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COURSE: CHEM 5130

SECTION: V1

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TOPIC: Heat Shock Protein 90 and its Inhibitors

SUB-TOPIC: Possible Structures of Inhibitors Binding to the ATP Active Site of HSP90

DATE: 21 December 2015

ABSTRACT:

Forty one HSP90 inhibitors were selected for computational docking on to HSP90. Several (about 327) structures of these inhibitors were prepared using LigPrep. These were later docked onto five receptor grids prepared from crystal structures of the N-terminus of HSP90 using Glide. Inhibitors were docked onto the receptors via self-docking and cross docking. Four out of five of the self-docking results resembled their PDB structures. Also, one inhibitor, Geldanamycin failed to dock on one of the receptor grids.

Also, IC_{50} values of the inhibitors were obtained and compared with the Glide scores after the computational docking exercise. Initially, it was assumed that inhibitors with low IC_{50} values produced low (more negative) glide scores. However, this was not so. Some ligands such as 17-AAG with an IC_{50} value of 1.27 had a Glide score of -4.571 when bound to the receptor grid of PDB code 1OSF and Geldanamycin had an IC_{50} of 0.281 and a glide score of -4.119.

Finally, after computational docking, it is seen that inhibitors with more negative Glide scores and that also have amine groups tend to have a protonated nitrogen atom before they bind with the HSP90 protein even if it is not shown by the crystal structure provided. One such inhibitor is 17-DMAG. Also, from both LigPrep and Glide, it is seen that ligands change the structures by tautomerization and protonation and deprotonation and even folding before they bind themselves to a protein. This helps in giving a possible prediction of a crystal structure. This is seen in Herbimycin when it binds to the N-Terminus of HSP90.

INTRODUCTION:

Heat Shock Protein 90 (HSP90) plays important biological roles in the functioning of living cells. It assists in proper folding of other proteins and acts as "heat absorbers" for them (Young, Moarefi, & Hartl, 2001). A heat shock protein stabilizes folded proteins which would otherwise unfold when subjected to high temperatures, thereby preventing cellular damage. About 200 different proteins rely on HSP90 for stability and correct folding and are as client proteins (Hong, et al., 2013). These are grouped as chaperones, transcription factors and kinases. Examples of each include p23, Sse1, Sse2 (chaperones), BBX, CAR (transcription factors) and AXL and FLT4 (kinases) (Picard, 2002).

At the same time, however, HSP90 plays significant roles in cancerous cells which includes the stabilization of growth factor receptors and mutant proteins as well as assisting in tumor growth. These include mutant forms of cKIT, HER2 and BCR-ABL (Hong, et al., 2013). HSP90 protects cancerous cells from becoming destabilized thus allowing them to perform their functions effectively. Unlike normal cells, cancerous cells are more sensitive to HSP90. As they rely on HSP90 for growth and survival, antitumor drugs have been used to target this protein and inhibit its activity. Inhibiting this protein results in cancer cells becoming more easily destroyed (Issacs, Xu, & Neckers, 2003). To achieve this, HSP90 inhibitors are administered to the patient. These inhibitors are given in combination with other drugs in order to perform their functions effectively. One example of this is Alvespimycin (17-DMAG) which is administered in combination with trastuzumab, a client protein inhibitor (Hong, et al., 2013).

Several HSP90 inhibitors including Tanespimycin (17-AAG), Alvespimycin (17-DMAG) and IPI-504 are used to inhibit this protein. These are derived from Geldanamycin, another HSP90 inhibitor. This

inhibitor was not used in clinical trials due to its high hepatotoxicity (Hong, et al., 2013). However, many more inhibitors have been synthesized and were tested against this protein. As a result, possible crystal structures of these compounds need to be predicted. The purpose of this project is to investigate by means of computational docking, different ligands that function as inhibitors of HSP90 and predict the structures of the complexes they form when bound to HSP90.

BACKGROUND:

Heat Shock Protein 90 or HSP90 is a chaperone protein that is found in a wide range of living cells of organisms including bacteria and eukaryotes. HSP90 weighs 90 kilo-Daltons and is a part of a group of heat shock proteins whose function is to protect cells from heat when they are exposed to such conditions. When cells are unstressed, HSPs make up about 1 to 2 percent of the total protein content. Under stress however, this number increases to about 4 to 6 percent (Crevel, Bates, Huikeshoven, & Cotterill, 2001).

The general HSP90 protein consists of three major domains: N-terminus, the middle domain and the C-terminus. There is also a charged linker domain which connects the middle domain to the N-terminus. Like other proteins, HSP90 consists of alpha helixes, beta plated sheets and random coils. HSP90 exists as homodimers, where the molecules can exhibit open and closed configurations (Karagöz & Rüdiger, 2015). In the open configuration, the molecules come into contact at the C-terminuses and the N-terminuses are away from each other while in the closed configuration, the N-terminuses of the molecules come close to each other. Adenosine triphosphate (ATP) is required for HSP90 to perform its activity. It binds to the N-terminus of the protein (which has a high affinity for ATP) and allows the protein to exhibit a closed configuration. Client proteins are now able to bind to

the protein. After the client binds to the protein, ATP undergoes hydrolysis which leads to the open configuration of HSP90 and the client protein being released (Hong, et al., 2013). At the same time, ATP breaks down into adenosine diphosphate (ADP) and a phosphate ion (PO_4^{3-}).

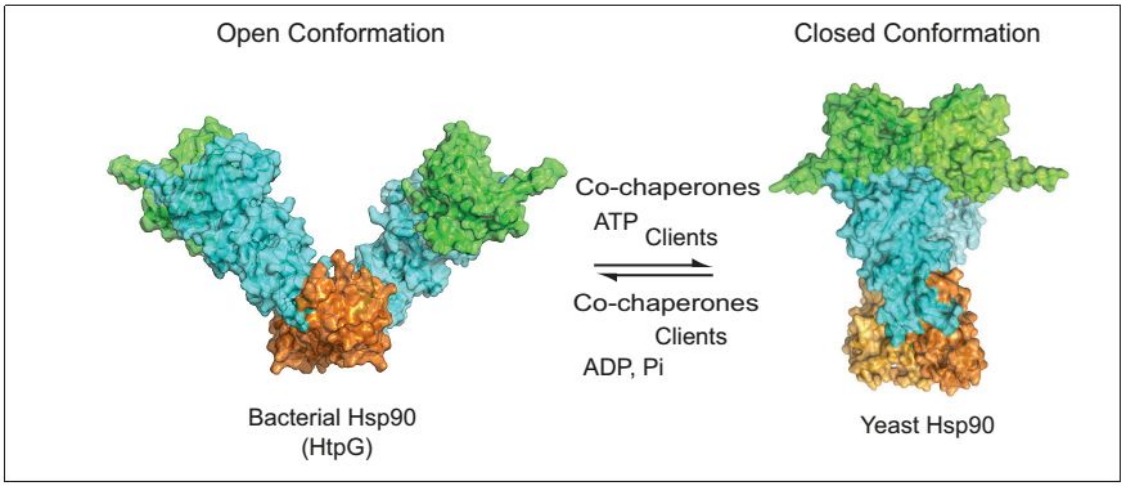

Figure 1: Open and closed configurations of HSP90 showing a simplified way of the interchanging between the two configurations. The bacterial HSP90 is in its open form while the yeast HSP90 is in its closed form. The domains are outlined in different colours: N-terminus (green), middle domain (blue), C-terminus (orange) (Li & Buchner, 2013).

HSP90 is found in a wide range of organisms from bacteria such as yeast to mammals including humans. In humans, HSP90 exists as five different isoforms. These are HSP90α_1 (HSP90AA1), HSP90α_2 (HSP90AA2), HSP90β (HSP90AB1), Endoplasmin/GRP-94 (HSP90B1) and TNF Receptor Associated Protein (TRAP1) (Chen, Piel, Gui, Bruford, & Monteiro, 2005). The first three are located in the cytosol while the last two are located in the endoplasmic reticulum and the mitochondria respectively. HSP90α and HSP90β proteins are very similar in structure but the β isoform has a shorter C-terminus than its α-counterparts. Likewise, the β isoform is constitutively expressed while its counterparts are inducible. HSP90β is one of the most studied proteins because of its antiapoptotic (apoptosis inhibiting) characteristic and binding activities with inhibitors, the latter being the reason

why it is a major target for cancer killing drugs (Law).

HSP90 performs several functions in cells. It is a major chaperone protein that is responsible for protein folding, a vital process for life. Proteins usually follow a folding pathway and acquire a three dimensional structure that circles around a hydrophobic core (Karagöz & Rüdiger, 2015). A second function of HSP90 is the stabilization of the tertiary structure of the 26S proteasome required for proteolysis or protein degradation. Proteolysis describes the process by which damaged, unnecessary and incorrectly folded proteins are destroyed by proteasome. Thirdly, HSP90 binds steroid receptors such as the glucocorticoid receptor which depends on the protein to perform its function (Pratt, Morishima, Murphy, & Harrell, 2006).

Genetic instability and mutations result in the formation of cancerous cells which lead to formation of tumors (Issacs, Xu, & Neckers, 2003). These are characteristic signs of cancer. Like normal cells, cancerous cells rely on HSP90 to function. Cancerous cells are exposed to stress environments when they are targeted by anti-tumor drugs and high levels of radiation from chemotherapy. As a result, they respond to these stresses by producing more HSP90 proteins, from as little as 2 times more to 10 times more than that of their normal levels (Issacs, Xu, & Neckers, 2003). In addition to acting as heat absorbers, HSP90 also performs other functions for cancerous cells including the stabilization of mutant forms of proteins such as v-Src and p53 (anti-tumor protein) as well as the vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS), both of which are required for stable tumor growth.

In order to destroy these cancerous cells, the protein responsible for their stability has to be inhibited. Like ATP, HSP90 inhibitors bind to its N-terminus. As a result, it a major site of study by medicinal chemists. These inhibitors displace ATP from the terminus, thus they are powerful enough to hinder

this protein's functions in cancerous cells (Hong, et al., 2013). Several inhibitors of HSP90 being studied in clinics include natural derived compounds such as geldanamycin, herbimycin and radicicol. Geldanamycin proved to be the most potent inhibitor but is not used because of its high toxicity (Hong, et al., 2013). Tanespimycin or 17-allylamino, 17-demethoxygeldanamycin (17-AAG), a derivative of geldanamycin with a smaller level of toxicity has a similar binding ability to that of its main counterpart (Bagatell & Whitesell, 2004). Hence it is used more often for cancer treatment. Several other smaller and cheaper synthetic inhibitors are used for treatment. Examples include CCT018159, PU3 and KF58333 (Bagatell & Whitesell, 2004).

Over the years, scientists have tried to find out more information on how these inhibitors bind to the protein as well as their crystal structures. In addition to that, they have developed HSP90 binding assays to measure binding affinities of inhibitors to HSP90. One such assay that is used is the fluorescence polarization assay. For this assay, a fluorescence probe (example: VER-00045864) is attached to the binding site of the protein. Fluorescence polarization increases when the probe is bound to the protein. Upon addition of the inhibitor, the inhibitor competes with the probe for the binding site. As the ligand displaces the probe, the fluorescence polarization signal decreases. From these readings, as well as the known concentrations of the ligands, IC_{50} values of these compounds can be determined (Howes, et al., 2006).

METHOD:

Forty one HSP90 inhibitors were researched in this study and their main structures were built in the Maestro program (Schrödinger, LLC) and placed in their respective groups. Next, with the use of LigPrep, various structures such as tautomers and protonation states of these inhibitors were created

and these were also grouped. Thirdly, PDB ID of known crystal structures of five of these inhibitors were downloaded unto the Maestro program. These structures were used to create receptor grids on which the various structures of HSP90 inhibitors would be docked to. Finally, with the use of the Glide Docking program, (Schrödinger, LLC) these inhibitors were docked on the five receptor grids that were prepared. After docking, results and structures were analyzed. Variations of the inhibitors were prepared using LigPrep (Schrödinger, LLC) at a PH range of 7±2, ligands were docked onto the receptors using the Glide program using the flexible docking algorithm.

RESULTS:

HSP90 Inhibitors Prepared

Number of HSP90 inhibitors prepared – 41

Table 1: HSP90 Inhibitors used in this study with their respective IC_{50} values and key of references.

HSP90 INHIBITORS USED FOR COMPUTATIONAL DOCKING			
Group	Name of Inhibitor	FP IC_{50} (μM)	References
Natural Derived Inhibitors	Geldanamycin	0.281	1, 2, 3
	Tanespimycin (17-AAG)	1.270	1, 3
	Alvespimycin (17-DMAG)	0.058	3, 4, 5
	Herbimycin	Unknown	4
	Radicocol	0.060	1, 3
Synthetic Inhibitors	NMS-E973	< 0.010	6
	AT-13387	0.018	7
	SNX2112	0.030	5
	NVP-AUY922	0.021	8, 10
	BIIB021	0.0017 (K_i Value)	9
Pyrazole Inhibitors	Pyrazole Inhibitor 1 (CCT018159)	0.280	10
	Pyrazole Inhibitor 2 (VER-49009)	0.025	10
	Pyrazole Inhibitor 3	0.258	10
	Pyrazole Inhibitor 4	0.600	10
Aminomethyl-Functionalized Diarylpyrazoles Inhibitors (AFDP Inhibitors)	AFDP Inhibitor 1	0.146	10
	AFDP Inhibitor 2	0.115	10
	AFDP Inhibitor 3	0.035	10
	AFDP Inhibitor 4	0.027	10
	AFDP Inhibitor 5	0.057	10
	AFDP Inhibitor 6	0.142	10
	AFDP Inhibitor 7	0.222	10
	AFDP Inhibitor 8	1.000	10
	AFDP Inhibitor 9	2.630	10
	AFDP Inhibitor 10	0.728	10
	AFDP Inhibitor 11	1.290	10
	AFDP Inhibitor 12	0.431	10
	AFDP Inhibitor 13	0.914	10
	AFDP Inhibitor 14	1.660	10

HSP90 INHIBITORS USED FOR COMPUTATIONAL DOCKING			
Group	Name of Inhibitor	FP IC_{50} (μM)	References
	AFDP Inhibitor 15	0.231	10
Aminomethyl-Functionalized Diarylisoxazoles Inhibitors (AFDI Inhibitors)	AFDI Inhibitor 1	0.021	10
	AFDI Inhibitor 2	0.014	10
	AFDI Inhibitor 3	0.039	10
	AFDI Inhibitor 4	0.021	10
	AFDI Inhibitor 5	0.064	10
	AFDI Inhibitor 6	0.019	10
	AFDI Inhibitor 7	0.028	10
	AFDI Inhibitor 8	0.039	10
	AFDI Inhibitor 9	0.018	10
	AFDI Inhibitor 10	0.127	10
	AFDI Inhibitor 11	0.343	10
	AFDI Inhibitor 12	0.239	10

Key to references:

1. (Howes, et al., 2006)
2. (Stebbins, et al., 1997)
3. (Bagatell & Whitesell, 2004)
4. (Jez, Chen, Rastelli, Stroud, & Santi, 2003)
5. (Chandarplaty, et al., 2008)
6. (Fogliatto, et al., 2013)
7. (Symth, et al., 2012)
8. (Jensen, et al., 2008)
9. (Lundgren, et al., 2009)
10. (Brough, et al., 2008)

2D structures of these inhibitors are shown in the attached PDF file. IC_{50} values are in μM.

HSP90 N-Terminus Receptor Grids Prepared:

Number of receptor grids prepared – 5

PDB ID Codes used:

1. 1YET - Geldanamycin Bound to the Hsp90 Geldanamycin-Binding Domain (Stebbins, et al., 1997).
2. 1OSF - Human Hsp90 in complex with 17-desmethoxy-17-N,N-Dimethylaminoethylamino-Geldanamycin (Jez, Chen, Rastelli, Stroud, & Santi, 2003).
3. 2BSM - Novel, Potent Small Molecule Inhibitors of the Molecular Chaperone Hsp90 Discovered Through Structure-Based Design (Brough, et al., 2008).
4. 2VCJ - 4,5 Diaryl Isoxazole Hsp90 Chaperone Inhibitors: Potential Therapeutic Agents for the Treatment of Cancer (Brough, et al., 2008).
5. 4B7P - Structure of HSP90 with NMS-E973 inhibitor bound (Fogliatto, et al., 2013).

Two other PDB ID codes that were found but their crystal structures were not used for docking were 2BT0 where CCT018159 was bound to HSP90 and 2VCI where NVP-AUY922 was bound to HSP90 (Brough, et al., 2008).

Table 2: Number of inhibitors prepared by LigPrep for analysis

NUMBER OF INHIBITORS PREPARED BY LIGPREP FOR DOCKING		
Group	Number of Inhibitors Submitted to LigPrep	Number of Inhibitors Prepared by LigPrep
Natural Derived Inhibitors	5	71
Synthetic Inhibitors	5	16
Pyrazole Inhibitors	4	32
Aminomethyl-Functionalized Diarylpyrazoles Inhibitors (AFDP Inhibitors)	15	153
Aminomethyl-Functionalized Diarylisoxazoles Inhibitors (AFDI Inhibitors)	12	55

DISCUSSION:

From the 41 inhibitors submitted to LigPrep, a total of 327 inhibitors were prepared by LigPrep. These were prepared in different pH solutions ranging from 5 to 9. As a result, the program prepared inhibitors that had different bond angles (folded structures), tautomers, and protonation and deprotonation states. Of the 71 inhibitors prepared from the natural group, 64 of them were derived from Alvespimycin (17-DMAG). This was due to the numerous protonation states in the nitrogen atoms attached to the carbon 17 on the main chain of the structure. Another group that had a large number of inhibitors prepared was the AFDP Inhibitors group (Table 2). The reason for this was because the inhibitors prepared for experimental analysis consisted of a common backbone structure with varying structures on the nitrogen atom situated on different carbons on the benzene ring (Brough, et al., 2008).

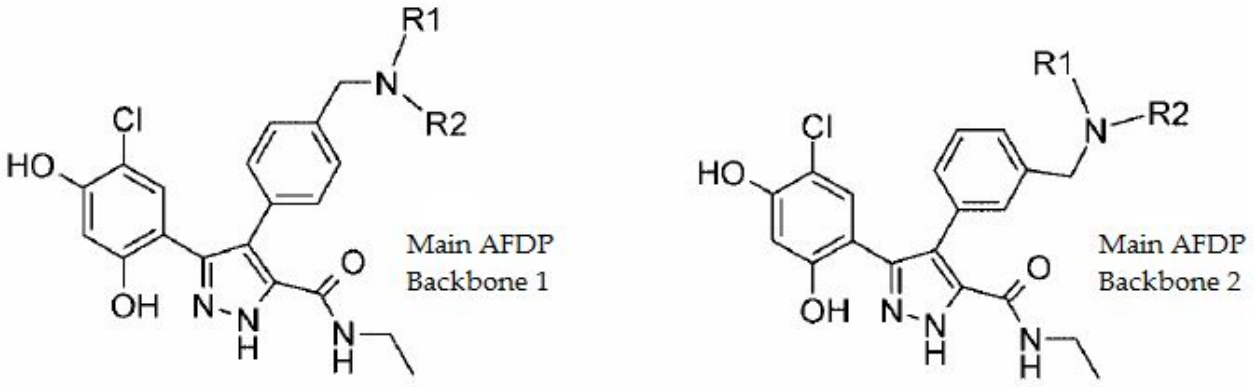

Figure 2: Backbones of Aminomethyl-Functionalized Diarylpyrazoles Inhibitors in which the R groups were modified (Brough, et al., 2008). The structure on the left was used to prepare AFDP inhibitors 1 to 6, while the second structure was used to prepare AFDP inhibitors 7 to 15.

The AFDP Inhibitors had so many structures prepared because of the varying tautomer ligands from these two backbone structures as well as the deprotonation states of the phenol groups. Likewise, some

of the groups attached to the R groups on the nitrogen had other nitrogen atoms and alcohol functional groups which were protonated and deprotonated respectively.

After performing computational docking of the inhibitors, the results produced gave ideas on how well the Glide Docking program docked the inhibitors on to the receptors, relationships between the Glide score and the IC_{50} of the values and possible structures and different states the inhibitors can exhibit when bound to the receptor grids. These results can be divided into four areas.

The first area looked at the effectivity of the Glide Docking program. To check this, the five ligands of known crystal structures were re-docked onto the same receptor grids (self-docking) that were prepared from proteins with PDB IDs. The PDB IDs that were used for this study were 1YET, 1OSF, 2BSM, 2VCJ and 4B7P. The ligands obtained from these codes were Geldanamycin (from 1YET), 17-DMAG (from 1OSF), VER-49009 (from 2BSM), AFDI Inhibitor 4 (from 2VCJ) and NMS-E973 (from 4B7P). Ligands of these compounds were prepared and re-docked on to their respective receptor grids (self-docking). Of the five ligands, four of them closely resembled their original crystal structures with VER-49009 giving the best result.

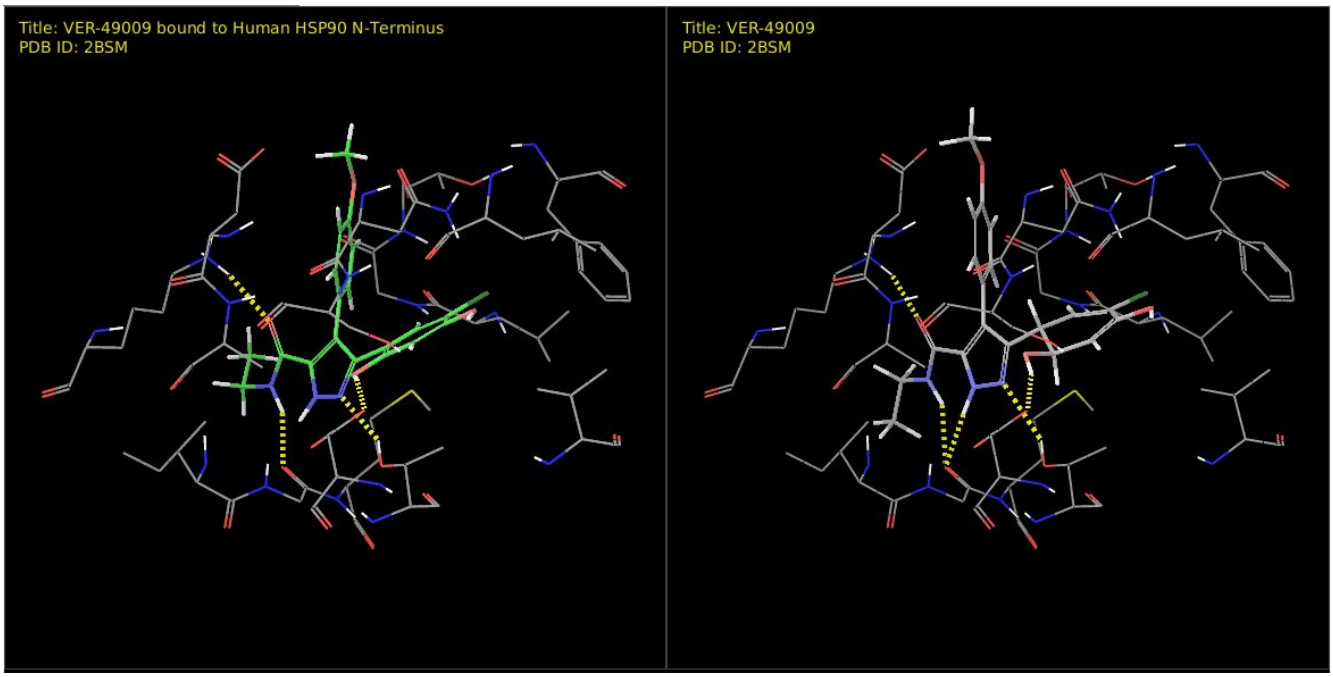

Figure 3: VER-49009 bound to N-terminus of HSP90. On the left is the PDB structure of the inhibitor and on the right is the result from self-docking. The ligand docked here had a low Glide score of -7.727.

Although the result for VER-49009 was really good, there was something interesting. The protonation species prepared by LigPrep that gave the best correspondence with the crystal structure is one in which the original aromatic ring on the right of the pyrazole is hydrogenated. A picture of the isolated ligand shows this (Figure 4).

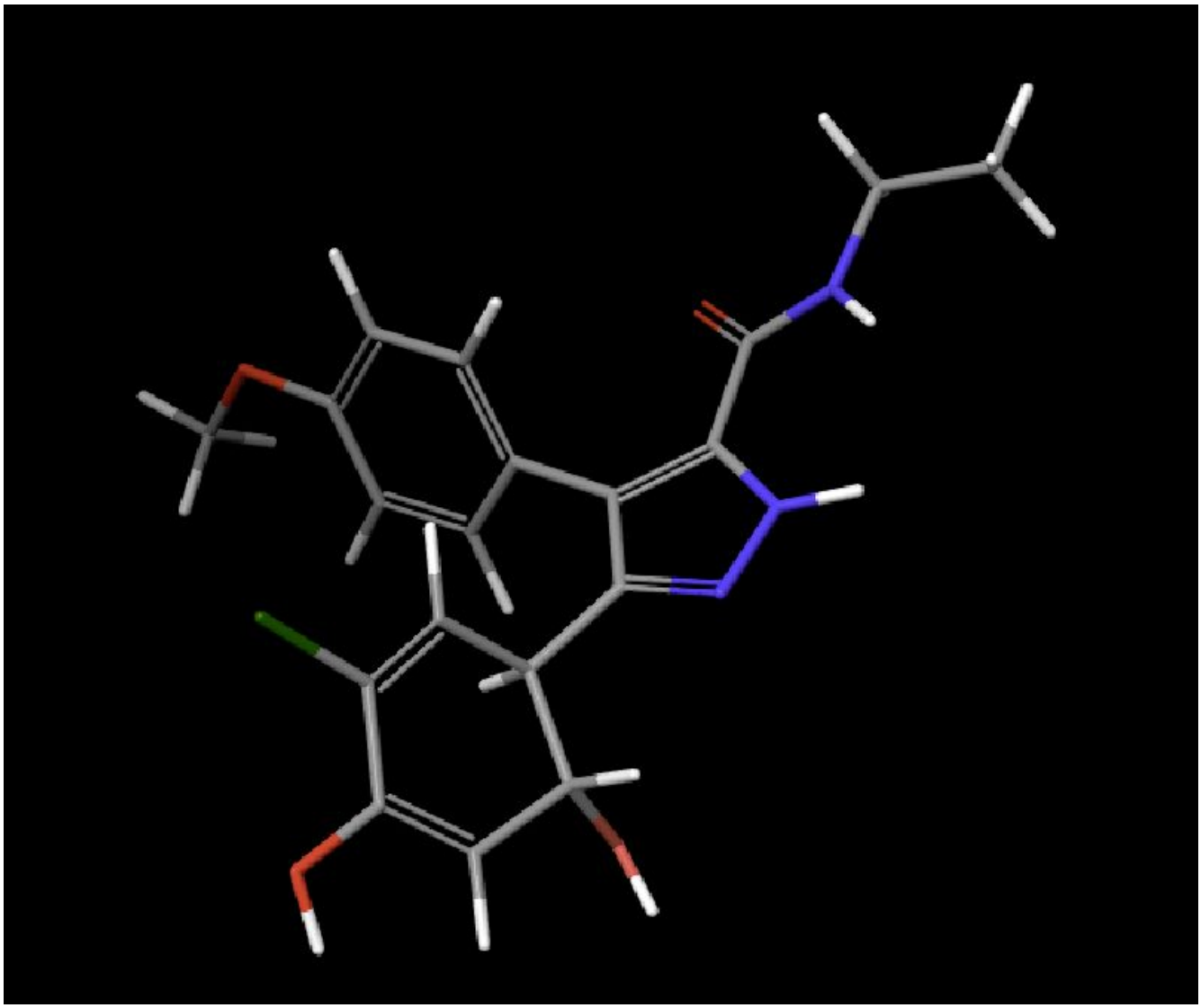

Figure 4: Close up diagram of VER-49009 inhibitor. Here the hydrogens of the aromatic ring with the chlorine atom are bonded singly. The double bond is not present as it was in the PDB structure. This implies that the ligand may not fully retain the aromatic structure when bound to the receptor.

Another good result was 17-DMAG binding on its receptor grid from 1OSF. The amine group on carbon 17 of the main chain was slightly shifted. The worst result was Geldanamycin when it was docked to its receptor grid. It was shifted completely. While many of the functional groups were positioned the same way, the difference for Geldanamycin might have been because of how Glide interpreted the ligand-receptor interactions.

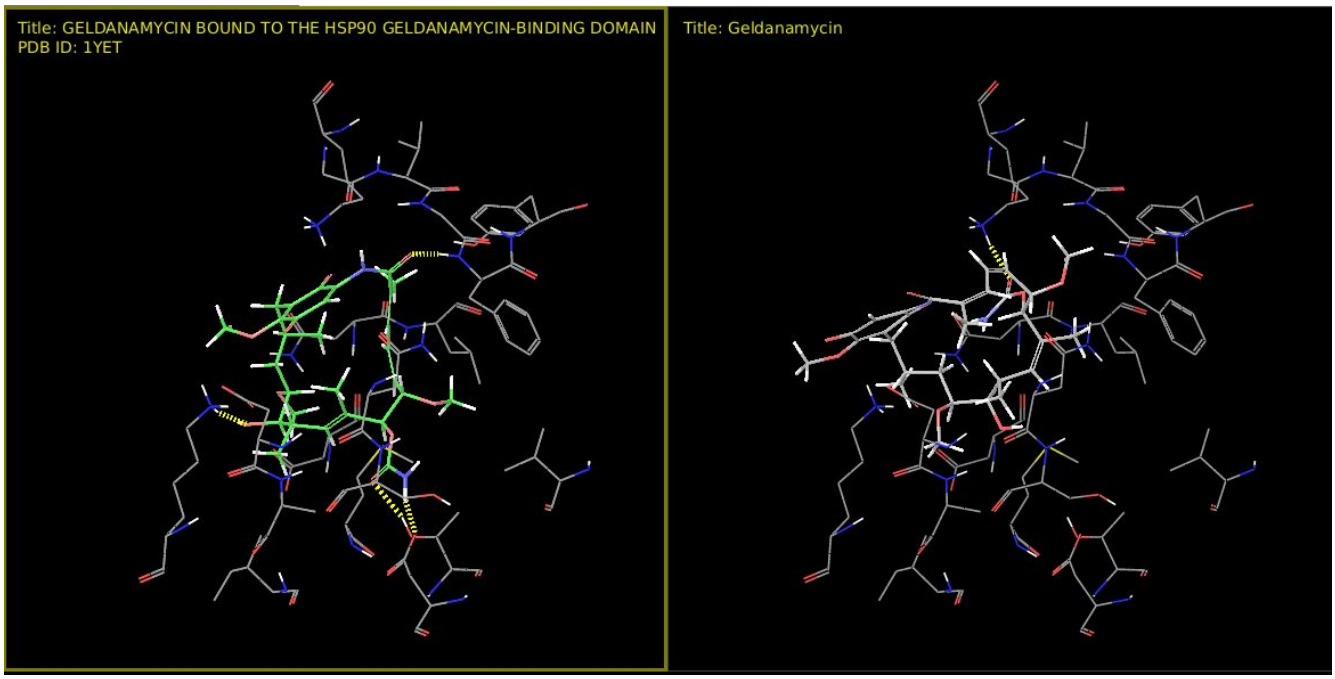

Figure 5: Geldanamycin bound to N-terminus of HSP90. On the left is the PDB structure of the inhibitor and on the right is the result from self-docking. The position of the docked ligand was completely different from that of the crystal structure. In the PDB structure, the inhibitor has three hydrogen bonds with the amino acids while on the self-docking structure, there is only one hydrogen bond present. The ligand was shifted completely.

Glide was then used to dock all of the prepared ligands on each of the processed receptor grids (cross docking). All of them were docked on each of the receptors with the exception of one. Geldanamycin was not docked on the receptor grid created from the 2BSM PDB ID code. That was due to Glide not being able find a way to dock the inhibitor on the receptor grid.

The second area focused on how well the glide scores related with the IC_{50} values of the compounds. It was expected that the lower the IC_{50} of the molecule, the better it would be for the molecule to bind and interact with the receptor and thus a low Glide score. However, this was not the case. There was no observed correlation between the two sets of data. One example of this was the docking of 17-AAG

and Geldanamycin on the 1OSF receptor grid. Geldanamycin had an IC_{50} of 0.281 with the lowest glide score of -4.007 while 17-DMAG had a glide score of -5.017 with an IC_{50} of 1.270. Similar results were seen with the other groups of ligands and the other receptor grids of the proteins. Table 3 summarizes some of these results.

Table 3: Glide Scores and FP IC_{50} for inhibitors bound to the receptor grid prepared from the 1OSF PDB structure

LOWEST GLIDE SCORES AND FP IC50 OF INHIBITORS			
Receptor from PDB ID code:			1OSF
Group	Inhibitor	Glide Score	FP IC_{50} (μM)
Natural Derived Inhibitors	Geldanamycin	-4.119	0.281
	Tanespimycin (17-AAG)	-4.571	1.27
	Alvespimycin (17-DMAG)	-8.409	0.058
	Herbimycin	-4.737	Unknown
	Radicocol	-5.511	0.06
Synthetic Inhibitors	NMS-E973	-6.675	< 0.010
	AT-13387	-6.817	0.018
	SNX2112	-6.327	0.03
	NVP-AUY922	-6.271	0.021
	BIIB021	-5.707	0.0017 (K_i Value)
Pyrazole Inhibitors	Pyrazole Inhibitor 1 (CCT018159)	-6.773	0.28
	Pyrazole Inhibitor 2 (VER-49009)	-6.553	0.025
	Pyrazole Inhibitor 3	-7.567	0.258
	Pyrazole Inhibitor 4	-6.788	0.6

The third area of analysis looked at the likely protonation states of the inhibitors bound to HSP90. Crystallographic structures generally do not have this information since hydrogen atoms can not be easily located. Docked ligands show that the ligands with amine groups and low Glide scores dock with a protonated nitrogen atom in the amine group. Overall, they have formal charges of +1. Alvespimycin (17-DMAG) is an example of this.

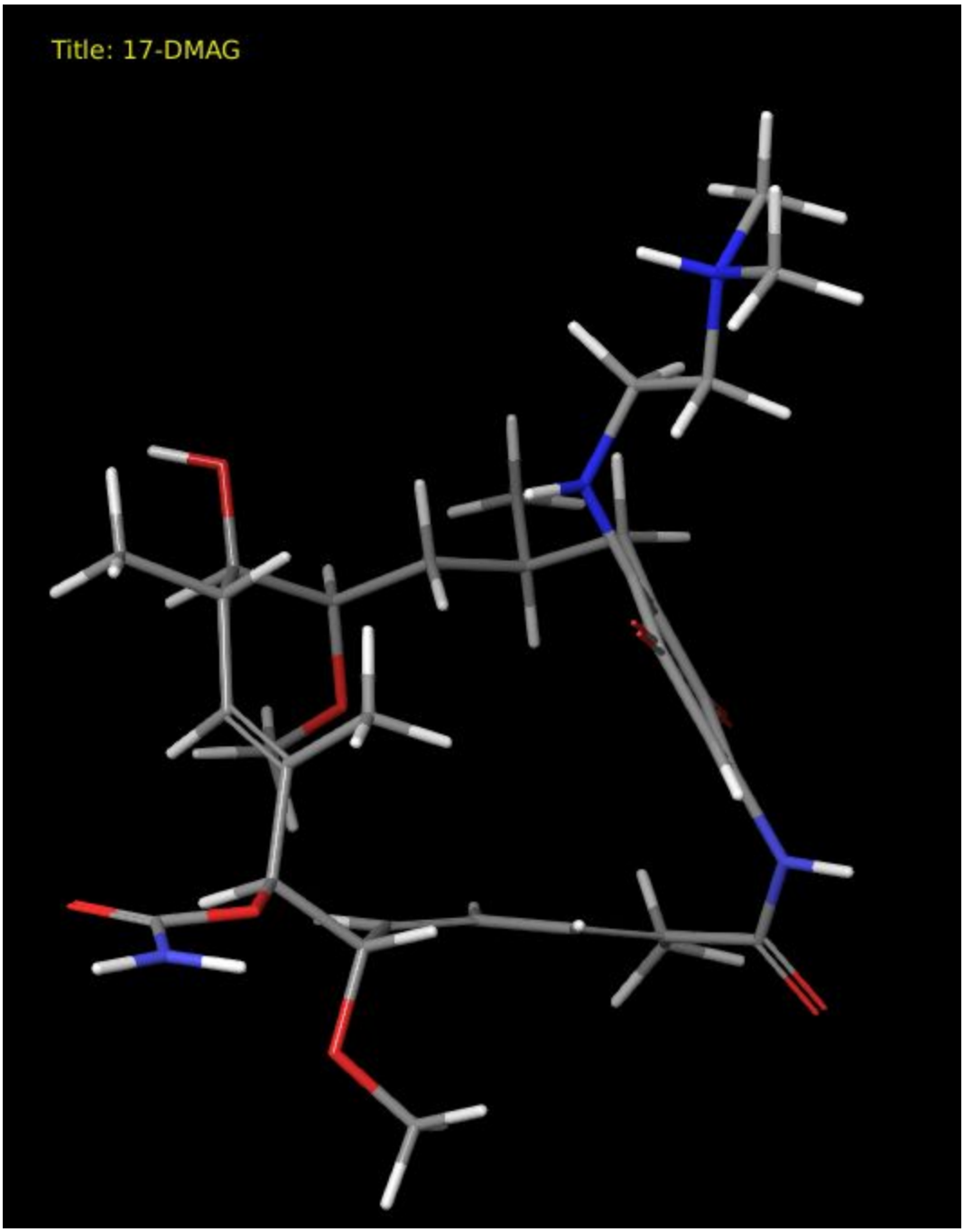

Figure 6: Protonated form of 17-DMAG. The protonated amine nitrogen is located on the upper right hand of the photo.

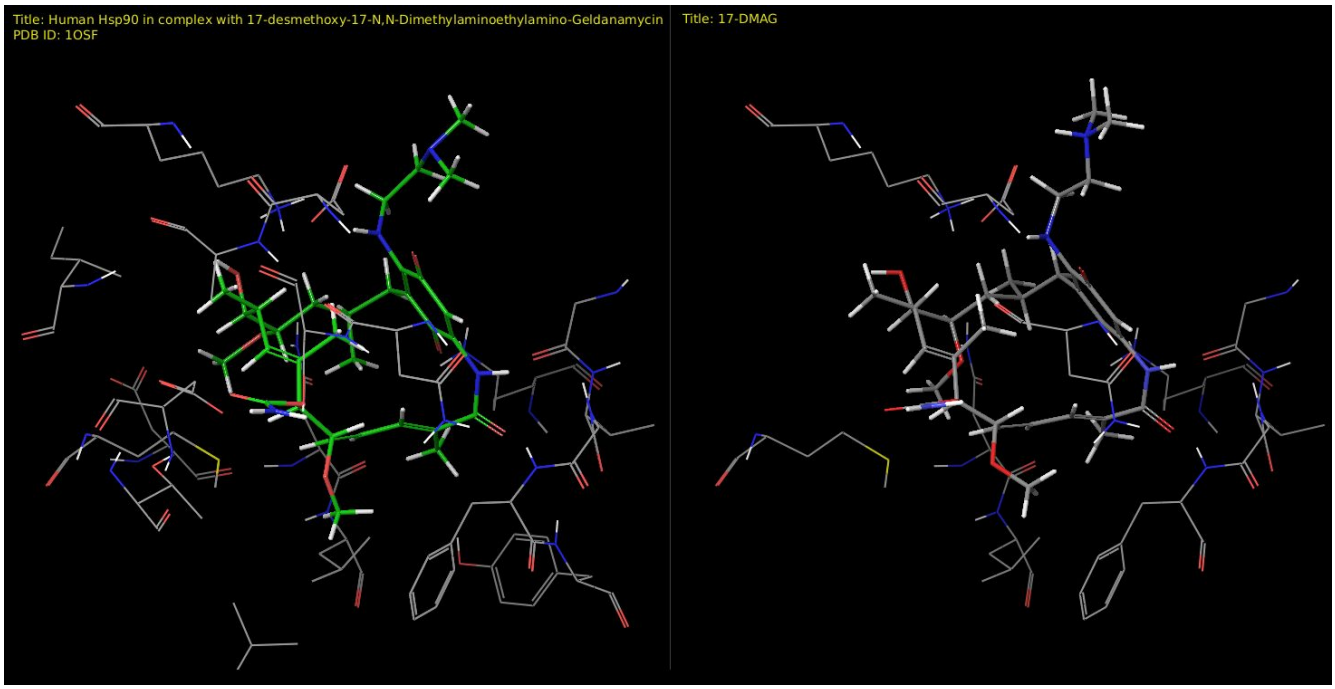

Figure 7: 17-DMAG bound to N-terminus of HSP90. On the left is the PDB structure of the inhibitor and on the right is the result from self-docking. The position of the docked ligand is the same as that of the crystal structure. In the PDB structure, the nitrogen at the upper right hand corner is deprotonated while on the right hand side, it is protonated.

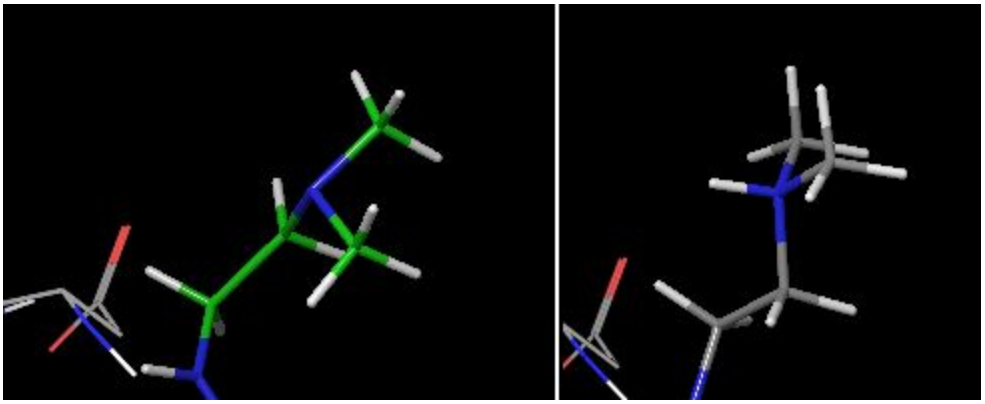

Figure 8: Close up of the nitrogen atom in the PDB structure (left) and the Glide docking structure (right).

The protonated nitrogen is possibly the reason for the shift in the docking position of the compound after self-docking.

The fourth and final area deals with the prediction of possible bound poses of inhibitor-HSP90 complexes with no available crystal structure. As several ligands were docked, there are several possibilities of the way the ligands can bind. One example of this is Herbimycin. This is also an HSP90 inhibitor derived from Geldanamycin. An interesting point to note here is that the compound undergoes three processes: deprotonation, tautomerization and reprotonation. It loses its hydrogen from the amine linkage. After losing the H atom, it forms a tautomer where there is a "–N=" bond that is formed between the carbon chains with the carbon double bonds to oxygen and the oxygen at the aromatic ring becomes a phenol group with the displaced hydrogen. It still retains an overall formal charge of 0.

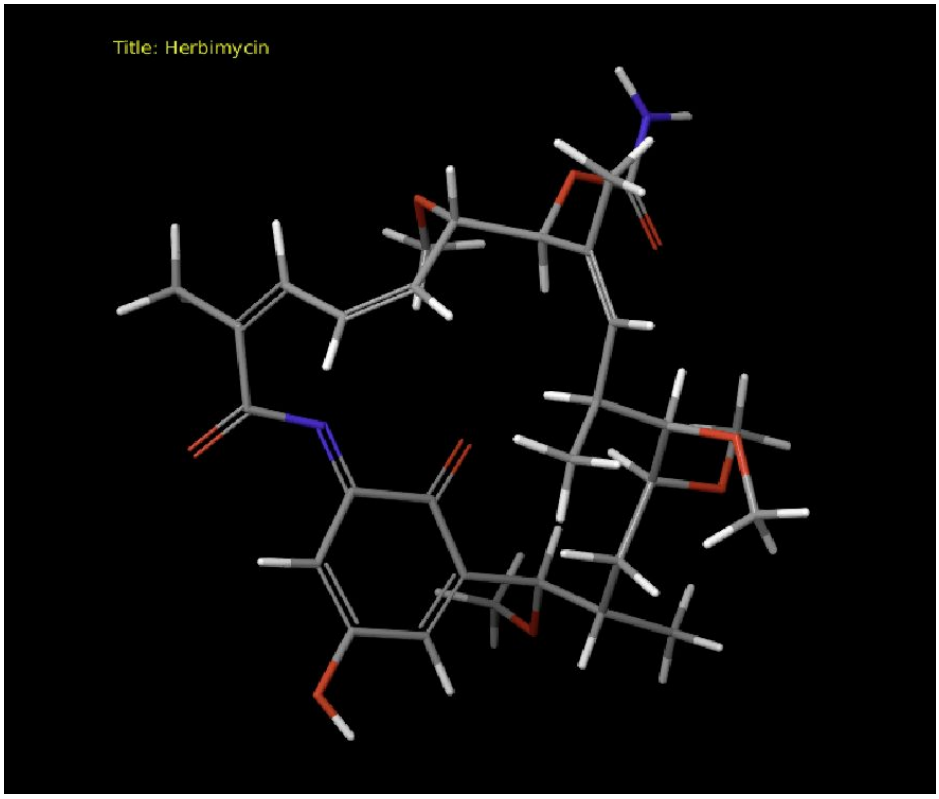

Figure 9: Tautomerized form of Herbimycin prepared by LigPrep. The "—N=" bond linkage can be easily seen.

The model therefore suggests that a tautomerized form of this compound binds HSP90. A possible bound structure of this compound is shown below.

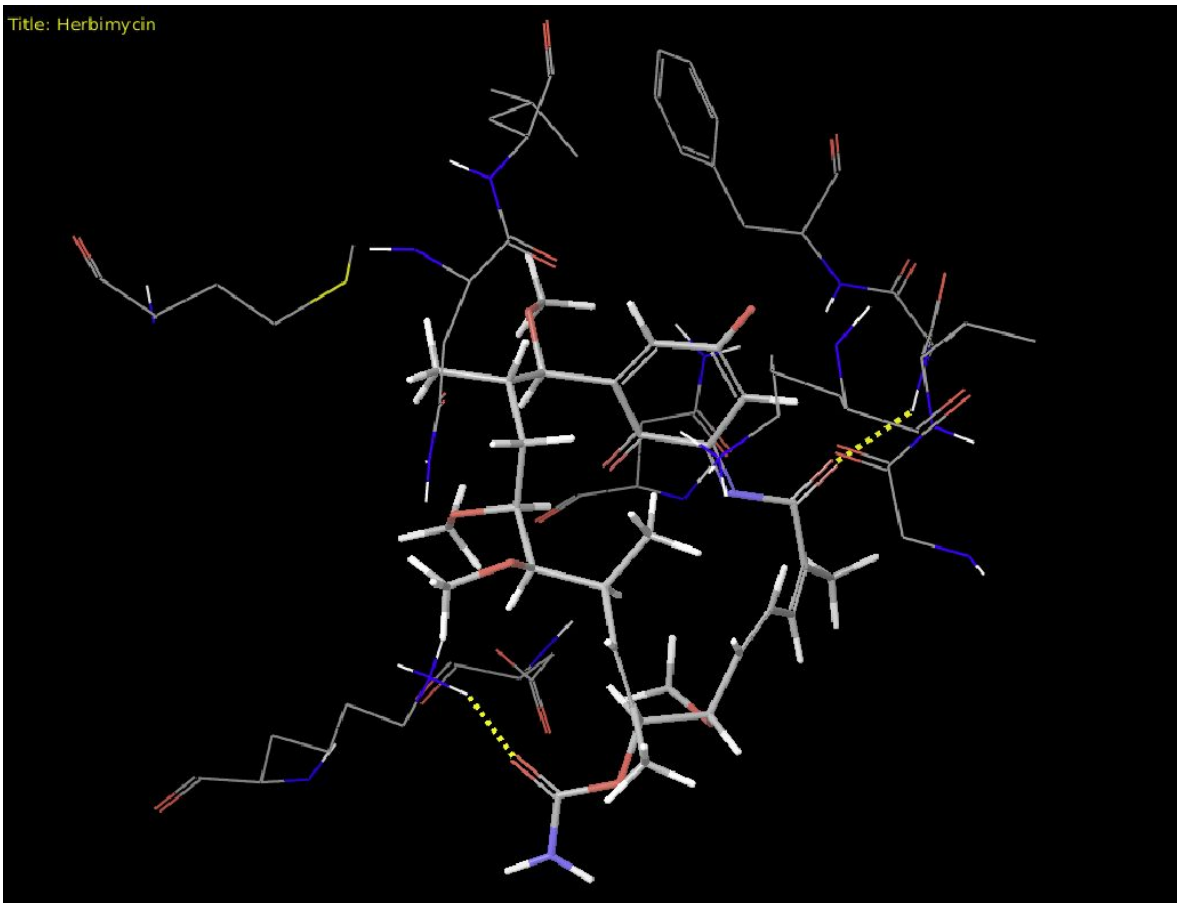

Figure 10: Prediction of the structure of Herbimycin bound to the N-terminus of HSP90. The hydrogen bonds between the inhibitor and the receptor can be easily seen.

CONCLUSION:

After carrying out the docking exercises, it can be seen that there are a lot of possible structures that can be made when a ligand is sent to LigPrep for preparations. This includes different protonation states, tautomers and folded structures. All of these can be docked unto different receptor grids prepared from known crystal structures. Glide gave good self-docking results for four of the five crystal structures. This means that Glide has done a good job in self-docking. Cross-docking experiments were less successful. An example of this is Geldanamycin not being docked on the receptor grid for 2BSM. Also, from docking the inhibitors, it can be seen that the low glide scores do not correspond with low

IC_{50} values. Inhibitors with high IC_{50} values like 17-AAG have lower Glide scores than those with lower IC_{50} values.

Crystal structures illustrate how a ligand docks to the HSP90 receptor. However, they do not directly inform on the protonation state of the bound ligand. When the ligands are isolated and the hydrogens are added, it means that the when it is time to be prepared (sent to LigPrep), it can form a state in which the ligand undergoes some process prior to docking. This gives one a better idea as to what happens to these inhibitors before they interact with the protein. This is seen when 17-DMAG is docked onto HSP90. It takes on a particular protonated state not easily predicted from the crystal structure.

Finally, Glide can give predictions on the pose of inhibitors bound to the receptor. This is particularly useful in cases where no experimental crystal structure is available. From analysis, it shows that the Herbimycin undergoes a special type of tautomerization which involves a deprotonation and protonation process that produces the "—N=" bond before binding onto HSP90.

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