OPINION

Natural and synthetic probable inhibitors of endoribonuclease Nsp15 encoded by Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Computer modeling approach.

p-coumaric acid, Curcumin and their Boronic acid derivatives.

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Abstract

Based on the structural and biochemical characterization of endoribonuclease Nsp15 in crystal structure, I am providing probable inhibitors of this enzyme. In this report I intent to signal that is possible to inhibit these enzymes by the use of naturaral occurring compound and their boronic acid derivatives, compounds with boronate $B(OH)_2$ groups. Boronic acids are atracted to serine side chains in the active site of serine proteases. Actual lab tests need to be conducted, nevertheless I venture here to propose four compounds: *p*-coumaric acid, Curcumin and their boronic acid derivatives with the use of computer modeling. I used AutoDock Vina, UCSF Chimera 1.12, DeepView / Swiss-Pdb Viewer, Sulp3.0, PubChem, MDL Isis Draw 2.5, OpenBabelGUI 2.2.3, and Microsoft word pad. As hardware I used a VAIO loptop with Intel Core i5 with Window 8.0, and a Dell loptop Inspiron E 1405 with an Intel Core Duo with Windows Xp.

Introduction

The crystal structure 5YVD corresponds to Nonstructural protein 15 (Nsp15) that is encoded by coronavirus (CoV). This protein is crucial in the life cylcle of coronavirus (CoV) responsible for Middle East respiratory syndrome CoV (MERS-CoV). (Zhang *et al.*, 2018).

p-coumaric acid occurs in the plant world in abundance and it shows biological activity. (Hoffmann, 2003). Other names: Phloretic acid, desaminotyrosine. IUPAC name: 3-(4-hydroxyphenyl)propanoic acid. Also an IUPAC name: 3-(4-hydroxyphenyl) propanoic acid. CID in PubChem: 63542 (PubChem).

Curcumin shows antioxidant and anti-inflamatory activity, it is found in tropical spices. (Hoffmann, 2003). Other names are: Natural yellow 3, tumeric yellow, tumeric, curcuma, Indian saffron, diferuloylmethane, and the IUPAC name (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione. The CID: 965516 in PubChem. (PubChem).

We need to predict the binding of substrate to enzyme in an efficient manner before conducting an actual experiment in the lab (Oleg *et al.*, 2009). For this purpose I used AutoDock Vina, which provides me of a score based on energy minimum per conformation of the ligand in the active site. This way I know which docking attempt is the preferred one by Vina. Needless to say that I do not let the computer do all the thinking. There could be a more energetic conformation that could work better in an actual experiment. We must use our judjement.

The visualization programs let me make determinations as stated above, whether it is plausible or not the structure in question. For this purpose I used UCSF Chimera 1.12, which interfaces with AutoDock Vina, and provides me with the first glance at the results of the docking procedure. The next program is Sculpt 3.0 an old version that I still use because it lets me convert the docked ligand into pdb file for use with Sculpt 3.0 and Swiss-PdbViewer 4 1.0. Sculpt also let me visualize the active site determined by a surface at a distance of 10 Angstroms from the ligand. And finally Swiss-PdbViewer 4 1.0 that puts together both pdb(s) files that of the ligand and the receptor into one pdb file.

Procedures

From PubMed Protein structures a crystal structue 5YVD was downloaded to process.

5YVD is a hexamer therefore I isolated one chain – the A chain.

Taking as an active site of the molecule where the glycerol molecule is shown. In AutoDock Vina we need to specify a center for a box that engolfs the active site. I made it the center the coordinates of the C2 of glycerol: 86.487, 154.530, and 40.433. I made the box 20, 20 and 20 Å in dimention. I kept these parameters constant throughout the docking.

Next I selected the inhibitor from Medical Herbalism (Hoffmann et al, 2003).

I decided for *p*-coumaric acid (Figure 1) because it resembles the boronic acids I used as inhibitors of betalactamase from *Mycobacterium tuberculosis* (Barquero 2013).

I also selected Curcumin after p-coumaric proved to work because cucumin (Figure 2) resembles *p*-coumaric structue but double.

I used then the program PubChem from Ncbi (PubChem) to draw and find the structure from database. Hidrogen then were added.

Then the structure was downloaded into Chimera 1.12 and minimized. (Dunbrack, 2002)

I downloaded into UCSF Chimera 1.12 both the ligand and the receptor.

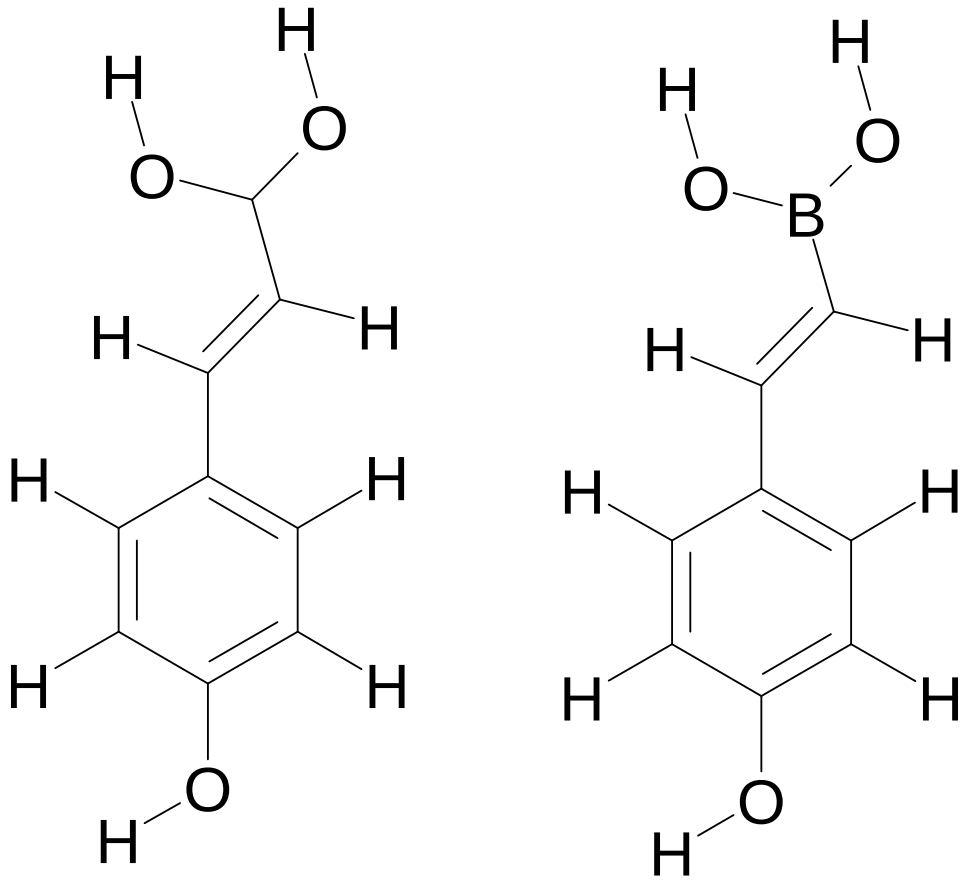
I run a predock (Dunbrack, 2002) interface and then proceeded with the docking with the interface of AutoDock Vina.

The results were recorded as pdb.

The pdb of the ligand was transformed into pdb written by Sculpt 3.0 by open it in Sculpt and then saving it tex with suffix pdb from Sculpt. This file is then unified to the pdb of the receptor produced after docking in Swiss-PdbViewer 4 1.0 by saving the (project) in PdbViewer.

Now the file of both the ligand and the rceptor unified by PdbViewre is opened by Sculpt and saved as pdb file. Then open with wordpad to change the number of the ligand to the next number in the amino acid sequence of the receptor. In this case was 342.

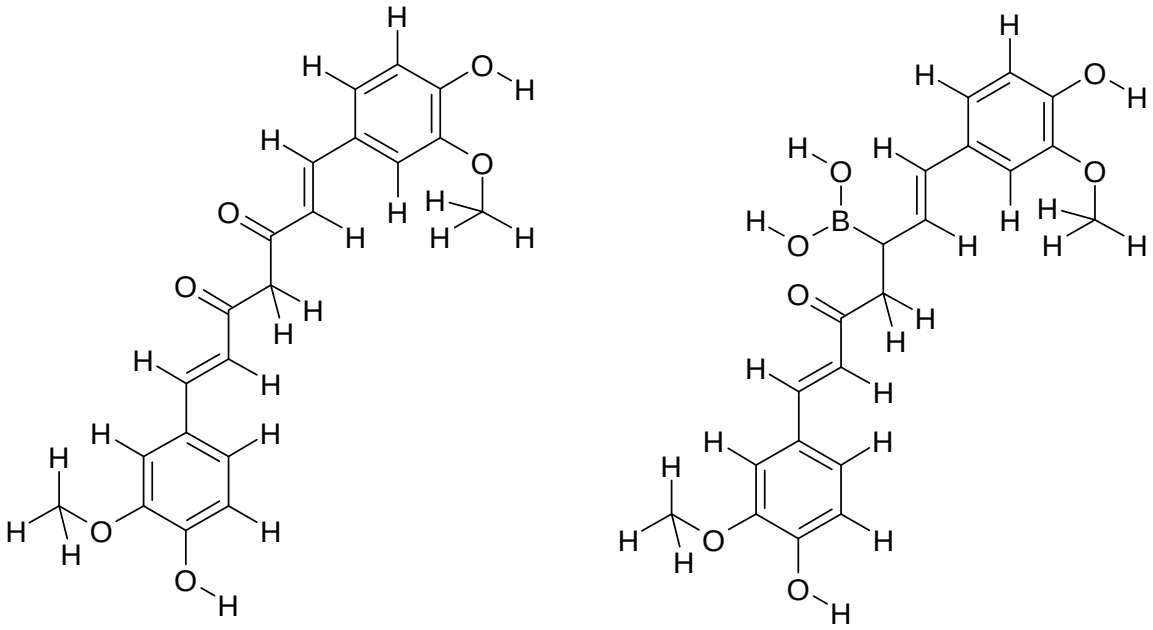
Now the file can be easily visualized In Sculpt and PdbViewr. In Sculpt I found out the distance from ligand to the backbone of the amino acids from 5 to 10 Angstroms away. And in PdbViewer I found Hidrogen bonds and their distances to the receptor.



a) *p*-coumarici acid

b) *p*-coumarici acid boronic acid derivative

Figure 1. Side by side comparison of *p*-coumarici acid and its boronic acid derivative. Drawn with MDL ISIS Draw 2.5. (MDL ISIS Draw 2.5).



a) Curcumin b) Curcumin boronic acid derivative (CurcuminB)

Figure 2. Side by side comparison of curcumin and its boronic acid derivative. Drawn with MDL ISIS Draw 2.5. (MDL ISIS Draw 2.5).

For ease of communication I will call the structure in b from figure 2: CurcuminB

Note that because my version of Vina doesn't recognize boron, carbon has been used instead. Doing so, for the purpose of docking, both structures in *p*-coumaric acid and its derivative would be the same. In the case of Curcumin and CurcuminB, they are not the same and therefore both structures were docked.

Results

For *p*-coumeric acid

File Compounds Column Selection Chimera HBonds Movie

S	Score	RMSD l.b.	RMSD u.b.	HBonds (all)	HBond Ligand Atoms	HBond Receptor Atoms
V	-5.1	0.0	0.0	3	3	2
V	-5.1	0.071	1.398	3	3	2
V	-4.9	1.643	5.095	1	1	1
V	-4.4	3.635	5.035	2	1	2
V	-4.3	2.425	5.088	0	0	0
V	-4.2	3.861	4.569	0	0	0

Figure 3. Vina results for docking p-cumeric acid and Nsp15. UCSF Chimera 1.12 interfaced with AutoDock Vina.

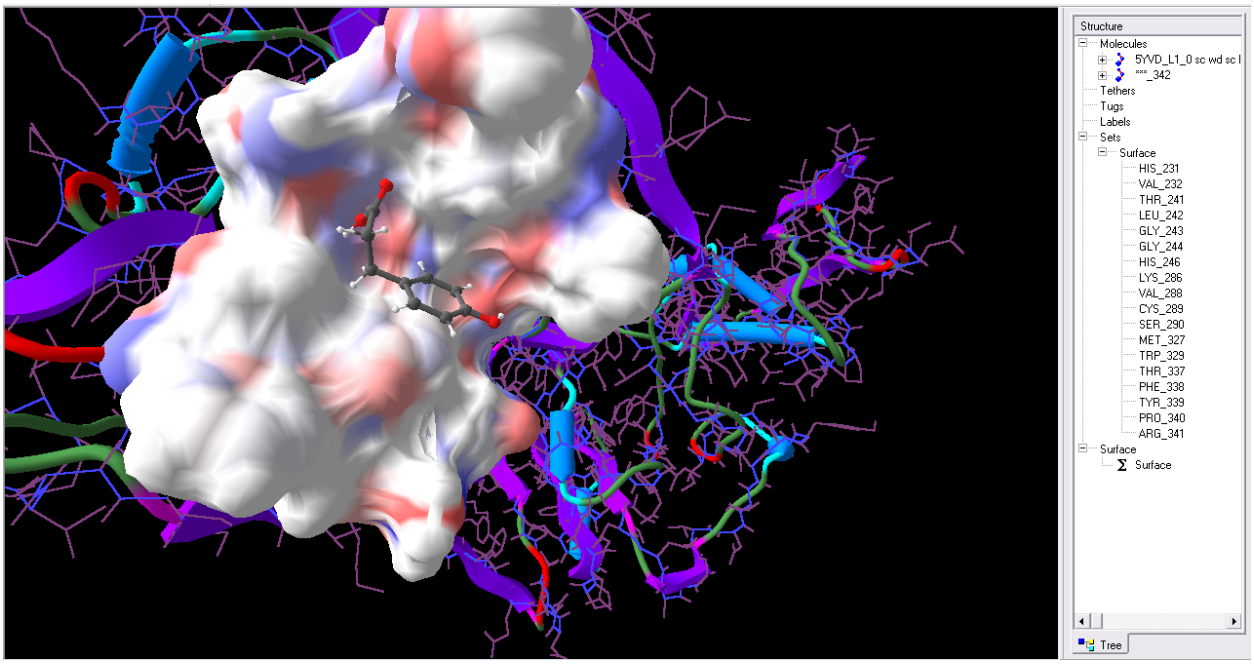


Figure 4. *p*-coumaric acid in the active site of Nsp15 (5YVD A). Sculpt 3.0
The list of amino acids in the active site are determined by putting a surface at 10.00 Angstroms from the ligand.

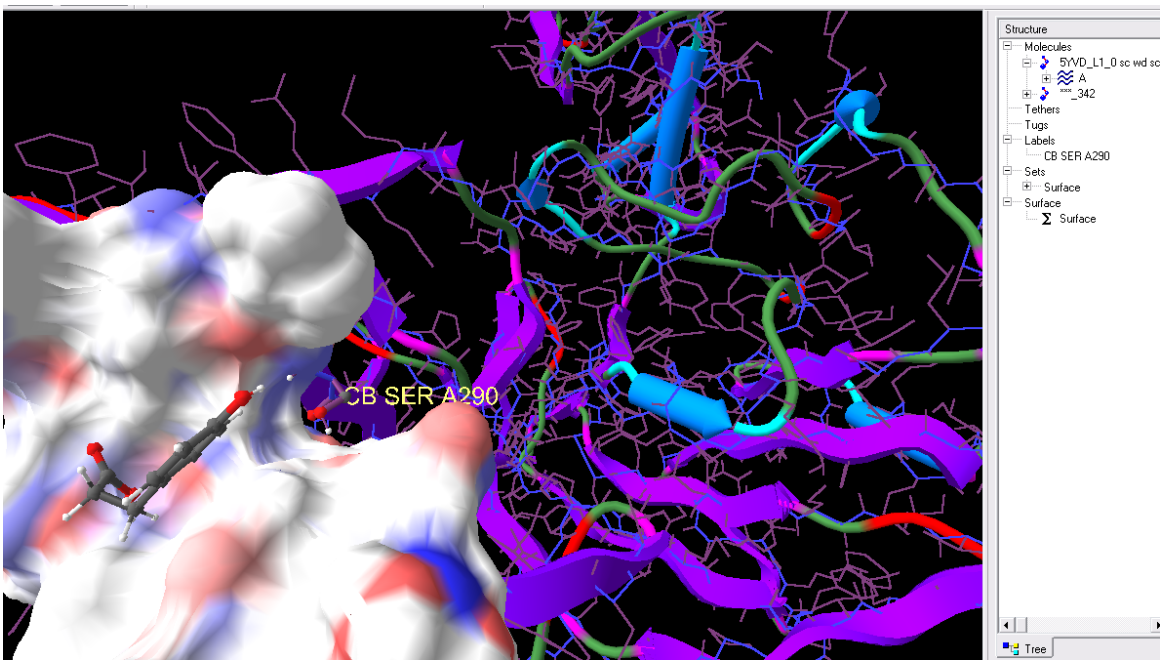


Figure 5. *p*-coumaric acid in the active site of Nsp15 (5YVD A). Sculpt 3.0
The surface was removed from Ser290 which is part of the active site. Ser290 appears at 8.00 Angstroms from the ligand.

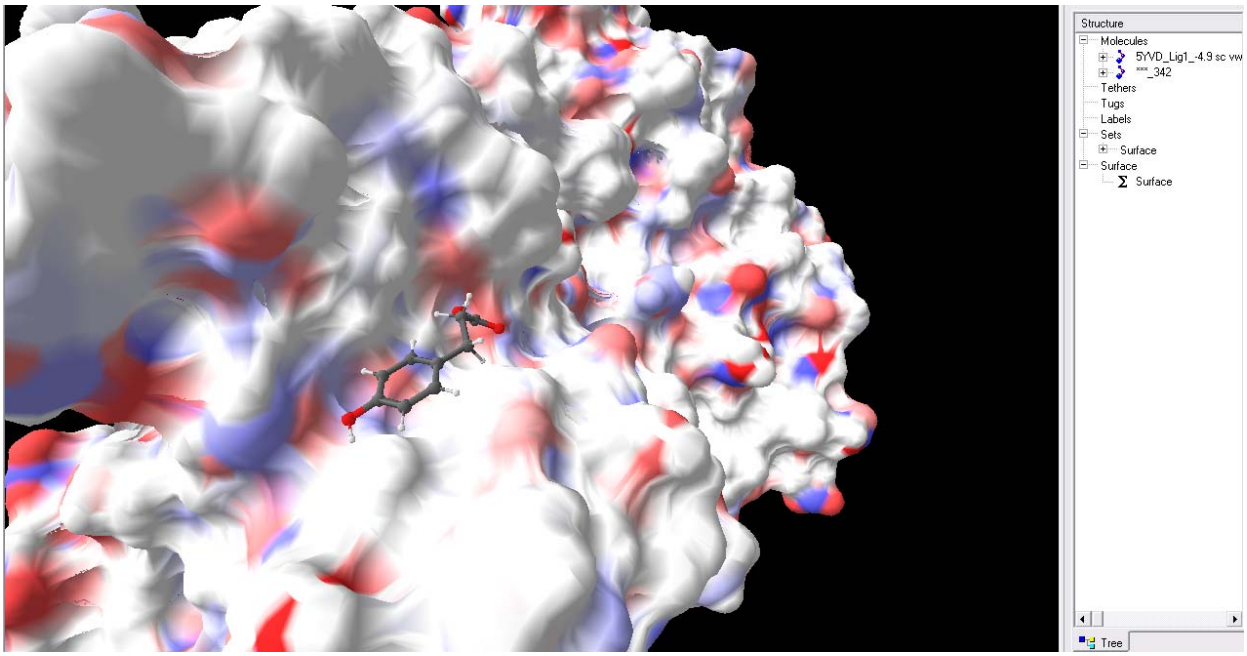


Figure 6. *p*-coumaric acid in the active site of Nsp15 (5YVD A). Sculpt 3.0
Vina free energy -4.9. Ligand closer to Ser290

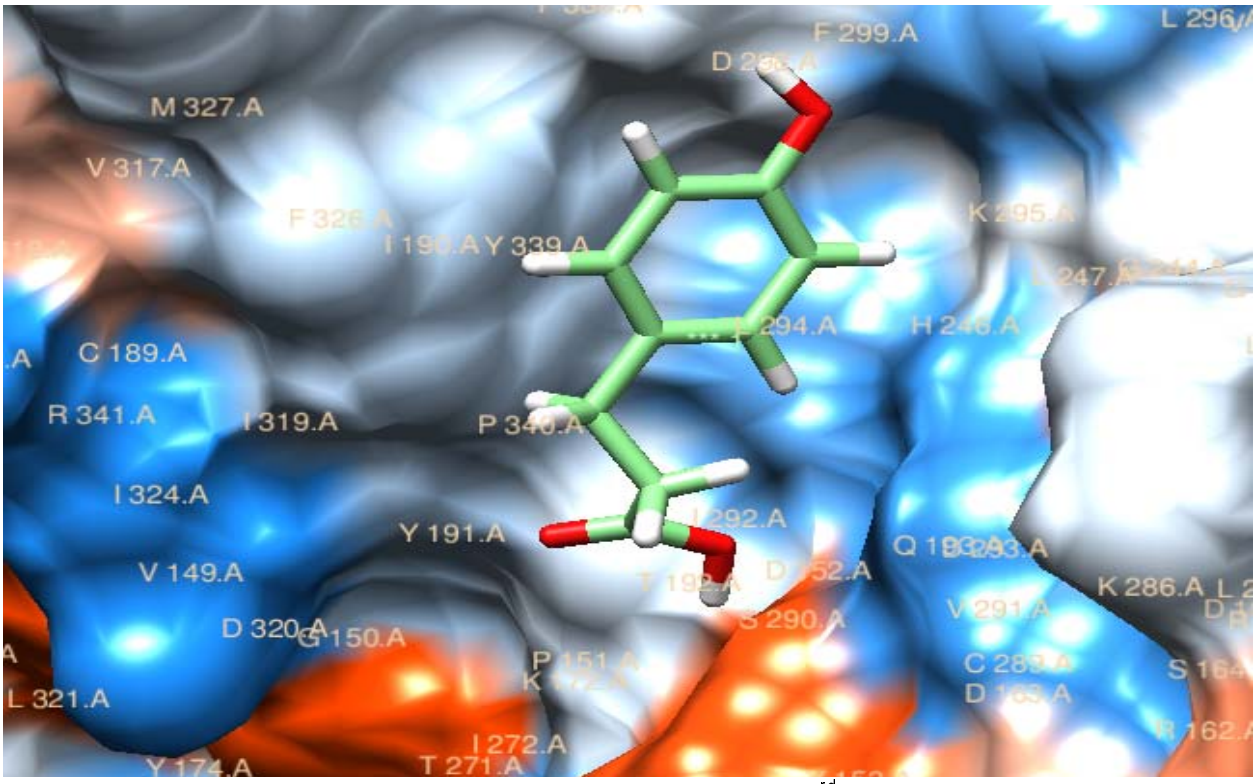


Figure 7. *p*-coumaric acid in Nsp15 (5YVD A). Vina free energy -4.9 (3rd in the list with 1 Hydrogen bond). UCSF Chimera 1.12
Shown here to indicate that one end of the ligand is close to Ser290.

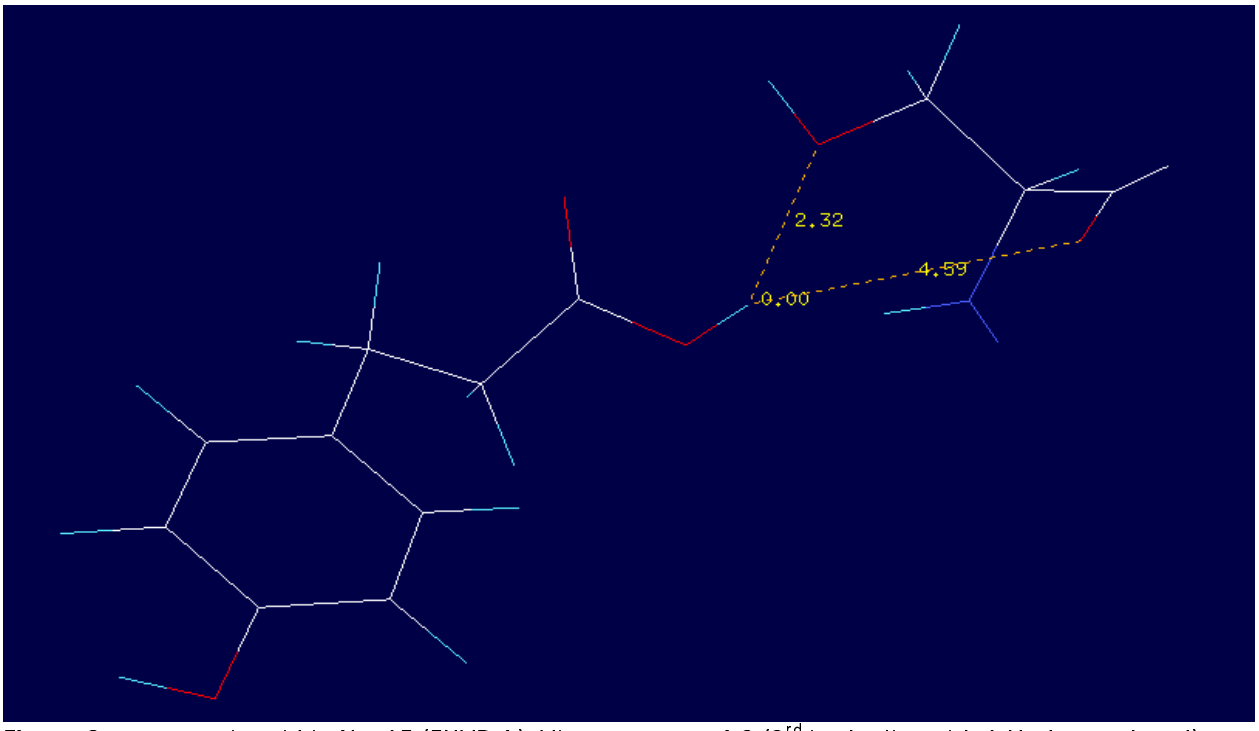


Figure 8. *p*-coumaric acid in Nsp15 (5YVD A). Vina energy = -4.9 (3rd in the list with 1 Hydrogen bond). UCSF Chimera 1.12

Results for Curcumin

File Compounds Column Selection Chimera HBonds Movie

S	Score	RMSD l.b.	RMSD u.b.	HBonds (all)	HBond Ligand Atoms	HBond Receptor Atoms
V	-6.3	0.0	0.0	3	2	3
V	-6.2	4.901	8.009	4	2	4
V	-6.1	4.111	6.138	4	2	3
V	-6.1	1.67	5.899	3	2	3
V	-6.1	3.257	7.581	3	3	3
V	-6.0	1.602	7.115	4	2	4

Figure 9. Results from docking for Curcumin and Nsp15 (5YVD A). Done in UCSF Chimera 1.12 and AutoDock Vina. I chose for analysis the one with the lowest energy -6.3.

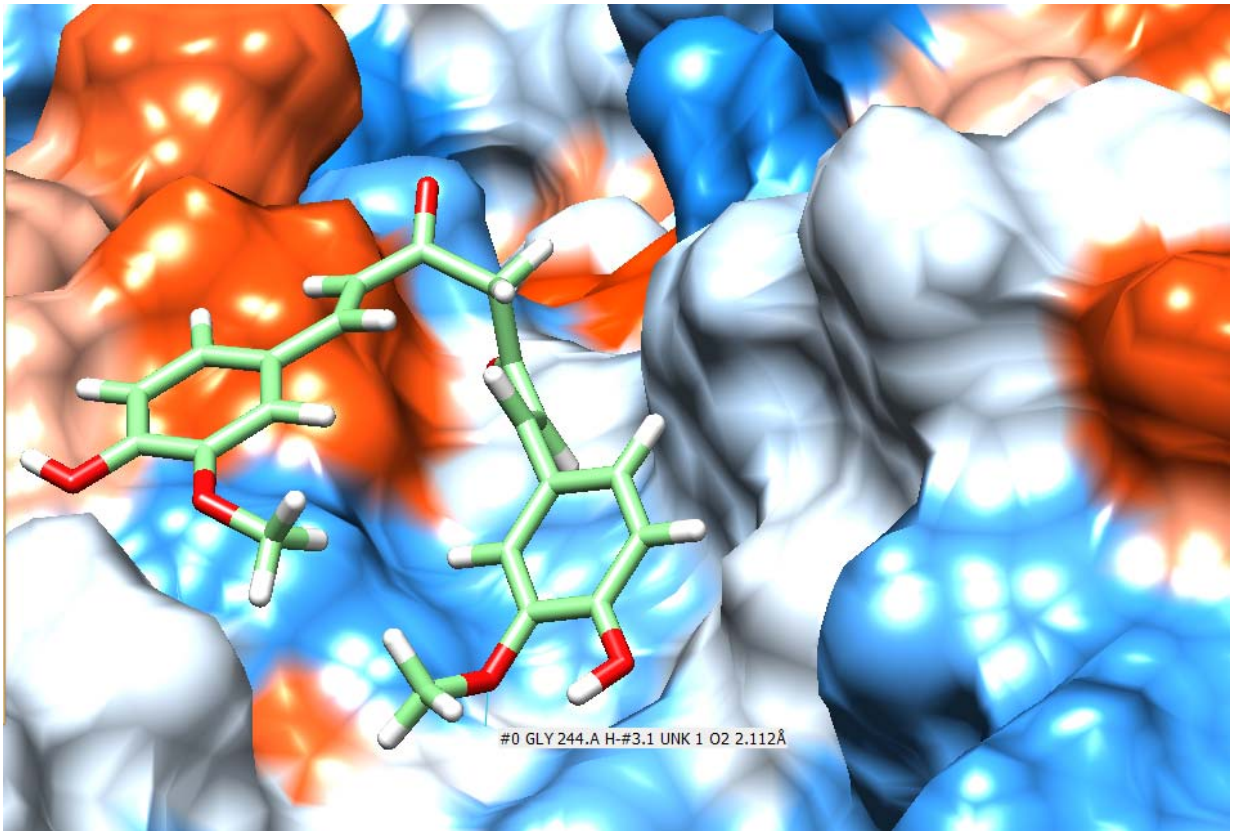


Figure 10. Results from docking for Curcumin and Nsp15 (5YVD A). Done in UCSF Chimera 1.12 and AutoDock Vina. UCSF Chimera 1.12

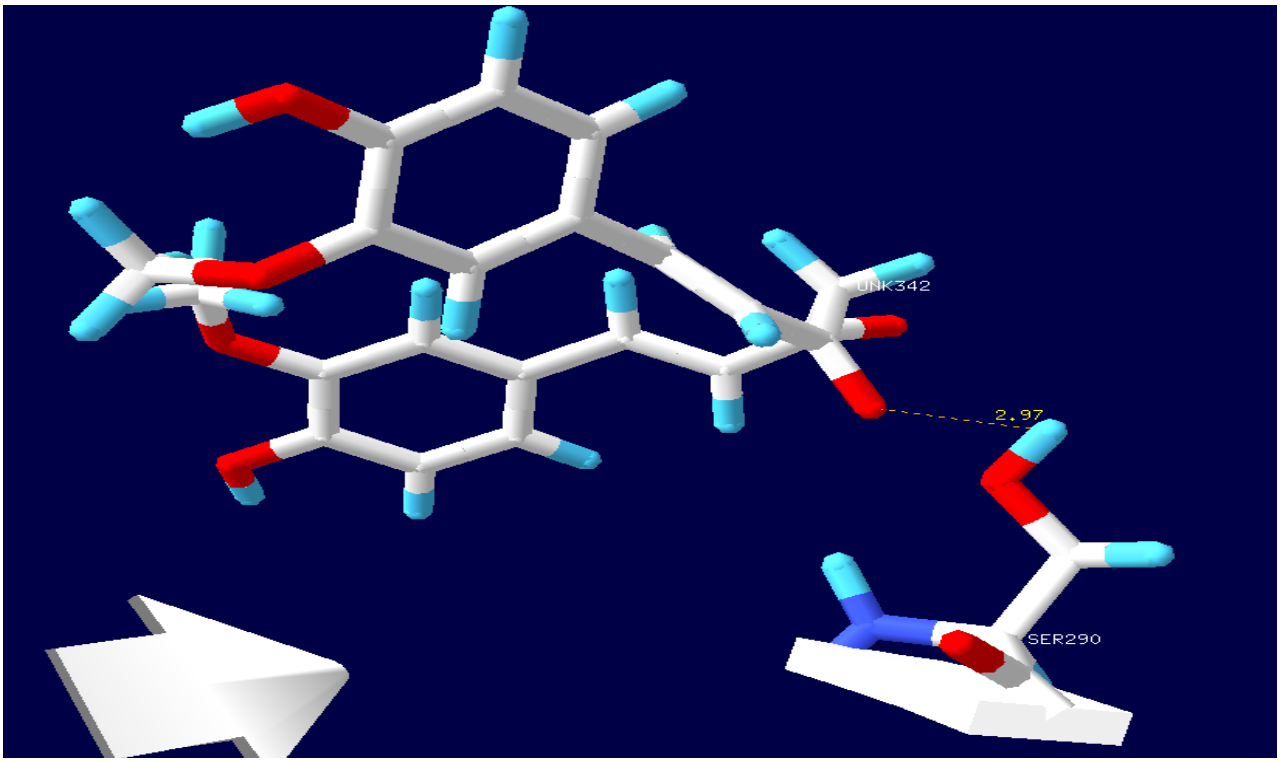


Figure 11. Curcumin in the active site of Nsp15 (5YVD A). Swiss-PdbViewer 4 1.0. Distance to Ser290 is 2.97 Å.

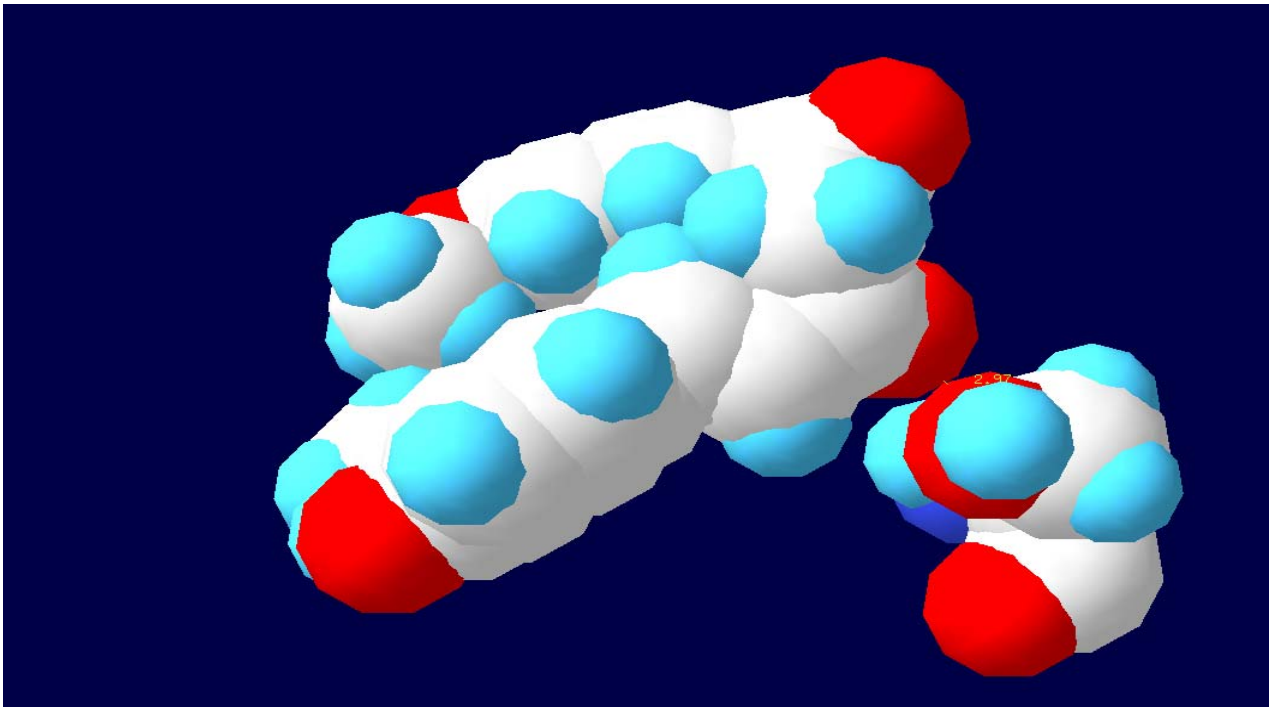


Figure 12. Curcumin in the active site of Nsp15 (5YVD A). Swiss-PdbViewer 4 1.0. Distance to Ser290 is 2.97 Å. Space filling model. On the left Curcumin, on the right bottom Ser290.

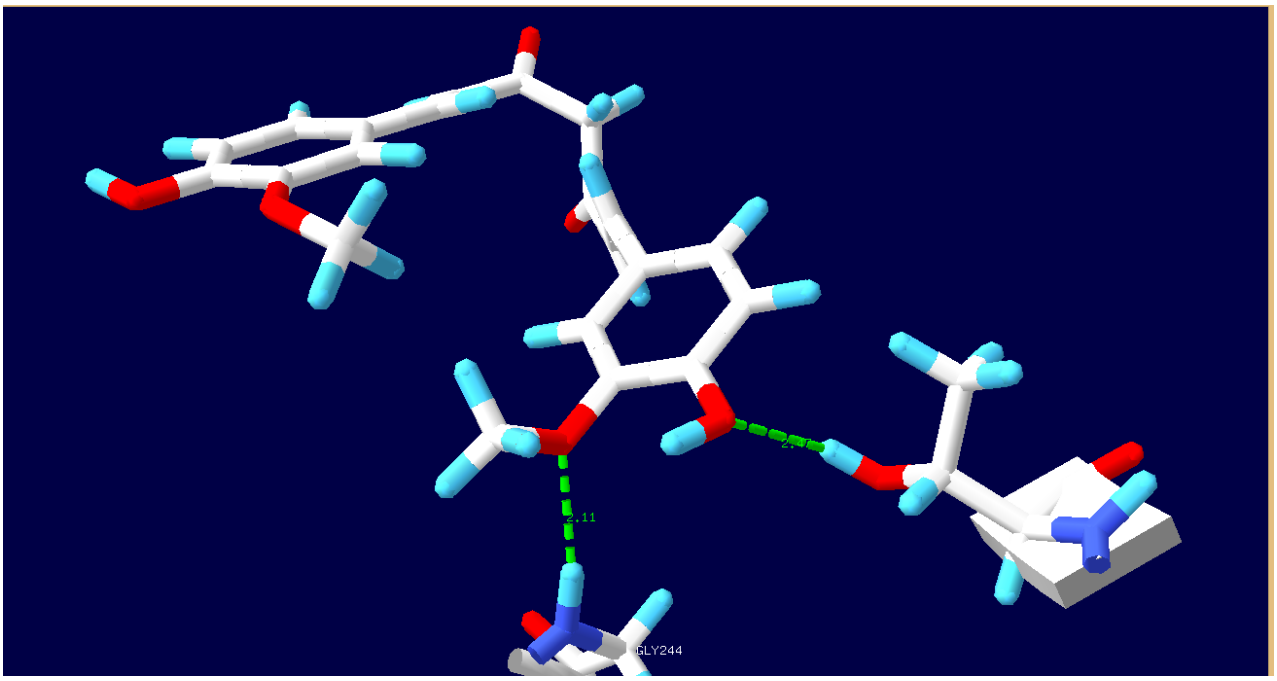


Figure 13. Curcumin in the active site of Nsp15 (5YVD A). Swiss-PdbViewer 4 1.0.
Ligand hydrogen bond to Gly244 2.11 Å and Thr337 2.47 Å

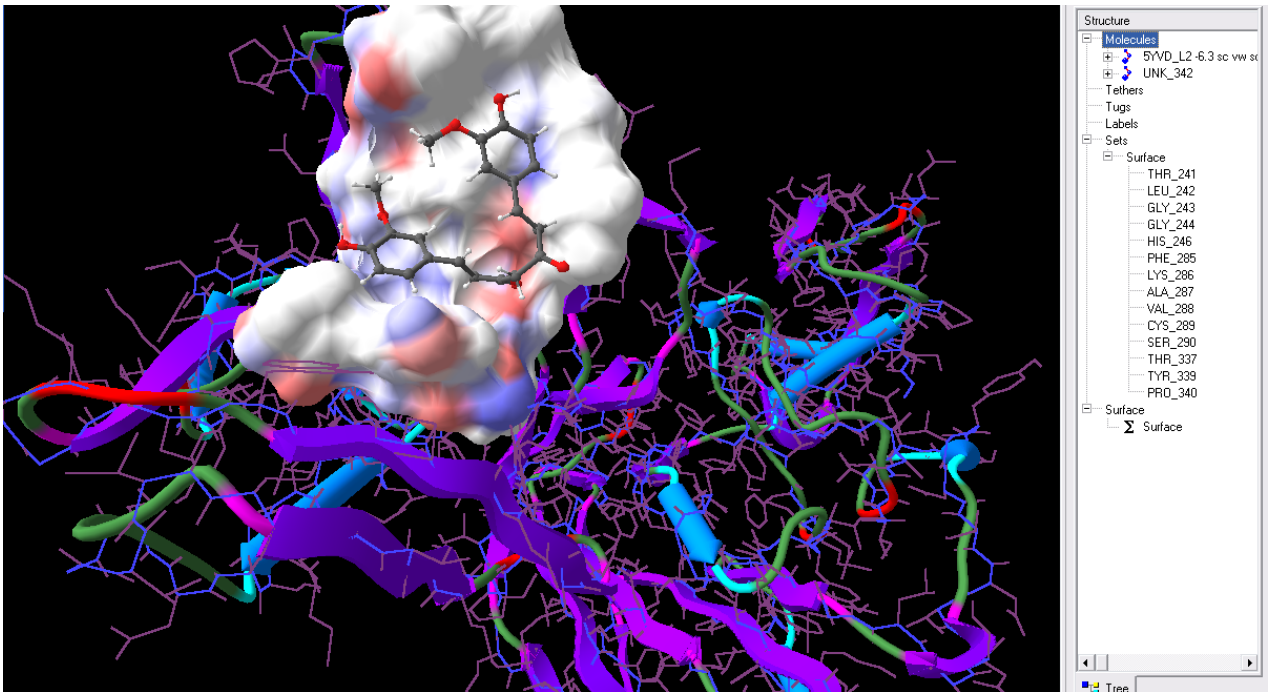


Figure 14. Curcumin in the active site of Nsp15 (5YVD A). Sculpt 3.0. Active site amino acids shown on right

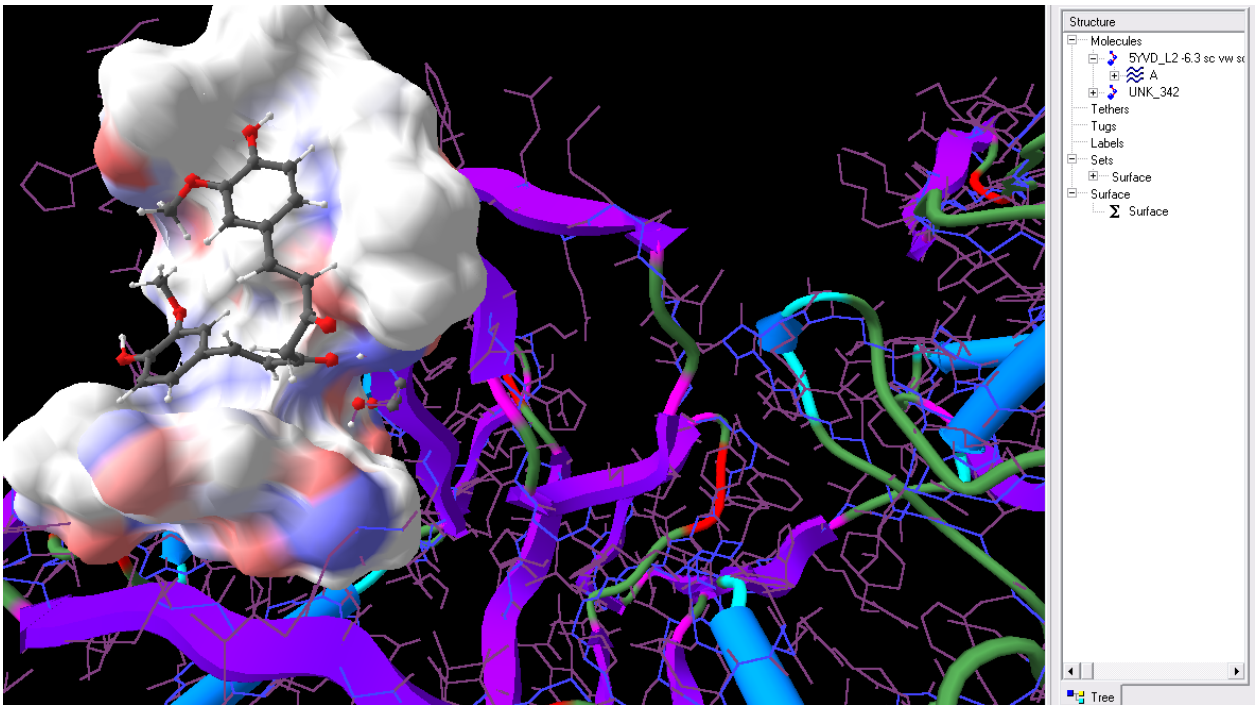


Figure 15. Curcumin in the active site of Nsp15 (5YVD A). Sculpt 3.0. Surface removed from Ser290 to show proximity.

Results for Curcurcumin boronic acid derivative (CurcuminB)

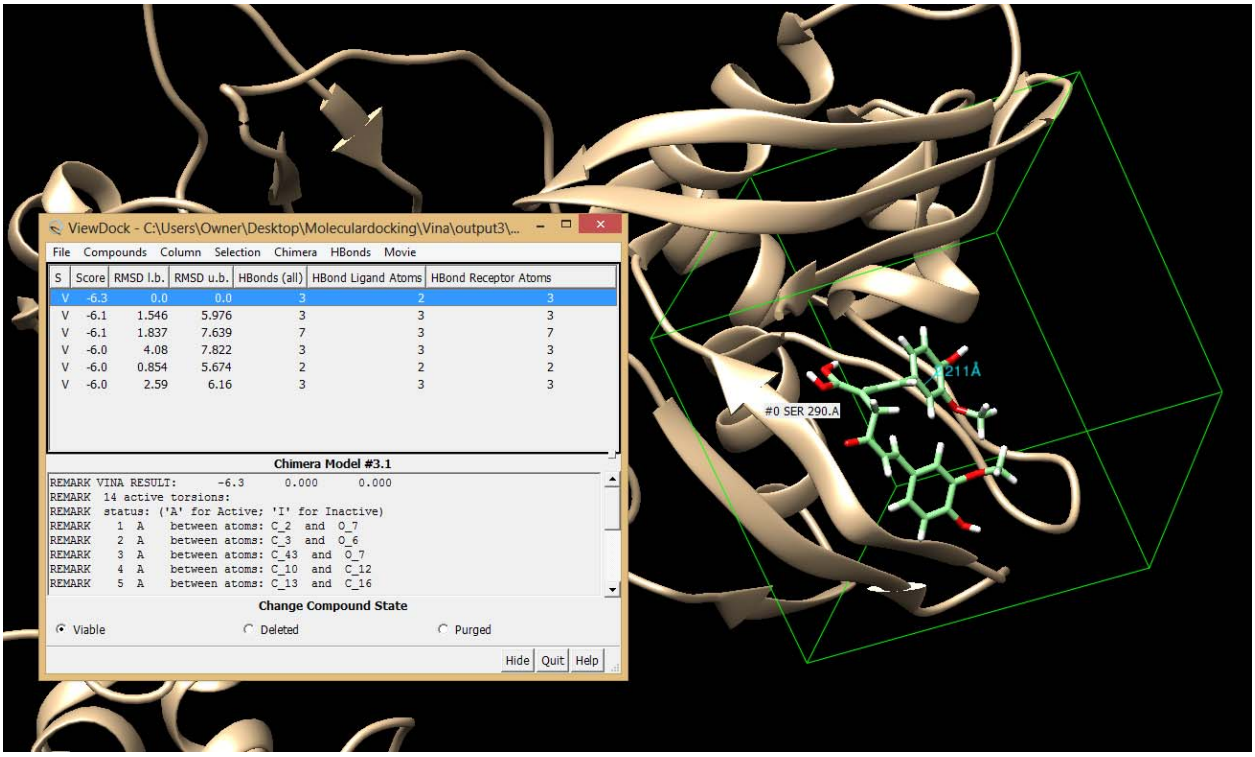


Figure 16. First take after CurcuminB was docked to 5YVD A with Vina. UCSF Chimera 1.12

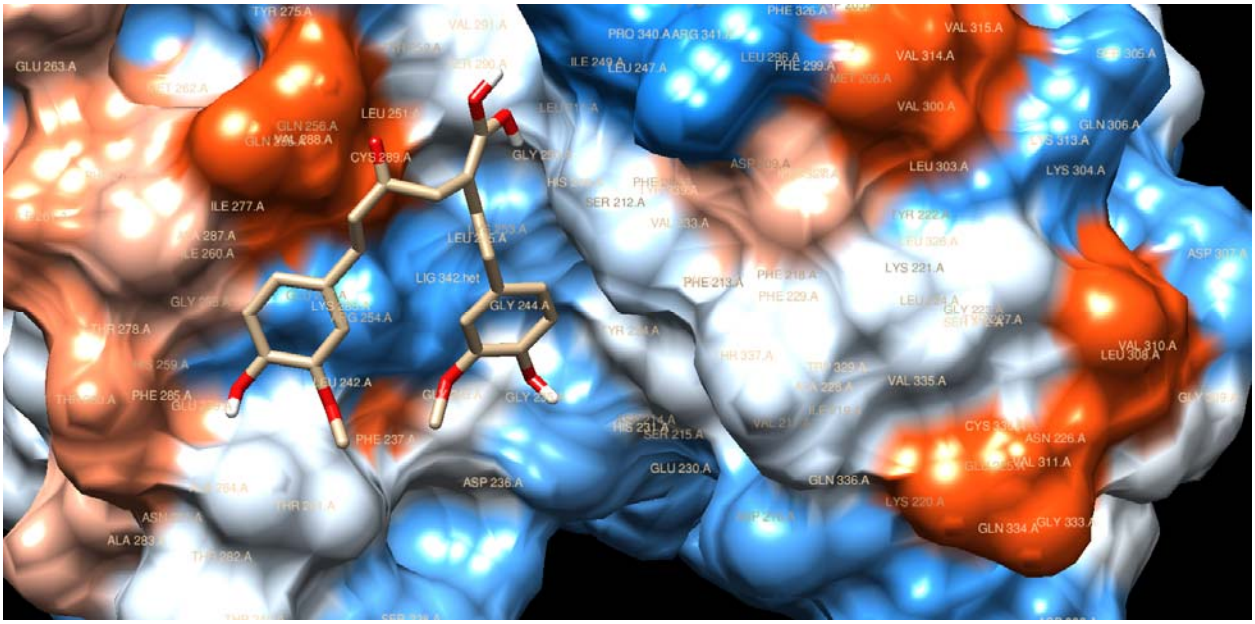


Figure 17. CurcuminB in the vacinity of Ser290 in 5YVD. UCSF Chimera 1.12.

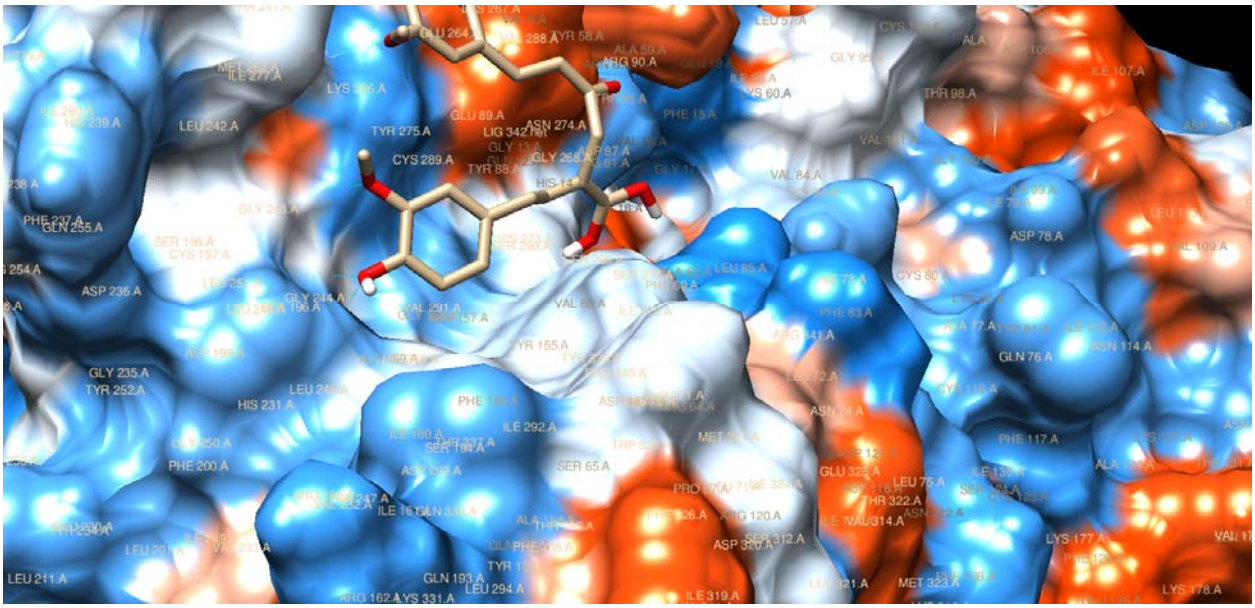


Figure 18. CurcuminB in proximity to a pocket formed by His231 and Arg341 from which form two Hydrogen bonds stabilizing the docking as if in a oxyanion hole. UCSF Chimera 1.12

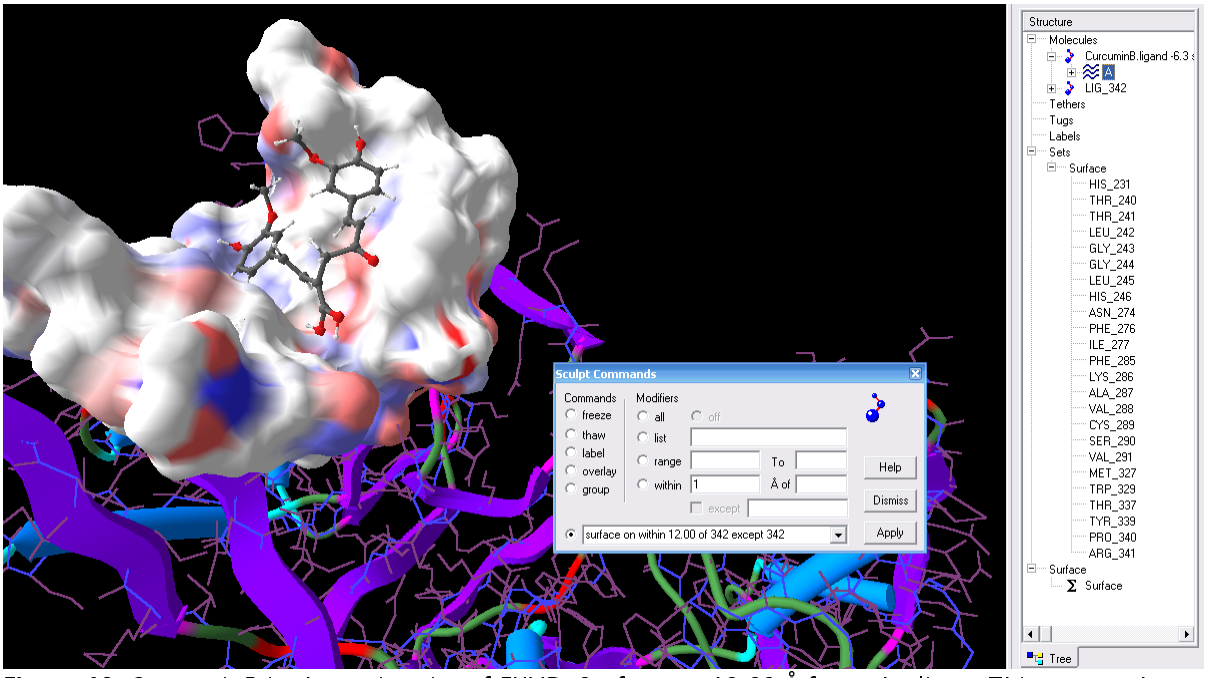


Figure 19. CurcuminB in the active site of 5YVD. Surface on 12.00 Å from the ligan. This was to show His231 and Arg341 that appeared at 11.00 and 12.00 Å respectively. Sculpt 3.0

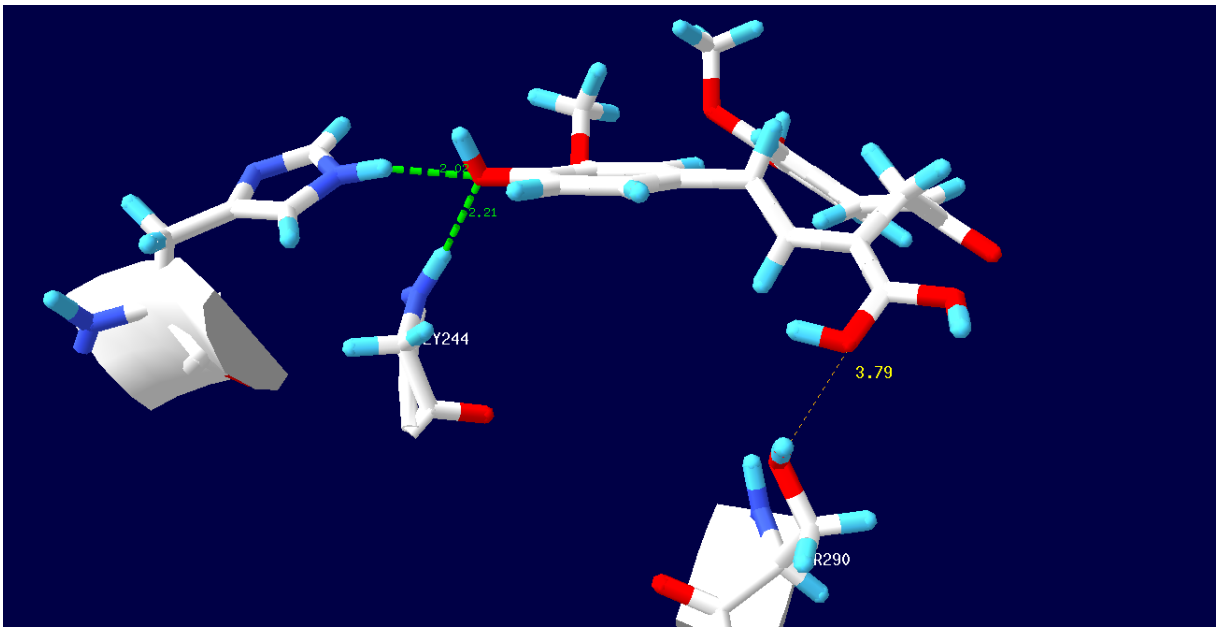


Figure 20. Distance from the Boron (C in simulation) to the sidechain Oxygen in Ser90. Also showing The oxyanion like stabilization pocket form between His231 and Gly244. Swiss-PdbViewer 4 1.0.

Conclusion

1. Both compound, *p*-coumaric acid and curcumin that are found in nature fit within reasonable paramerters in the active sites of this enzyme.
2. Both natural compound dock with high proximity to Ser290.
3. The enzyme could be a serine protease.
4. If we substitute one carbon atom or an oxygen, in the ligand, for a borate $B(OH)_2$ moiety we may convert the compounds into boronic acids.
5. The boronate moiety would then interact with Ser290 for a tighter interaction with the receptor enzyme. The inhibitor would be more efficient.
6. In CurcuminB, there is greater stabilization by the two hyddrogen bonds from Gly244 (2.211 Å), and His231 (2.017 Å) to the ligand acting as some sort of oxyanion hole. Therefore, CurcuminB should be consider a high probable inhibitor of Nsp15.

Future Development

More simiulations will be done as more enzyme crystal structures become available in NCBI protein database.

Aknowledgment

My special gratitud to my former mentor Dr. Manfred Philipp, Ph.D. retired professor from Lehman College and the Graduate Center/CUNY for his support in my endavor.

Appendix A.

Stuctures downloaded from internet

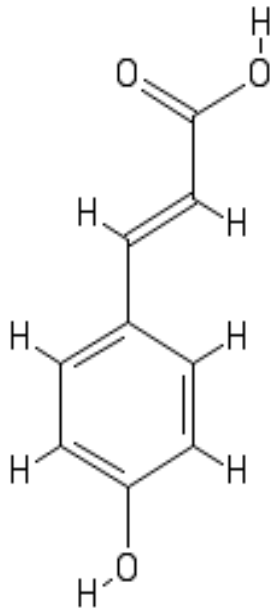


Figure A1. *p*-coumeric acid, (PubChem).
ID: 637542

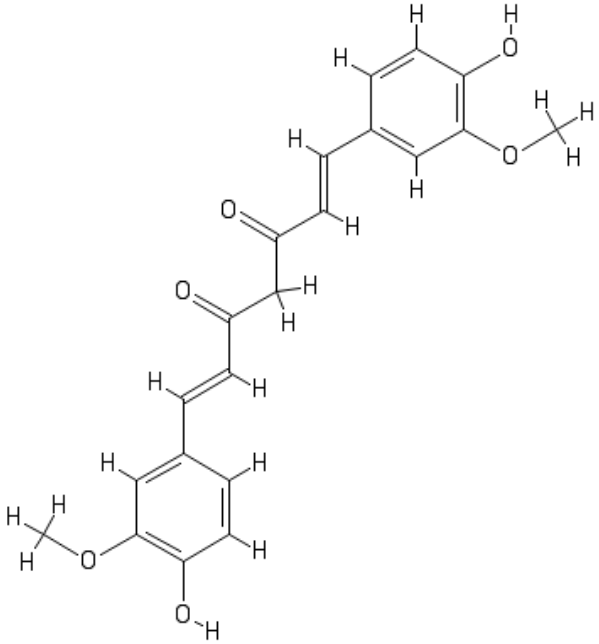


Figure A2. Curcumin. PubChem from NCBI.
CID: 969516 (PubChem).

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