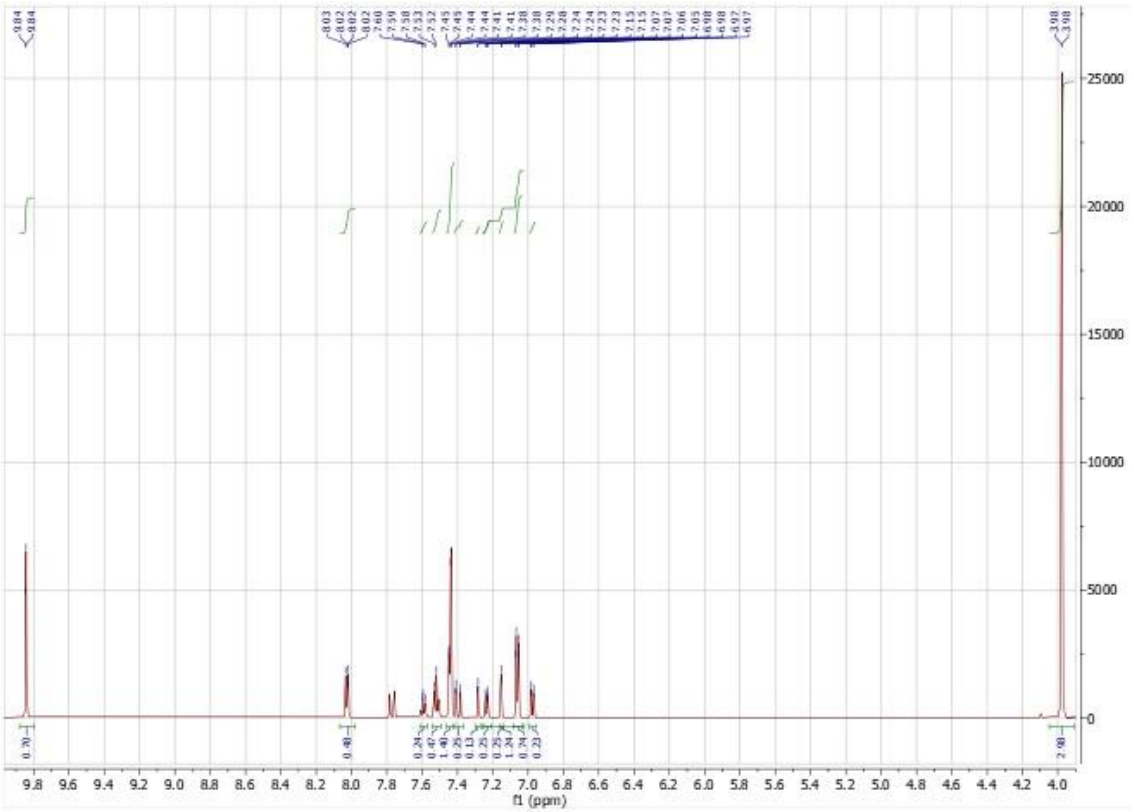

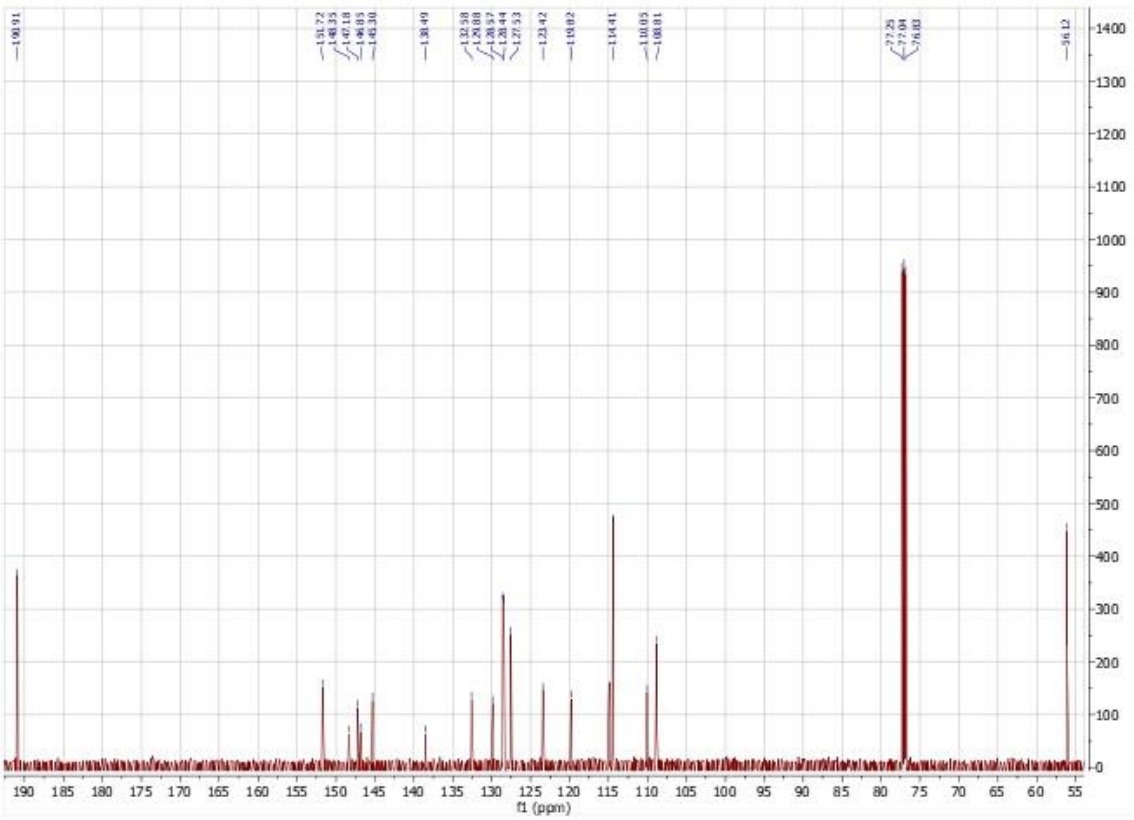

(*E*)-1-(4-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (7)