Mebendazole’s Conformational Space and its Predicted Binding to Human Heat-Shock Protein 90

Walter Fiedler¹, Fabian Freisleben¹, Jasmin Wellbrock¹, and Karl N. Kirschner^{*2}

¹Department of Oncology, Hematology and Bone Marrow Transplantation with Section Pneumology, Hubertus Wald University Cancer Center, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany

²Department of Computer Science, Hochschule Bonn-Rhein-Sieg, University of Applied Sciences, 53757 Sankt Augustin, Germany

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Abstract

Recent experimental evidence suggest that mebendazole, a popular antiparasitic drug, binds to heat shock protein 90 (Hsp90) and inhibit acute myeloid leukemia cell growth. In this study we use quantum mechanics (QM), molecular similarity and molecular dynamics (MD) calculations to predict possible binding poses of mebendazole to the adenosine triphosphate (ATP) binding site of Hsp90. Extensive conformational searches and minimization of the five tautomers of mebendazole using MP2/aug-cc-pVTZ theory level resulting in 152 minima being identified. Mebendazole-Hsp90 complex models were created using the QM optimized conformations and protein coordinates obtained from experimental crystal structures that were chosen through similarity calculations. Nine different poses were identified from a total of 600 ns of explicit solvent, all-atom MD simulations using two different force fields. All simulations support the hypothesis that mebendazole is able to bind to the ATP binding site of Hsp90.

1 Introduction

Heat shock protein 90 (Hsp90) is a chaperone protein that enables the functionality of numerous client proteins by facilitating their maturation and stabilizing their protein structure [1, 2]. Signalling proteins switch between conformational states depending on their activation. Due to this intrinsic structural instability, proteins being essential for the cell’s signalling network, such as kinases, hormone receptors, and transcription factors, are particularly dependent on HSP90 [3]. Dysregulation of cellular signalling networks is a central characteristic of cancer cells [4] and many client proteins of Hsp90 are critically involved in oncogenesis [5]. Accordingly, Hsp90 has been indicated to play a role in cancer [6–9]. Altered expression levels of Hsp90 have been found in patients with medulloblastoma [10] and breast cancer [11], while binding studies in cells show growth inhibition of various tumors [12–15]. The development of resistance is a major obstacle in cancer therapy [16]. As a highly specialized chaperone, Hsp90 has found to be crucial in enabling adaptability and heterogeneity of cancers [17] and growing evidence suggests Hsp90 inhibitors might be the key in limiting the ability of cancer cells to adapt and evolve drug resistance [16, 17].

Of specific interest is Hsp90’s N-terminal adenosine triphosphate (ATP) binding site [18] that shows the ability to bind many ligands. A substantial body of research exists that computationally investigates this site, primarily with the goal of understanding the binding of ligands [19–26] or for predicting new active compounds [27–38] for further study. It was also the focus of Drug Design Data Resource’s first Grand Challenge [39–46].

Mebendazole (methyl 5-benzoyl-2-benzimidazole-carbamate) is an established antiparasitic drug that has been indicated as a possible repurposing candidate for cancer treatment [47–53], including acute myeloid leukemia (AML) [54–56]. Mebendazole has been used as a medicine for several decades, has a well established pharmacology [57] and is listed as an essential medicine by the World Health Organization [58]. Experimentally, four small-molecule crystal structures of mebendazole exist in the Cambridge Crystallographic Data Centre database [59] that provide unique conformations for one protonated [60] and three neutral [61–63] species. An experimental crystal structure of mebendazole bound to a protein has never been reported. However, few computational predictions have been made for mebendazole’s binding to various proteins [64–67].

*Corresponding author: karl.kirschner@h-brs.de

Recently, Fiedler and colleagues experimentally showed that mebendazole is a potent Hedgehog signaling inhibitor that acts by inducing proteasomal degradation of glioma-associated oncogene (GLI) transcription factors [56]. GLI transcription factors drive a genetic program being fundamental for the pathophysiology of leukemia initiating cells that are responsible for therapy failure and tumor relapse due to their chemotherapy resistance [68]. Targeted inhibition of GLI1 and GLI2 mediates anti-leukemic effects in vitro and in vivo. Moreover, high GLI1 and GLI2 expression has been shown to represent a negative prognostic marker in AML [69]. Specifically, there is strong evidence that mebendazole binds to Hsp70 or Hsp90, and consequently inhibits their chaperone activity that is needed for maintaining the protein stability of GLI transcription factors and preventing their degradation. This mebendazole-induced inhibition leads to strong, does-dependent anti-leukemic effects on AML cells. In this contribution, motivated by this recent experimental finding, we predict the binding and dynamics of mebendazole to the ATP site of Hsp90 using quantum mechanics and molecular dynamics simulations. The resulting data indicates that mebendazole forms a stable interaction with Hsp90, with the likelihood of adopting different poses within the binding pocket.

2 Methodology

2.1 Quantum Optimization

Mebendazole Conformations There are five possible tautomers for mebendazole, as shown in Figure 1, which result in a large possible conformational space that needs sampling. Mebendazole can be separated into three distinct chemical units: a carbamate, an imidazole and a biphenyl ketone (i.e. benzophenone). The tautomerization occurs within the carbamate and imidazole groups. Mebendazole tautomers 1–3 have five flexible torsion angles, (Figure 2, labeled a-e) that define the conformational space to be explored. An additional torsion angle (labeled f) is required for tautomers 4 and 5 due to the alcohol functionality. Conceptually, two sets of iterations were performed for generating conformations. The first set consisted of setting [d,e] torsions to $[150^\circ, 150^\circ]$ and to $[-150^\circ, -150^\circ]$, and subsequent iterations through the remaining torsions. Analysis of the resulting geometries allowed us to reduce the angles sampled for the creation of the second set. For example, it was observed that torsion c sampled only 0° and 180° for tautomer 3, allowing us to remove the $\pm 60^\circ$, $\pm 90^\circ$, $\pm 120^\circ$ from its list. These torsion angles were systematically set to all possible combination using the values given in Table 1.

Table 1. Torsion angles (degrees) used for creating input conformations for QM optimizations.^a

Torsion	Set 1	Set 2
a	0, ± 60 , ± 90 , ± 120 , 180	0, ± 60 , ± 120 , 180
b	0, ± 60 , ± 90 , ± 120 , 180	0, ± 60 , ± 120 , 180
c	0, ± 60 , ± 90 , ± 120 , 180	0, 180
d	± 150	± 35
e	± 150	± 30
f	0, ± 60 , ± 90 , ± 120 , 180	0, ± 60 , ± 120 , 180

All input conformations were optimized using HF/6-31G(d) theory [70]. Frequency analyses were subsequently performed to verify that the resulting structures were potential energy minima. All structurally unique minima were further optimized at MP2/aug-cc-pVTZ (AVTZ) theory level [71,72], correlating only the valence electrons. Density-fitted algorithm [73–75] was utilized with the appropriate corresponding auxiliary basis sets [76,77]. All optimizations were performed using C1 molecular symmetry and until the following convergence criteria were met (i.e. gau.tight): Max. Force: 1.5×10^{-5} , RMS Force: 1.0×10^{-5} , Max. Disp.: 6.0×10^{-5} , RMS Disp.: 4.0×10^{-5} . The Psi4 software (v. 1.2.1) was used for all QM calculations [78].

2.2 Identification and preparation of protein targets and their bound ligands

The Protein Data Bank (rcsb.org) [79,80] was searched for HPS90 proteins, specifying X-ray structures only and human organism as the material source. All associated ligands found by the search were downloaded (a single SDF-formatted file) applying the default setting of RCSB (e.g. no hydrogen, exclude "instances with missing heavy atoms"). This list was further curated by removing small-sized and transition metal containing ligands (i.e. magnesium ions, chloride ions, sodium ions, nickle (II) ions, calcium ions, phosphate ion, sulfate ion, carbonate ion, acetate ions, acetic acid, formic acid, thiocyanate ion, dimethyl sulfoxide, ethylene glycol,

Figure 1: The five tautomers of mebendazole [62].

Figure 2: The flexible torsion angles (i.e. a–f) of mebendazole, represented by tautomers 1 and 4. The bond sequence used for defining the torsions are shown with thicker lines. Note that torsions a and b are defined using the same atoms for all tautomers.

ethane-1,2-diol, glycerol, cobalt hexamine (III)). This resulted in a final selection of 300 ligands. The resulting crystal and ligands ID codes can be found in Tables S1 and S2.

Ligand Preparation Open Babel [81, 82] was used to separate the crystallized ligands and convert them to PDB-formatted files. Hydrogen atoms were automatically added using either Open Babel or PyMOL [83]. Manual inspections were subsequently done and adjustment were made to correct any improperly add hydrogens. Adjustment were not made to assigned tautomer, or for correcting nitrogen hybridization (planar vs pyramidal).

Protein Preparation MolProbity was used to analyze, modify side-chain orientations (i.e. ASP, GLU, HIS) and add hydrogen to the protein crystals that were used in the MD simulations (i.e. 2WI6, 2BT0 and 4W7T;

see Results section) [84–87].

2.3 Similarity

To compute the similarity, one requires reliable geometries and electrostatics. AMBER’s antechamber program was used to generate AM1-BCC partial atomic charges for both crystallized ligands and the mebendazole tautomers - thus, providing a consistent methodology for electrostatic comparison. For mebendazole the MP2/AVTZ geometries were used, while for the crystallized ligands AM1 optimizations were performed. For ligands containing phosphate functional groups, a formal molecular charge of -2 was assigned. All other ligands were individually assigned a charge state of 0, -1 or +1. The ShaEP program was used to compute all pairwise similarities [88].

2.4 Molecular Mechanics and Dynamics

Partial Atomic Charges The partial atomic charges for the tautomer 1 and 2 of mebendazole were separately computed using the RESP [89,90] methodology as implemented in Psi4 [91]. Specifically, Connolly molecular electrostatic surfaces were generated at the HF/6-31G(d) theory level, using the MP2/AVTZ optimized geometries. A two-stage procedure was used. In the first stage, a 0.0005 a.u. restraint was used without enforcing charge equivalency between rotationally equivalent atoms. In the second stage aliphatic carbons and their hydrogens were refitted using a 0.001 a.u. restraint, and charge equivalency was enforced (i.e. phenyl ring atoms, methyl hydrogens). During both stages, four conformations were used with intermolecular constraint to ensure that the resulting single partial atomic charge set optimally reproduce all four conformations. For each tautomer, the four conformations with the lowest unique relative energies (i.e. conformations 1, 3, 5 and 7) were used (see Table 2) to compute the final averaged charge set. The resulting partial atomic charges can be found within the two mol2 files of SI material.

Molecular Dynamics The ff14SB [92] and FB15 [93] force fields were used to model the protein, while the gaff2 [94] force field was used for modeling mebendazol. For the validation of gaff2, the MP2/AVTZ optimized geometries were used as input into the gas-phase molecular mechanics optimizations. Explicit water periodic box simulations were also performed using the TIP3P and force balance water models, as recommended for use with the ff14SB and FB15 protein force fields, respectively. The SLTCAP software was used to compute the number of ions need for modeling a biological salt concentration of 150 mM [95]. In total, 32 Cl⁻ and 30 Na⁺ atoms, plus 9844 (2bt0 model) to 10121 (2wi6) water molecules, were added to the models using AmberTools19’s tLEaP algorithm [96]. Minimization were performed first by constraining (500.0 kcal·mol⁻¹·Å² harmonic constraint) the protein and mebendazol, followed by a minimization of the entire model to a convergence criterion of 0.5 kcal·mol⁻¹·Å⁻¹.

The models were heated from 0–310 K in 1.0 ns under constant volume conditions, followed by a 5 ns constant pressure equilibrium simulation using a Berendsen barostat and isotropic position scaling. Constant volume production simulations were subsequently ran for 110 ns. Langevin dynamics [97] with a collision frequency (i.e. gamma_{ln}) of 1.0 ps⁻¹ [98] was used for temperature regulation. A nonbonded cutoff of 8.0 Å was used in all explicit simulations, with Particle Mesh Ewald [99] used to for modelling the nonbonded interactions at longer distances. Covalently bound hydrogen atoms were constrained using the Shake algorithm [100]. A random number generator (i.e. ig=-1) was used to create the seed value for all Langevin dynamics. A 2 fs per iteration time step was employed. Molecular dynamics simulations were performed using AMBER16’s CUDA version of PMEMD [101,102].

MD Analysis The last 100 ns of each MD trajectory was analyzed using CPPTRAJ (v4.25.6) [103], with frame alignment performed via an RMSD calculation of the protein’s heavy atoms. Waters that interact with mebendazole were identified using CPPTRAJ’s ‘watershell’ command, with a default cutoff distances of 3.4 Å specified. The mean of the resulting number of waters was then used to remove all but the closest water using the ‘closest’, ‘outtraj’ and ‘parmsstrip’ commands.

Root-mean-squared fluctuations (RMSF) were computed for the protein’s backbone heavy-atoms and a mass-weighted average was done to provide a single value per amino acid (i.e. CPPTRAJ’s ‘atomicfluc’ command with ‘byres’ specified). The secondary structure was computed for each trajectory using the Define Secondary Structure of Proteins (DSSP) method [104] (i.e. CPPTRAJ’s ‘secstruct’ command).

Cluster analyses were performed on RMSD protein-aligned trajectories that clustered the mebendazole position within the binding pocket. Hydrogen atoms were excluded from the alignment and clustering, as well as 2–4 amino acids at the proteins’ termini due to their high fluctuations. Six independent hierarchical agglomerative clustering analyses were performed on each trajectory using integer epsilon values that incrementally ranged from 2.0 to 7.0. Based on their resulting pSF (pseudo-F statistic) [105] – as measure for clustering quality – the results using the best epsilon value for a given trajectory were focused upon. The resulting binding-pose

trajectories were subsequently used for the remaining analyses. The exception to this are the 4W7T trajectories where the clustering did not yield meaningful results (see below), and thus their last 100 ns were used.

The amino acids that closely interact with mebendazole in the binding pocket were identified using CPPTRAJ’s ‘mask’ command and a heavy-atom cutoff 3.4 Å. The resulting list was filtered to identify only the amino acids that interacted with mebendazole for at least 50% of the frames for a given trajectory.

Spatial distribution analyses were performed on using the ‘grid’ command, which creates a histogram of the spatial positioning of desired molecular species using a defined gridded cube. A cell spacing of 0.5 Å was specified and the resulting density was normalized. Spatial distributions were analyzed for the following three chemical groups: mebendazole, binding pocket amino acids and waters. Care was taken to ensure that analyses were performed specifically on trajectory portions that correspond to the most populated ligand binding motifs.

The binding energies (ΔE_{bind}) of mebendazole to Hsp90 were estimated through Molecular Mechanics/Poisson Boltzmann Surface Area (MM/PBSA) calculations using the MMPBSA.py [106] program. In all MM/PBSA calculations the entropy ($T\Delta S$) was not computed, and thus our binding energies take the form $\Delta E_{\text{bind}} = \Delta E_{\text{MM}} + G_{\text{Solv}}$, where ΔE_{MM} is the molecular mechanics energy difference between the complex and uncomplexed species. For each pose originating from the 2WI6 and 2BT0 simulations, ΔE_{bind} was computed using 100 equally spaced pose-specific frames, as identified during the cluster analysis. For the poses originating from the 4W7T simulations, the last 70 ns of the trajectories were used as input. Note that the minimum possible time separating the sampled trajectory frames was 168 ps, which occurred 2BT0-f14SB pose 1. MM/PBSA was performed using both force field, which gave identical results. All water and salt ions were removed from the frames before computing ΔE_{bind} . An implicit ionic strength of 0.1 mM was specified in all MM/PBSA calculations, the atomic radii were set to mbond2 [107], and with all other parameters set to MMPBS.py’s default values.

3 Results

3.1 QM Mebendazole Optimizations

Since all 5 tautomers of the drug (Figure 1) are possible candidate for binding to a protein’s active site, conformational searches and subsequent QM optimizations were performed on all tautomers. All energetically unique structures occur in conformational isomeric pairs (see Table 2) that primarily arise from the different carbamate orientations relative to the benzophenone functionality. To clearly identify the conformations with respect to the tautomers, we will use the following nomenclature: $T_{\text{conf. num.}}^{\text{taut. num.}}$.

The gas-phase conformational search using HF/6-31G(d) theory resulted in 94 pairs of conformational isomers (i.e. 188 spatially unique conformations). Subsequent MP2/AVTZ optimizations resulted in a total of 76 isomer pairs, whose relative energies are provided in Table 2. The more stable MP2/AVTZ conformations (i.e. < 5 kcal·mol⁻¹) occur when mebendazole exists in its tautomer 1 (T^1) and 2 (T^2) form. In comparison to the three crystal structures of neutral mebendazole, T_1^2 and T_2^2 , the most stable conformation pair, corresponds to Caira [61], Ferreira [63] and coworkers’ crystal structures. In contrast, T_1^1 , one conformation from the second most stable pair, corresponds to Martins et al.’s crystal structure [62].

The agreement in geometries between the crystal and MP2/AVTZ structures are very good as seen in Table 3’s RMSD values. The poorest agreement is between T_1^2 and the oldest crystal structure, published in 1998, with a heavy-atom RMSD of 0.24 Å. For T_2^2 and T_1^1 , their structures have heavy-atom RMSD values of 0.07 and 0.08 Å to their respective crystal conformation. Collectively, the MP2/AVTZ results clearly demonstrates the complexity of the potential energy surface (PES) that mebendazole resides upon, with 152 unique conformations whose relative energies can extend 32 kcal·mol⁻¹ above the global minima. It is encouraging to see that the conformations of the most stable minima computed using a moderate electron correlation level with an extensive basis set coincides with experimental solid-state results, with very good agreement in the geometries themselves.

3.2 Similarity Calculations

The similarity between all mebendazole tautomers and their QM optimized conformations were computed (11,476 pairwise calculations), whose resulting index values (max=0.92; min=0.45; avg=0.65) are shown in Figure 3. This figure illustrates the wide variety of geometric and electrostatic topologies that mebendazole could present to a protein for binding.

Since we have computed an extensive collection of reliable mebendazole conformations and 287 human Hsp90 crystal structures with diverse ligands bound to its ATP site, molecular similarity can also be used a) to make initial predictions for how mebendazole might bind to Hsp90 and b) to create initial structures for MD simulations. Towards these goals, the similarity index between the eight most stable mebendazole conformations (i.e. T_{1-4}^1 , T_{1-4}^2 ; Table 2) and the 300 ligands extracted from 287 crystal structures were computed. The choice to use the eight most stable mebendazole conformations, whose relative energies are within 0.6 kcal·mol⁻¹, was made based on the assumption that they are the most likely ones to be populated at body temperature.

Table 2. Relative conformational energies (kcal·mol⁻¹) across all tautomers computed at the MP2/AVTZ theory level.

Taut. 1		Taut. 2		Taut. 3		Taut. 4		Taut. 5	
Conf.	Rel. E.	Conf.	Rel. E.	Conf.	Rel. E.	Conf.	Rel. E.	Conf.	Rel. E.
1, 2	0.166	1, 2	0.000	1, 2	5.152	1, 2	8.697	1, 2	8.551
3, 4	0.610	3, 4	0.272	3, 4	5.351	3, 4	9.147	3, 4	8.936
5, 6	3.820	5, 6	3.465	5, 6	5.420	5, 6	10.906	5, 6	10.809
7, 8	4.352	7, 8	3.932	7, 8	5.498	7, 8	11.215	7, 8	11.100
9, 10	7.370	9, 10	7.296	9, 10	7.170	9, 10	21.644	9, 10	21.476
11, 12	7.725	11, 12	7.450	11, 12	7.488	11, 12	21.983	11, 12	21.858
13, 14	17.177	13, 14	16.967	13, 14	7.569	13, 14	22.323	13, 14	21.867
15, 16	17.311	15, 16	17.072	15, 16	7.605	15, 16	22.585	15, 16	22.238
17, 18	17.830	17, 18	17.459	17, 18	11.003	17, 18	23.308	17, 18	23.186
19, 20	17.945	19, 20	17.535	19, 20	11.093	19, 20	23.325	19, 20	23.208
				21, 22	11.297	21, 22	23.495	21, 22	23.296
				23, 24	11.356	23, 24	23.585	23, 24	23.336
						25, 26	23.792	25, 26	23.554
						27, 28	23.835	27, 28	23.574
						29, 30	23.955	29, 30	23.697
						31, 32	24.304	31, 32	23.794
						33, 34	25.194	33, 34	25.106
						35, 36	25.427	35, 36	25.262
						37, 38	25.617	37, 38	25.437
						39, 40	25.835	39, 40	25.619
						41, 42	31.760	41, 42	31.718
						43, 44	32.262	43, 44	31.997

Table 3. All-atom (heavy-atom) RMSD between MP2/AVTZ optimized and experimental mebendazole structures for equivalent conformations.

	T ₁ ¹	T ₁ ²	T ₂ ²
Crystal (1998) [61]	0.265 (0.236)		
Crystal (2009) [62]	0.126 (0.071)		
Crystal (2010) [63]	0.265 (0.077)

Figure 3: Similarity indexes (structure & electrostatic) computed between all MP2/AVTZ//MP2/AVTZ mebendazole conformations.

Figure 4: The structure overlay of mebendazole (brown) with the resulting three most similar Hsp90 ligands (gray) as determined by Shape (index=0.86), Electrostatic (index=0.59) and Shape & Electrostatic (index=0.69) criteria. Apart from the imidazole's hydrogen, only the heavy atoms are displayed for simplicity.

Figure 5: An RMSD overlay of the 2WI6 (orange), 2BT0 (purple), and 4W7T (blue-green) N-terminal domain of Hsp90. The ATP binding site with mebendazole (tautomers 1 and 2) replacing the crystal ligands as specified from the similarity calculations. The surface colors highlight the amino acids that are within 4 Å (i.e. half of the MD nonbonded cutoff) of mebendazole. Shown are the mebendazole and protein structures with hydrogen atoms added and after molecular mechanics minimization.

The resulting indexes reveal that the most similar structures are T_3^2 and 2WI6's ligand ZZ6 (index=0.86), T_2^1 and 2BT0's ligand CT5 (index=0.59), and T_2^1 and 2BT0's ligand CT5 (index=0.59), respectively based on 1) shape, 2) electrostatic and 3) shape & electrostatic criteria. The resulting structural overlay of mebendazole conformations with 2WI6, 2BT0 and 4W7T's ligands can be seen in Figure 4.

2WI6 [13], 2BT0 [108] and 4W7T [109] are well resolved crystal structure with resolutions of 2.18, 1.90 and 1.80 Å, and have chemically distinct ligand bound to Hsp90's ATP binding site [18]. A heavy-atom RMSD fit of these three crystallized proteins show that the structures are within 0.96–1.51 Å of one another, indicating that the overall protein structures are similar. Even though the ligands bind to the ATP binding pocket within in each protein, the binding pockets' topologies differs substantially (Figure 5).

Using the resulting coordinates from the similarity calculations and replacing the crystallized ligand with the corresponding QM optimized mebendazole's conformations, one can obtain a prediction of how mebendazole might bind to Hsp90. As seen in Figure 5, the resulting "docked" mebendazole occupies a different starting orientation within each binding pocket, and consequently forms different nonbonded contacts with different amino acids. The mebendazole's tautomers 1 and 2 have the carbamate functionality pointing out of the 2BT0 and 2WI6 binding pockets, respectively, while for 4W7T the tautomer 1 is rotated ca. 180° and is deeply inserted into the pocket. These three resulting mebendazole-Hsp90 protein complexes were used as the starting structure for all-atom MD simulations.

3.3 Mebendazole's Force-Field Validation

Using the QM relative energies for mebendazole's tautomer 1 and 2, we can gauge how well Gaff2 performs at modeling the drug's conformational space. As seen in Table 4, Gaff2 reproduces the relative stability of the low energy conformations very well, with a maximum deviation of $-0.23 \text{ kcal}\cdot\text{mol}^{-1}$ (T_3^1 & T_4) occurring for conformations that have a relative energy of $< 3.4 \text{ kcal}\cdot\text{mol}^{-1}$. For the conformations with MP2/AVTZ relative energies between 3.4 – $7.4 \text{ kcal}\cdot\text{mol}^{-1}$, Gaff2 performs less well with deviations ranging from 2.6 – $4.8 \text{ kcal}\cdot\text{mol}^{-1}$.

Table 4. Relative molecular mechanics conformational energies^a (kcal·mol⁻¹) for tautomers 1 and 2, along with selected QM theory levels. Relative values are computed independently for each tautomer. (Conformation numbers are currently ordered according to the raw data - this will change. Differences are between Gaff2 and AVTZ results.)

Tautomer 1				Tautomer 2			
Conformation	MP2/AVTZ	Gaff2	Diff. ^b	Conformation	MP2/AVTZ	Gaff2	Diff. ^b
1, 2	0.000	0.000	0.000	1, 2	0.000	0.000	0.000
3, 4	0.443	0.209	-0.234	3, 4	0.272	0.403	0.131
5, 6	3.654	8.198	4.544	5, 6	3.465	8.025	4.560
7, 8	4.186	8.577	4.391	7, 8	3.932	8.766	4.834
9, 10	7.204	10.142	2.938	9, 10	7.296	10.257	2.961
11, 12	7.559	10.149	2.590	11, 12	7.450	10.498	3.048
13, 14	17.011	16.659	-0.352	13, 14	16.967	16.421	-0.546
15, 16	17.144	16.659	-0.485	15, 16	17.072	16.421	-0.651
17, 18	17.663	17.195	-0.468	17, 18	17.459	17.318	-0.141
19, 20	17.779	17.195	-0.584	19, 20	17.535	17.318	-0.217
Avg. of Abs. Diff. ^c			1.843				1.899
Tautomer Diff. ^d		0.166	7.762			0.000	0.000

^a All relative energies were computed from full optimizations performed at the specified theory levels.

^b Energy difference computed between Gaff2 and MP2/AVTZ relative energies.

^c Averages of the absolute differences are computed using the data show, but excludes the global minimum of each tautomer.

^d The energy difference between the respective global minimum of tautomer 1 (i.e. conformations 1 and 2) and 2 (i.e. conformations 1 and 2).

For the highest energy conformations, Gaff2 is unable to reproduce several of the minima found by MP2/AVTZ (i.e. multiple input geometries are optimized to the same minima by Gaff2). Therefore, concerning the force field’s performance in MD simulations, the low energy conformations are expected to be modelled well, while the results involving higher energy conformations would be less reliable.

3.4 MD Simulations

The similarity calculations were subsequently used to guide our choice for which protein structure and mebendazole tautomer to use for creating our MD models. Since sampling is critical in MD simulations, we choose to use all three similarity results (Section 3.2) for creating the MD input models of mebendazole’s binding to Hsp90. Consequently, six simulations were performed, which will be referred to as 2WI6-FB15, 2WI6-ff14SB, 2BT0-FB15, 2BT0-ff14SB, 4W7T-FB15 and 4W7T-ff14SB.

All simulations resulted in trajectories with stable nonbonded interactions between mebendazole and the ATP binding site. A dissociation of the ligand from the binding site was never observed during any of the 100 ns simulations. The protein structure itself was also stable, maintaining the crystals’ tertiary structures. The simulations also resulted in a good general consensus of the protein’s secondary structure (see Figure S6, with local deviation occurring in the residues that start and end the individual secondary elements. Consequently, all six simulations provided well-behaved and stable trajectories for further analysis into the binding of mebendazole to Hsp90.

Binding Pocket Poses Protein alignment of the individual frames within a given trajectory, followed by clustering mebendazole’s position allows for the identification of its possible binding poses within the ATP binding site. From these analyses, a total of nine distinct and well populated poses were observed under physiological conditions across all simulations, as detailed in Table 5 and shown in Figure 6. Seven poses position mebendazole’s terminal phenyl ring into the binding pocket with its carbamate functionality exposed to bulk water, each arising from the 2WI6 and 2BT0 simulations. The two remaining poses, arising from the 4W7T simulations, position the carbamate into the binding pocket, with the phenyl and ketone functionality solvent exposed.

While an exact one-to-one comparison between mebendazole’s QM-optimized conformations with those adopted in the MD simulations – due to the temperature effects, immersion into solution and nonbonded interaction with amino acids – can not be achieved, insights can be gained through their general comparison.

The mebendazole conformations adopted in 2BT0-FB15 pose 0, 2BT0-FB15 pose 1 and 2BT0-ff14SB pose 1 correspond to the MP2/AVTZ conformation pair $T_{3,4}^1$ ($\Delta E = 0.44$ kcal·mol⁻¹, Table 4), while 2BT0-ff14SB pose 0 adopts the lower energy conformation T_1^1 . Similarly in the 2WI6 simulations, the drug closely adopts a spatial arrangement that matches the MP2/AVTZ conformation pair T_3^2 and T_4^2 ($\Delta E = 0.447$ kcal·mol⁻¹). Considering that thermal energy at body temperature is ca. 0.6 kcal·mol⁻¹, it is reasonable that these conformations are well populated during the MD simulations.

A different result is obtained from the 4W7T simulations. In both resulting poses, mebendazole adopts a conformation that was not optimized by the QM calculations. These new conformations place $T_{1,2}^1$ ’s amide hydrogen aligned on the same side as the imidazole’s hydrogen and in-plane with the conjugated ring. Gas-phase MM optimization of the new conformations confirms them as stationary points on GAFF2’s PES, with a relative energy of 13.1 kcal·mol⁻¹ above the tautomer’s $T_{1,2}^1$ minima. These stabilities are within the range of similar QM amide-imidazole aligned conformations that have relative energies of ca. 17 kcal·mol⁻¹. Note that in these QM conformations, mebendazole’s ester group is out-of-plane with the imidazole ring. Additional QM calculations were performed in order to isolate these two new conformations, but both HF/6-31G(d) and MP2/AVTZ theory levels were unable to optimize them to a stationary point on their gas-phase PESs. Consequently, these conformations are unlikely to exist for isolated mebendazole. Coupling this with the conformation’s high relative energies predicted by Gaff2, if these conformations (i.e. 4W7T-FB15 pose 0 and 4W7T-ff14SB 0) exist within the ATP binding site, there must be an induced fit mechanism that includes substantial stabilization through nonbonded interactions and solvation effects.

Table 5. The nine top binding poses identified from the MD simulations. The total number of amino acids that form short-range, nonbonded interactions (i.e. within 3.4 Å) with mebendazole for at least 50% of the simulated time for each pose are provided, which are individually listed in Tables 6 and S3. Also included are the overall percentage that each pose sampled in their entire trajectory, as determined from the clustering analysis.

MD Model	Pose	Number of Amino Acids	Sampled (%)
2WI6-FB15	0	11	88
2WI6-ff14SB	0	10	63
	1	12	21
2BT0-FB15	0	10	49
	1	13	38
2BT0-ff14SB	0	13	75
	1	11	17
4W7T-FB15	0	15	100
4W7T-ff14SB	0	16	100

The nine poses differ in mebendazole’s relative orientations within the binding pocket, resulting in nonbonded contacts to a different set of amino acids as detailed in Tables 6 and S3. In total, 25 amino acids were identified to make stable, short-ranged nonbonded contacts with mebendazole. Of these amino acids, only 10–16 are involved for a given pose. Those that most frequently participate in binding, across all simulations, are the following residues: Asn51, Ala55, Ile96, Gly97, Met98, Asn106, Leu107, Phe138, Thr184 and Val186. Consequently, we predict that these ten amino acids are the ones most likely to be involved in binding mebendazole to the ATP binding site of Hsp90. Interestingly, 4W7T-ff14SB pose 0 is the only one that makes contacts with all of these ten amino acids.

Several Hsp90 studies have also indicated many of these amino acids as important for ligand binding, and thus lends support to the predictive nature of the MD simulations performed here. Most notably, a 2010 survey of 40 crystallized Hsp90-ligand complexes found Asn51, Ala55, Met98, Phe138 and Thr184 (plus Lys58, Asp93 and Gly97) make frequent contacts to bound ligands [28]. A recent pharmacophore model identified Ala55, Lys58, Asp93, Ile96, Met98 and Thr184 as important in binding Hsp90 inhibitors [37]. Of the MD predicted poses, 2BT0-ff14SB pose 0 and 2BT0-FB15 pose 1 form interactions with all 6 of the pharmacophore’s amino acids (Table S3).

A comparison of binding pocket topology can be achieved by selectively mapping different colors to the amino acids categorized by the a) pharmacophore [37], b) most frequently (Table 6) and c) least frequently (Table S3) involved mebendazole binders that were identified from the MD simulations. As seen in Figure 6, the binding site amino acid composition and their positions differ significantly across the nine poses, arising primarily from the spacial placement of amino acids’ side chains. Generally consistent across the poses are the positions of pharmacophore amino acids (red) that are deep in the binding pocket, and the most frequent (cyan) amino

Figure 6: The major binding poses predicted by molecular dynamics simulations. All images were created by aligning the proteins, allowing for a consistent orientation of the binding site. The 2WI6 (blue-green) and 2BT0 (purple) structures are the best representative of the centroid identified in the cluster analysis, while those for 4W7T (orange) represent structure with the lowest RMSD value to the average structure from their respective 100 ns trajectories. The pharmacophore (red [37]), most frequent (cyan, Table 6), and less frequent (olive green, Table S3) amino acids are highlighted if indicated they fall within 3.4 Å of mebendazole in a given pose. Note that if a residue is categorized as both a pharmacophore and either as most/least frequent amino acid, then it is colored red.

Table 6. The percentage of time for the most frequently involved amino acids that form short-range, nonbonded interactions (i.e. ≤ 3.4 Å) with mebendazole. The percentages are computed for the trajectory portion that corresponds to each identified binding mode. The values in parentheses are the binding pose populations computed in the last 100 ns. All interactions that occur for over 50% for a given pose are reported.

MD Model	Pose	Asn51	Ala55	Ile96	Gly97	Met98	Asn106	Leu107	Phe138	Thr184	Val186
2WI6-FB15	0	99.7	98.0	100.0	99.2	100.0	99.9	99.9	99.8	94.2	
2WI6-ff14SB	0	94.0				99.9	95.8	99.9	99.9	96.4	91.7
	1	99.8	98.6	98.9	97.7	99.9	99.2	99.8	97.8	84.8	
2BT0-FB15	0	99.9				97.4		99.9	99.7	91.8	
	1	99.9	95.9	99.8	98.8	100.0		79.6	76.8	99.0	98.6
2BT0-ff14SB	0	99.9	100.0	99.5	89.5	100.0		81.5	69.4	100.0	85.9
	1	99.1	55.0			98.1		100.0	97.7	87.5	80.3
4W7T-FB15	0	97.1	99.9	77.2	84.8	100.0	91.0	94.3		100.0	99.8
4W7T-ff14SB	0	98.6	99.6	60.1	50.9	98.9	86.5	96.9	74.3	100.0	99.9

acids forming the top and left-side of the binding pocket. The shape and composition of the binding pockets' entrance varies significantly across the poses, best exemplified by the differences in the less frequent (olive green) interacting amino acids. Thus, taken together and assuming that the poses sampled by the different MD models are meaningful, the binding site is dynamically labile, which is supported by the large number of Hsp90-ligand crystal structures deposited in the Protein Data Bank [79].

To further investigate the protein's flexibility, an analysis of the RMSF of the amino acids' backbone atoms (ie. C, C $_{\alpha}$ and N) were performed. As shown in Figure 7, the fluctuations across all six MD models show very similar patterns, with differences mainly seen in the RMSF's magnitude. The majority of the binding pocket amino acids reside in the more dynamically stable regions of the protein fold. These observations supports the above conclusion that the changes seen within the binding pocket topology are largely due to the local amino acid side chain motion.

The protein residues with the largest fluctuations occur in helices and loops that are not directly involved in mebendazole's binding, while the β -sheets provide local regions of dynamic stability (Figure 7). Interestingly, the "lid" region (i.e. Gly108–Tyr139) displays significant fluctuations, indicating that it is dynamically labile as suggested by the open and closed state seen in crystallography [110–112]. However, at the timescales studied herein, and in the absence of a second Hsp90, the large-scale movement to a lid-closed state is not observed. The fluctuation observed herein are consistent with other recently reported long (200 and 100 ns) MD simulation of Hsp90-ligand complexes [25, 38].

Insight into the role that water has in mebendazole's binding can be gained through an SDF analysis of the first solvation shell of the drug (see Figures S4–S5). As seen in Table 7, the binding poses predicted by both 4W7T simulations – which position mebendazole's phenyl groups near the binding pocket's entrance – have the least waters (i.e. 12 molecules) forming the drug's first solvation shell. This is followed by the 2WI6-FB15 trajectory, with 15 waters solvating mebendazole. The remaining trajectories provide poses that are notably more solvated, with 19-22 water composing the first solvation shell.

Table 7. The average number of waters that are within 3.4 Å of mebendazole for each MD simulation.

	2WI6	2BT0	4W7T
FB15	15.2 $\pm$ 2.5	22.2 $\pm$ 4.8	12.1 $\pm$ 2.0
ff14SB	21.0 $\pm$ 4.2	19.4 $\pm$ 3.1	12.1 $\pm$ 2.2

A majority of the waters interact with mebendazole where it is exposed to bulk solvent, or in the space formed between the drug and a single side of the binding pocket. Most of the water molecules translates fairly uninhibited, creating a large globular and continuous spacial occupancy, as exemplified in Figure 8. Consequently, it is difficult to isolate specific waters within these globular regions that unequivocally bridge an individual amino acid and mebendazole. Apart from these globular density regions, there are localized areas where 1-2 waters do form isolated and well resolved occupancy densities. The most frequently observed bridging water occurs with Asn51, occurring in seven of the poses (i.e. absent in the 2WI6-ff14SB simulation). Other bridging waters that were observed in 1-2 poses involved the amino acids Asp54, Asp93, Asp102, Ans106, Lys112 and Thr184. For the remaining instances, water molecules form a bridge between amino acids and do not form

Figure 7: The root-mean-square fluctuations ($\text{\AA}$) for the most populated binding poses predicted by molecular dynamics simulations. The dash lines indicate the 10 binding pocket amino acids that most often form the close nonbonded interactions with mebendazole (see Table 6). To represent where the generally low (blue) and high (red) fluctuations occur, 4W7T-FB15 pose 0's fluctuations are mapped onto the representative structure; the magnitude at Ser68 and Gly69 was reduced to $3.5 \text{ \AA}$ to allow for the lid fluctuations to be seen better. The secondary structure representation is estimated from the DSSP calculation across all trajectories (see Figure S6)

notable interaction with mebendazole.

Table 8. MM/PBSA relative binding energies (ΔE_{bind}) of the different poses that mebendazole adopts within the ATP binding pocket of Hsp90.

	FB15		ff14SB	
	Pose 0	Pose 1	Pose 0	Pose 1
2WI6	8.9 $\pm$ 4.0		6.2 $\pm$ 3.9	12.6 $\pm$ 4.5
2BT0	0.3 $\pm$ 3.4	9.2 $\pm$ 3.8	4.6 $\pm$ 3.9	8.0 $\pm$ 4.0
4W7T	0.0 $\pm$ 4.7		6.3 $\pm$ 5.1	

In an attempt to provide a ranking of the poses, MM/PBSA binding energies (ΔE_{bind}) were computed. Here, we are interested only in the relative trends provided by the MM/PBSA calculations, and do not expect that the absolute computed energies reflect those that could be obtained experimentally. As seen in Table 8, when two poses are identified for a given protein model, the most populated pose (i.e. pose 0) has the more favorable binding energy. Thus, providing some confidence that the computed trends are reliable. Of the poses predicted, 2BT0-FB15 pose 0 and 4W7T-FB15 pose 0 have the lowest binding energies, both falling within the standard deviation of one another. If one disregards the solvation contribution, and only consider the interaction energy arising from nonbonded interactions (i.e. Lennard-Jones and electrostatics forces), both 4W7T-FB15 poses provide the most favorable interactions (pose 0 = -81 kcal·mol⁻¹; pose 1 = -70 kcal·mol⁻¹), followed by 2WI6-FB15 pose 0 (-54 kcal·mol⁻¹) and 2WI6-ff14SB pose 1 (52 kcal·mol⁻¹). Consequently, both energetic analyses, with and without solvation effects, reveal that 4W7T-FB15 pose 0 is the most favorable.

Thus, 4W7T-FB15 pose 0 appears to be a good candidate for the pose that could be resolved experimentally through crystallography. This pose make close contact between mebendazole and 15 amino acids, with nine out of the ten most frequently involved amino acids being included in the binding. Only 4W7T-ff14SB pose 0 has more, with one additional amino acids that interact with mebendazole. However, some uncertainty exists in this prediction because the mebendazole conformation that is present does not exist on the gas-phase MP2/AVTZ PES.

4 Conclusions

An extensive search of mebendazole conformational space, across all five tautomer forms, was performed in the gas phase using the MP2/AVTZ theory level. A total of 152 conformations were identified as stationary points on the molecule’s PES. The most stable conformations coincide with the known crystal structures. Similarity calculations computed between the eight most stable mebendazole conformational predicted by QM to 300 crystallized ligands bound to Hsp90 revealed three possible poses that mebendazole could adopt within the ATP binding site. Using the results of the similarity calculations, six MD models were generated that differ by the choice of protein crystal used and in the force field employed for modeling the protein.

Clustering the resulting MD trajectories lead to nine possible binding poses. Trajectory analyses of the drug’s nonbonded interactions with the Hsp90 predict that Asn51, Ala55, Ile96, Gly97, Met98, Asn106, Leu107, Phe138, Thr184 and Val186 are most likely to be involved in stabilizing the binding complex. The protein backbone atoms that comprise the ATP binding pocket has a high degree of dynamic stability in the presence of mebendazole, with the largest backbone fluctuations occurring away from the binding pocket at Ser68 and in the lid region.

The MD simulations presented here indicate that mebendazole can adopt several dynamically stable poses within the ATP binding pocket of Hsp90. Based on MM/PBSA calculations, 4W7T-FB15 pose 0 is predicted to be the most experimentally relevant binding pose. However, this conformation adopted by mebendazole in this pose and that of 4W7T-ff14SB pose 0 does not exist on the gas-phase MP2/AVTZ PES. Consequently, these poses are either artifacts arising from the molecular mechanics’ force field, or there is an induced fit mechanics that occurs during mebendazole binding to the ATP pocket. Aside from these two poses, the conformations adopted by mebendazole exist on the MP2/AVTZ PES, giving us confidence that one or more of the poses predicted here are physiologically and experimentally relevant. This supports the use of mebendazole as a future therapeutic option as an Hsp90 inhibitor for the treatment of diverse cancers and as a potential new therapeutic strategy to limit the ability of cancer cells to adapt and evolve drug resistance.

Figure 8: The spatial distribution of mebendazole's first solvation shell (blue isosurface) for the primary poses computed from the FB15 force field. Two orientations are shown in each case, and the isosurface encapsulates a normalized occupancy density (molecule/ $\text{\AA}^3$) of 80%.

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6 Conflicts of Interests

WF has an advisory and consulting role for Amgen; an advisory role for ARIAD/Incyte, Novartis, Pfizer; Celgene, Morphosys, AbbVie, Jazz Pharmaceuticals, and Servier; receives royalties from Amgen; receives support for meeting attendance from Amgen, Gilead, Servier, Jazz Pharmaceuticals, and Daiichi Sankyo; and research funding from Amgen and Pfizer.

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Figure S1: A schematic of the workflow used in this study.

8 Supplementary Information

Table S1. The PDB ID code [80] for the crystal structure of Hsp90 human proteins that are bound with ligands.

1BYQ	1ELW	1OSF	1UY6	1UY7	1UY8	1UY9	1UYC	1UYD	1UYE
1UYF	1UYG	1UYH	1UYI	1UYK	1UYM	1YC3	1YET	2BSM	2BT0
2BYH	2BYI	2BZ5	2CCS	2CCT	2CCU	2FWY	2FWZ	2H55	2JJC
2QF6	2QFO	2QG0	2QG2	2UWD	2VCI	2VCJ	2WI1	2WI2	2WI3
2WI4	2WI5	2WI6	2WI7	2XAB	2XDK	2XDL	2XDS	2XDU	2XDX
2XHR	2XHT	2XHX	2XJG	2XJJ	2XJX	2XK2	2YE2	2YE3	2YE4
2YE5	2YE6	2YE7	2YE8	2YE9	2YEA	2YEB	2YEC	2YED	2YEE
2YEF	2YEG	2YEH	2YEI	2YEJ	2YI0	2YI5	2YI6	2YI7	2YJW
2YJX	2YK2	2YK9	2YKB	2YKC	2YKE	2YKI	2YKJ	3B24	3B25
3B26	3B27	3B28	3BM9	3BMY	3D0B	3EKO	3EKR	3FT5	3FT8
3HEK	3HHU	3HYY	3HYZ	3HZ1	3HZ5	3INW	3INX	3K97	3K98
3K99	3MNR	3NMQ	3O0I	3O5L	3O5R	3OW6	3OWB	3OWD	3QTF
3R91	3R92	3RKZ	3RLP	3RLQ	3RLR	3T0Z	3T10	3T1K	3T2S
3TUH	3VHA	3VHC	3VHD	3WHA	3WQ9	4AWO	4AWP	4AWQ	4B7P
4BQG	4BQJ	4CWF	4CWN	4CWO	4CWP	4CWQ	4CWR	4CWS	4CWT
4DRH	4DRI	4DRJ	4DRK	4DRM	4DRN	4DRP	4EEH	4EFT	4EFU
4EGH	4EGK	4FCP	4FCQ	4FCR	4HY6	4JFI	4JFJ	4JFK	4JFL
4JFM	4JQL	4L8Z	4L90	4L91	4L93	4L94	4LAX	4LAY	4LWE
4LWF	4LWG	4LWH	4LWI	4NH7	4NH8	4NH9	4O04	4O05	4O07
4O09	4O0B	4O33	4R3M	4TW6	4TW7	4TX0	4U93	4W7T	4W9O
4W9P	4W9Q	4Z1F	4Z1G	4Z1H	4Z1I	5BXJ	5CF0	5DIT	5DIU
5DIV	5F5R	5FNC	5FND	5FNF	5GGZ	5HPH	5J20	5J27	5J2X
5J64	5J6L	5J6M	5J6N	5J82	5J86	5J8M	5J9X	5LNY	5LNZ
5LO0	5LO1	5LO5	5LO6	5LQ9	5LR1	5LR7	5LRL	5LRZ	5LS1
5M4E	5M4H	5M4K	5M4M	5M4N	5M4P	5NYH	5NYI	5OBK	5OCI
5OD7	5ODX	5T21	5UC4	5UCH	5UCI	5UCJ	5VYY	5XQD	5XQE
5XR5	5XR9	5XRB	5XRD	5XRE	5Y3N	5Y3O	5ZR3	6B99	6B9A
6CEO	6CYG	6CYH	6FCJ	6GP8	6GPH	6GPO	6GPP	6GPR	6GPT
6GQ6	6GQR	6GQS	6GQU	6GR1	6GR3	6GR5			

Table S2. The ID code for the ligands found in the crystal structures listed in Table S1

ADP	LGA	XQI	YKC	P54	H05	RL1	3JP	70L	9QY
TRS	A56	VXX	YKE	1PE	6LV	RL3	H71	70M	8CF
KOS	A13	2FY	YKI	FK5	T62	X29	94M	70O	8CO
MPD	A51	2A7	YKJ	MEX	TV2	S36	4KP	72K	8CR
PU3	A94	2AE	B2J	BSM	W2D	S46	ANP	72Y	8CU
PU4	A91	2GA	B2K	MEY	HAJ	FK5	NPO	73J	8DU
PU5	2GG	2D3	B2L	05S	G3R	I63	FJS	73S	9ZC
PU6	2GJ	2D4	B2T	06H	IK9	FJ2	5B8	73Y	JX1
PU7	2EQ	2A9	B2X	06J	RAP	FJ3	5BG	73Z	8MF
PU8	ZZ2	2K4	BXZ	06T	RAP	FJ4	5BH	7F9	8M9
PU9	ZZ3	XQ0	CXZ	3RP	RAP	FJ5	ANP	7FX	9J0
PU1	ZZ3	ADE	SNX	3RQ	I63	FJ6	IEE	7F9	NEC
PU2	ZZ4	2EC	PYU	3RR	0MC	E0G	IQ5	TF3	PA7
PU0	ZZ5	ANP	PY9	ATP	0MC	2LC	TQL	7FW	D57
PUZ	ZZ6	XQG	MO8	ACP	0MD	2LC	6TN	TF3	N5O
PUX	2KL	2KU	MOJ	ANP	HH6	2Q8	ANP	7FV	N5A
PU3	VHD	XQI	BD0	AGS	EFT	2Q9	6FJ	TF3	D4W
4BC	XDK	XQK	819	TUH	EFU	FGH	6FF	7FE	APC
GDM	2DL	XQK	37D	MES	0OY	2R6	6DL	TF3	A12
BSM	MT0	ZZ3	42C	VHA	RDC	2QA	6G7	9EK	CMP
CT5	LGA	YI0	37D	VHC	42C	3PG	6GC	2EQ	ADP
2D7	MT0	YI5	42C	VHE	2N6	TZN	6FJ	9QN	CMP
2DD	WOE	6QM	Z64	WHA	0TM	JR9	6FF	9R8	A12
AB4	C0P	BZ8	JZB	YUK	FJ1	37L	6GV	H0T	ADP
4BH	C0Y	YJW	JZC	99B	1KT	37K	6GW	9RZ	APC
2E1	T5M	YJX	4CD	99A	1KU	384	6DL	74E	A12
2D9	XJG	YJW	1RC	592	JFK	990	6GC	83S	CMP
H64	L81	YJX	PFT	9UN	1KY	3JC	70K	871	APC
H71	XJX	YK9	SD1	50Q	1KZ	3JQ	70Z	874	AN2
DZ8	ADP	YKB	7PP	XKL	VJ6	3JR	70N	KU3	ADP

Figure S2: Similarity indexes, computed using shape & electrostatic criteria, between the eight lowest energy (MP2/AVTZ//MP2/AVTZ) mebendazole conformations and 300 ligands extracted from 287 crystals.

Figure S3: The spatial distribution of mebendazole (orange isosurface), binding pocket amino acids (purple) and two orientations of the close waters (blue) for the 2WI6 simulations, showing a normalized occupancy density (molecule/ $\text{\AA}^3$) of 80%.

Figure S4: The spatial distribution for the 2BT0 simulations.

Figure S5: The spatial distribution for the 4W7T simulations.

		16																
Amino Acid	EVETFAFQAE	IAQLMSLIIN	TFYSNKEIFL	RELISNSSDA	LDKIRYESLT													
2WI6, fb15	EEEE	HH	HHHHHHHHTT	S	S	GTGHH	HHHHHHHHHH	HHHHHHHHTT										
2WI6, ffl4sb	EEEE	HH	HHHHHHHHHH	S	GGGHH	HHHHHHHHHH	HHHHHHHTTT											
2BT0, fb15	EEEE	HH	HHHHHHHHTS	S	S	GGGHH	HHHHHHHHHH	HHHHHHHTTT										
2BT0, ffl4sb	EEEE	HH	HHHHHHHHHH	S	GGGHH	HHHHHHHHHH	HHHHHHHHHT											
4W7T, fb15	EEEE	HH	HHHHHHHHHT	S	S	GGGGG	HHHHHHHHHH	HHHHHHHTTT										
4W7T, ffl4sb	EEEE	HH	HHHHHHHHHH	S	TTHHH	HHHHHHHHHH	HHHHHHHTTT											
		→	→	→	→	→	→	→	→	→	→	→						
		66																
Amino Acid	DPSKLDGKE	LHINLIPNKQ	DRTLTIIVDTG	IGMTKADLIN	NLGTIAKSGT													
2WI6, fb15	GGGGTT	EEEEETT	TTEEEEE	S	HHHHH	HTTTTTHHHH												
2WI6, ffl4sb	TTHHHTSS	EEEEETT	TTEEEEE	S	HHHHH	HHTSSTHHHH												
2BT0, fb15	GHHHTT	EEEEETT	TTEEEEE	S	HHHHHT	GGG	HHHHH											
2BT0, ffl4sb	TTHHHT	EEEEETT	TTEEEEE	S	HHHHHT	GGG	HHHHH											
4W7T, fb15	GGGGHT	EEEEETT	TTEEEEE	S	S	HHHHH	TTTSTTSSH											
4W7T, ffl4sb	GGGGHT	EEEEETT	TTEEEEE	S	HHHHH	TTTT	SS	HH										
		→	→	→	→	→	→	→	→	→	→	→						
		116																
Amino Acid	KAFMEALQAG	ADISMIGQFG	VGFYSAYLVA	EKVTVITKHN	DDEQYAWESS													
2WI6, fb15	HHHHHHHHTT	GGGGGTTT	GGGGGGGTE	EEEEEEE	T	TS	EEEE											
2WI6, ffl4sb	HHHHH	TT	GGGGGTTT	GGGGGGGTE	EEEEEEE	T	TS	EEEE										
2BT0, fb15	HHHHHHHHTT	GGGGGGGT	GGGGGGGTE	EEEEEEE	T	TS	EEEE											
2BT0, ffl4sb	HHHHHHHHTT	GGGGGTTT	GGGGGGGTE	EEEEEEE	T	TS	EEEE											
4W7T, fb15	HHHHHHHHTT	GHHHHHT	SGGGGGGTE	EEEEEEE	T	TS	EEEE											
4W7T, ffl4sb	HHHHHHHHTT	GGGGGTTT	GGGGGGGTE	EEEEEEE	T	TS	EEEE											
		→	→	→	→	→	→	→	→	→	→	→						
		166																
Amino Acid	AGGSFTVRTD	TGPEPMGRGTK	VILHLKEDQT	EYLEERRIKE	IVKKHSQFIG													
2WI6, fb15	SSSEEEEE	SS	SEEE	EEEE	TT	G	GGG	HHHHH	HHHHHTTT	S								
2WI6, ffl4sb	STSEEEEE	S	SEEE	EEEE	GGG	GGGSHHHHHH	HHHHHTTT	S										
2BT0, fb15	SSSEEEEE	TTS	SEEE	EEEE	GGG	GGGSHHHHHH	HHHHHTTT											
2BT0, ffl4sb	TTSEEEEE	S	SEEE	EEEE	GGG	GGG	HHHHH	HHHHHSTT	S									
4W7T, fb15	SSSEEEEE	SS	SEEE	EEEE	GGG	GGGSHHHHHH	HHHHH	S	S									
4W7T, ffl4sb	SSSEEEEE	S	SEEE	EEEE	GGG	GGGSHHHHHH	HHHHHTTT											
		→	→	→	→	→	→	→	→	→	→	→						
		216																
Amino Acid	YPITLFVEK																	
2WI6, fb15	S	EEE																
2WI6, ffl4sb	S	EE																
2BT0, fb15	S	EEE																
2BT0, ffl4sb	S	EE																
4W7T, fb15	S	EE	S															
4W7T, ffl4sb	S	EE																
		→	→	→	→	→	→	→	→	→	→	→						

Sheets: E (Parallel Beta), B (Anti-parallel Beta)

Helix: G (3-10), H (Alpha), I (Pi/3-14)

Turn: T (Turn), S (Bend), None

Sheets: E (Parallel Beta), B (Anti-parallel Beta)
Helix: G (3-10), H (Alpha), I (Pi/3-14)
Turn: T (Turn), S (Bend), None

Figure S6: Average secondary structure assignment of the Hsp90 protein for each MD simulation using the DSSP method [104].

Table S3. The percentage of time for the least frequently involved amino acids that form short-range, nonbonded interactions (i.e. within 3.4 Å) with mebendazole.

MD Model	Pose	Leu48	Ser52	Asp54	Lys58	Ile91	Val92	Asp93	Asp102	Gly108	Thr109	Ile110	Lys112	Gly135	Val150	His154
2W16-FB15	0								88.7							65.6
2W16-ff14SB	0				63.6				89.8						92.2	
	1				64.8				73.6							71.1
2BT0-FB15	0									97.0	99.9	96.2		88.4	53.6	
	1		87.4					64.1							59.0	
2BT0-ff14SB	0		86.1	98.0	94.1			84.4								
	1									99.3	99.9	95.9			60.6	
4W7T-FB15	0	92.1	100.0			100.0		100.0	86.4				85.4			
4W7T-ff14SB	0	80.1	100.0			100.0	69.3	100.0						52.7		