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Screening of miscellaneous Hsp90 inhibitors using virtual co-crystallized pharmacophore

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ABSTRACT

*Based on previous studies on 1YET as heat shock protein (Hsp90), we explored the pharmacophoric features of geldanamycin co-cystallized in ATPase binding site of Hsp90 (1YET). The best phamacophore was selected and used as a searching tool to explore the national cancer institute (NCI) database. The captured hits were mapped on successful hypotheses and the best fitted compounds were selected. The inhibition of ATPase activity of Hsp90 was measured and expressed as percentage of inhibition. Compounds **1** and **2** showed 41.6% and 36.3% inhibition respectively. The captured hits were diverse with novel scaffold as Hsp90 inhibitors.*

Keywords: Co-crystallized structure, Hsp90, Cancer, Pharmacophore, Docking, Geldanamycin

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INTRODUCTION

Hsp90 is a molecular chaperone that plays a crucial role in the conformational maturation, stability, and function of protein substrates within the cell [1-6]. The interaction of ATP with its binding domain in Hsp90 leads to autophosphorylation of certain tyrosine residues, thus activating this kinase and provides the necessary energy for refolding of denatured proteins [1-6]. Previously screened hits show Hsp90 inhibitory effect [7-11]. Accordingly, we initiated an exploratory effort to evaluate a generated pharmacophore from co-crystallized structure, then the mapped compounds were assayed using malachite green assay [12,13]. The validity of Hsp90 as anticancer target for drug discovery was established by emerging clinical trials employing potent Hsp90 inhibitor 17-allylaminogeldanamycin and the natural Hsp90 inhibitors geldanamycin and radicicol [14-17]. However, despite the high cellular activity and clinical progression of 17-allylaminogeldanamycin, [18] it has several limitations, for example, poor solubility, hepatotoxicity, and extensive metabolism. These issues have led to significant efforts to identify novel rationally designed small molecular inhibitors of Hsp90. [19] The main focus of recent efforts toward the development of new Hsp90 inhibitors concentrate on structure-based ligand design [20-22] and high-throughput screening, [23] with a few ligand-based examples. [24,25] The continued interest in designing new Hsp90 inhibitors and lack of adequate ligand-based computer-aided drug discovery efforts combined with the drawbacks of structure based design and the significant induced fit flexibility observed for Hsp90 prompted us to explore the possibility of developing a pharmacophoric model derived from the virtual co-crystallized structure 1YET (from Protein Data Bank, PDB) [26].

MATERIALS AND METHODS

2.1 Molecular modeling

Pharmacophore mapping was performed using CATALYST (HYPOGEN module) and CERIUS2 software suites implemented in Discovery Studio 4.0 from Accelrys Inc. [36]. Structure drawing was performed employing ChemDraw Ultra 7.0 [37].

2.1.1 Receptor-ligand pharmacophore generation

Structure-based pharmacophore model utilizes the interactions between receptor-ligand complexes to generate a hypothesis [27]. As deposit of X-ray crystal structures in PDB is growing rapidly, the structure-based methods have become increasingly important. The information about the protein structure is a good source to bring forth the structure-based pharmacophore and used as first screening before docking studies. 1YET crystal structure had been selected for heat shock protein 90 to explore the pharmacophoric features of co-crystallized ligand (geldanamycin) [26]. The crystal structure was downloaded from the Protein Data Bank. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the website (http://www.rcsb.org). After downloading the desired crystal structure of Hsp90, (PDB code: 1YET, resolution: 1.9 Å). Hydrogen atoms were added to the protein, utilizing DS 4.0 templates for protein residues. Receptor-ligand pharmacophore generation algorithm was used within DS 4.0. The used parameters were set as follows: minimum number of features 4 while the maximum number of features was 10, the number of generated pharmacophoric models was 10 and the cocrystalized water was kept in the protein structure. The Receptor-Ligand Pharmacophore Generation protocol of Accelrys Discovery Studio v4.0 (DS), Accelrys, San Diego, USA, was applied to accomplish this task with default parameters. This protocol generates selective pharmacophore models based on receptor-ligand interactions. First, a set of features from the binding ligand is identified. The following predefined feature types are considered: hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (Hbic), negative ionizable (NI), positive ionizable (PI), ring aromatic (RA). Second, the pharmacophore models were ranked based on a measure of selectivity score and the top models are returned.

2.1.2 Molecular Docking

The 3D coordinates of Hsp90 were retrieved from the Protein Data Bank (Hsp90 PDB code: 1YET, resolution: 1.9 Å). Hydrogen atoms were added to the protein, utilizing DS 4.0 templates for protein residues. Gasteiger–Marsili charges were assigned to the protein atoms, as implemented within DS 4.0 [28]. The protein structures were utilized in subsequent docking experiments without energy minimization. Explicit water molecules were retained according to the required docking conditions (i.e., docking in the presence of explicit water molecules). Docking settings of LigandFit considers the flexibility of the ligand and considers the receptor to be rigid according to the LigandFit algorithm [29-31].

In the current docking experiments, the binding site was generated from the co-crystallized ligand (geldanamycin,GMD), and selected hits were docked into the binding site in the presence of explicit water molecules, employing the following docking configurations; Monte Carlo search parameters were as follows: number of trials 15000; search step for torsions with polar hydrogens = 30.0°. The RMS threshold for ligand-to-binding-site shape matching was set to 2.0 Å, employing a maximum of 1.0 binding-site partitions. The interaction energies were assessed employing the CFF force field (v.1.02) with a nonbonded cutoff distance of 10.0 Å and distance-dependent dielectric. An energy grid extending 3.0 Å from the binding site was implemented. The interaction energy was estimated with a trilinear interpolation value using soft potential energy approximations [29]. Rigid body ligand minimization parameters: 20 steepest descent iterations followed by 40 BFGS-minimization iterations were applied to every orientation of the docked ligand. The best ten poses were further energy minimized within the binding site for a maximum of 200 rigid body iterations. The protein – ligand interactions were examined by DS. Hit molecules which showed the expected interactions with the critical amino acids present in the active site of the protein, and comparable with the cocrystalized ligand.

2.1.3 Ligand pharmacophore mapping

The activity of any compound can be estimated from a particular hypothesis through equation (1) [32].

$$\text{Log (Estimated Activity)} = I + \text{Fit} \qquad (1)$$

Where, I = the intercept of the regression line obtained by plotting the log of the biological activity of the training set compounds against the Fit values of the training compounds. The Fit value for any compound is obtained automatically employing equation (2) [32-35].

Fit = Σmapped hypothesis features × W [1−Σ(disp/tol)2] (2)

Where Σmapped hypothesis features represents the number of pharmacophore features that successfully superimpose (i.e., map or overlap with) the corresponding chemical moieties within the fitted compound, W is the weight of the corresponding hypothesis feature spheres, disp is the distance between the center of a particular pharmacophoric sphere (feature centroid) and the center of the corresponding superimposed chemical moiety of the fitted compound; tol is the radius of the pharmacophoric feature sphere (known as Tolerance, equals to 1.6 Å by default) and $\Sigma\ (disp/tol)^2$ is the summation of $(disp/tol)^2$ values for all pharmacophoric features that successfully superimpose corresponding chemical functionalities in the fitted compound [32-35].

In order to compare the mapped feature with the docked pose, the screened hits were fitted against virtual cocrystalized Hsp90 pharmacophoric model Hypo1 using the "best fit" option within CATALYST in DS 4.0 [32] then the mapped compounds were docked in the ATP binding site of Hsp90 using the rigid docking option [31].

2.2 *In vitro* Experimental Studies

2.2.1 Reagents and Reference Samples

Active Hsp90 enzyme 10 µg (Sigma-Aldrich, Germany), malachite green 99% (Sigma-Aldrich, Germany), Ammonium molybdate 95% (Sigma-Aldrich, Germany), Sodium citrate 99%(Sigma-Aldrich, Germany), ATP 1mM solution (Sigma-Aldrich, Germany), Allylaminogeldanamycin 10 mg (Sigma-Aldrich, Germany), 15 NCI compounds from National Cancer Institute (USA).

2.2.2 Preparation of hit compounds for *in vitro* assay

The tested compounds were provided as dry powders in variable quantities (5-500 mg). They were initially dissolved in DMSO to give stock solutions of 0.02 M. Subsequently, they were diluted to the required concentrations with deionized water for enzymatic assay.

2.2.3 Quantification of Hsp90 activity in a spectrophotometric assay

The ATPase activity of Hsp90 was quantified by colorimetric measurement of released inorganic phosphate [12, 13]. Bioassays were performed as follows; in a 96-well clear plate, the reaction solution of total volume of 50µL contains 100mM Tris/HCl, pH 7.4, 6mM $MgCl_2$, 20mM KCl, 100µM ATP, 0.1mg/ml BSA and 50ng/well of human Hsp90 enzyme, 5 µL of tested compounds. The plate was sealed and the reaction was incubated at 37^{o}C for 24 hours. The reaction was stopped by the addition of 50µL of previously prepared malachite green solution (5.2% ammonium molybdate in H_2SO_4, 0.0812% malachite green, 2.32% polyvinyl alcohol and water in ratios of 1:2:1:2 respectively), followed by 10 µL of 10% Sodium citrate, left for 20 minutes and the absorbance at 630 nm was measured using a plate reader (Bio-Tek instruments ELx 800, Winooski, VT). The calibration curve was prepared using 5 different concentrations of phosphate ion (10-200µM). The final concentration of DMSO did not exceed 1.0%. Inhibition of Hsp90 was calculated as percent activity of the uninhibited ATPase control. Allylaminogeldanamycin was tested as positive control, while negative controls were prepared by adding the substrate after reaction termination.

Table 1: Pharmacophores generated from the co-crystallized Hsp90 structure (1YET)

Pharmacophore	Model	Number of Features	Feature Set*	Selectivity Score
Hypo1	Pharmacophore_01	7	AAAADDH	12.944
Hypo2	Pharmacophore_02	6	AAADDH	11.429
Hypo3	Pharmacophore_03	6	AAADDH	11.429
Hypo4	Pharmacophore_04	6	AAADDH	11.429
Hypo5	Pharmacophore_05	6	AAADDH	11.429
Hypo6	Pharmacophore_06	6	AAAADD	11.429
Hypo7	Pharmacophore_07	6	AAAADH	10.516
Hpo8	Pharmacophore_08	6	AAAADH	10.516
Hypo9	Pharmacophore_09	5	AADDH	9.9146
Hypo10	Pharmacophore_10	5	AAADD	9.9146

**A : Hydrogen bond aceptor, D : Hydrogen bond donor, H: Hydrophobe*

RESULTS AND DISCUSSION

Table 1 shows the generated pharmacophores using DS studio 4.0 . Hsp90 protein cocrystalized with geldanamycin (1YET, resolution 1.9A) was used to generate the pharmacophores. Hypo1 was selected because of 7 pharmacophoric features and it was the most selective pharmacophore with selectivity score equals 12.944 (table 1).

Table 2 : Pharmacophoric features and corresponding weights, tolerances and 3D coordinates of generated pharmacophore from co-crystallized Hsp90

Model	definition		Chemical Features						
			HBA1		HBA2		HBA3		
Hypo1	Tolerances		1.60	2.20	1.60	2.20	1.60	2.20	
	Coordinates	X	38.44	35.47	37.59	34.73	44.94	45.16	
		Y	-50.99	-50.76	-47.39	-47.08	-43.48	-41.91	
		Z	68.15	68.53	62.64	63.50	64.42	66.97	
			HBA4			HBA5	HBD	Hbic	Hbic
Hypo1	Tolerances		1.60	2.20	1.60	2.20	1.60	2.20 1.60	1.60
	Coordinates	X	42.11	44.05	39.59	38.30	36.73	34.04	42.48
		Y	-49.45	-51.73	-49.16	-48.80	-43.19	-44.27	-42.28
		Z	68.12	67.98	68.82	71.50	62.25	63.03	64.0

Hypo1 The pharmacophore was generated from the cocrystalized structure of Hsp90 (1YET, resolution 1.9A)

Table 3 : Exclusion spheres added to the generated pharmacophore

Feature	X	Y	Z	Radius
ExcludedVolume_1.14	36.095	-49.535	57.896	1.154
ExcludedVolume_1.15	44.741	-39.671	65.875	1.368
ExcludedVolume_1.16	40.522	-51.082	71.915	1.154
ExcludedVolume_1.17	46.725	-47.755	59.215	1.454
ExcludedVolume_1.18	40.066	-38.739	63.746	1.368
ExcludedVolume_1.19	40.814	-52.543	61.24	1.454
ExcludedVolume_1.20	35.533	-51.728	64.625	1.724
ExcludedVolume_1.21	37.936	-55.192	66.1	1.368
ExcludedVolume_1.22	43.528	-53.296	68.995	1.27
ExcludedVolume_1.23	35.524	-39.882	60.527	1.27
ExcludedVolume_1.24	36.39	-44.182	66.815	1.6
ExcludedVolume_1.25	43.344	-39.086	61.021	1.008
ExcludedVolume_1.26	45.402	-51.005	63.564	1.53
ExcludedVolume_1.27	37.958	-53.109	70.901	1.368
ExcludedVolume_1.28	35.133	-51.062	69.915	1.27
ExcludedVolume_1.29	40.796	-40.228	67.613	1.53
ExcludedVolume_1.30	42.585	-41.689	58.511	1.154
ExcludedVolume_1.31	35.489	-40.089	64.733	1.368
ExcludedVolume_1.32	36.402	-42.936	57.772	1.53
ExcludedVolume_1.33	42.257	-45.941	58.916	1.368

Hypo1 (Table 2, 3, figure 1A) was used as a searching tool for NCI database (257,000 compounds) out of which 683 compounds were captured and ranked according to the fit values. The highest ranked 40 compounds were ordered and tested in vitro for Hsp90 inhibition using malachite green assay. 15 tested compounds (**1**-**15**) (Figure 4, 5) showed Hsp90 inhibition as illustrated in table 4 while the rest compounds were inactive. Table 4 summarizes the fit values for the generated pharmacophore Hypo1, percentage of inhibition at 100 µM. Figure 1 shows the interaction of geldanamycin with the amino acids in the binding pocket; ASP93, LYS58, MET98, ASN51, LYS112, PHE138 and also with the bridging water HOH384, HOH521 and HOH520. These interactions are expressed by hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) and hydrophobe (Hbic) features. It is clear that forbiden areas for inteactions are filled with exclusion spheres which represent the steric clash and unfavourable interactions.

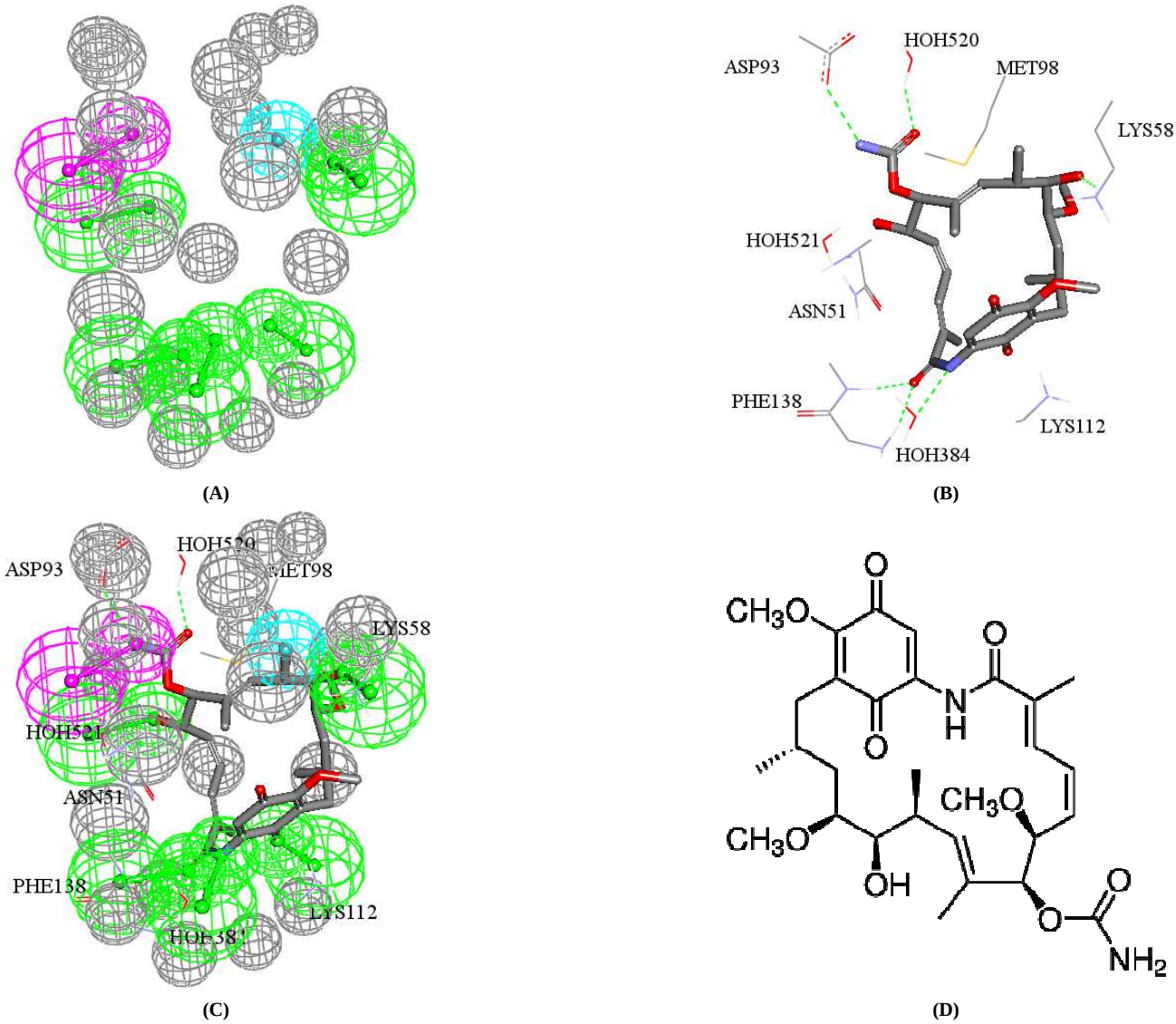


Figure 1 : (A) Hypo1, the best pharmacophore generated by DS studio 4.0, HBA as green vectored two spheres, HBD as pink vectored two spheres, Hbic feature as blue sphere, Exclusion volume as gray spheres, (B) Cocrystalized Geldanamycin in Hsp90 (1YET, resolution 1.9A), (C) Mapping of pharmacophore with co-crystallized Ligand GMD, (D) Chemical structure of geldanamycin.

Furthermore Figure 2 shows the possible interaction of cocrystalized structure (GMD) inside the binding pocket of Hsp90 (pdb: 1 YET) after 2D analysis using DS3.5 visualizer. It shows the cocrystalized compound (geldanamycin) inside the ATPase binding site of Hsp90, the corresponding amino acids that are involved in the interactions significantly are; ASP93, SER52, LYS58, ASN51, PHE138,GLY137, GLY135, ASP54, LYS112 (Hydogen bond interactions) in addition to MET98, LEU107, ASP102, ALA55 and ILE96 (Van der Waals interactions-hydrophobic interactions). Those amino acids form the binding pocket for the corresponding cocrystalized ligand (GMD). By comparing the mapping features of Hypo1 in Figure 1 (C); that could be explained as follows; methyl group mapped with hydrophobic feature corresponding to Van der Waals interaction with the hydrophobic part of MET98, LEU107, ASP102, amide group mapped with hydrogen bond donor corresponding to interaction of NH groups with carboxyl group of ASP93. Furthermore, oxygen group mapped with four hydrogen bond acceptor corresponding to interaction of oxygen in methoxy group with ASN51, oxygen of amide group with NH group of PHE138, NH group of amide with bridging water HOH384, oxygen of quinone with NH group of LYS112, oxygen of OH group with amino group of LYS58 as shown in figure 1 (B). Mapping of geldanamycin with Hypo1 as shown in figure 1 (C) correlated with the cocrystalized pose of geldanamycin within the binding pocket of Hsp90 (PDB: 1YET, resolution 1.9A). Docked poses of compound **1** and **2** inside the binding pocket of Hsp90 compared with the mapped features in Hypo1 as follows: phenyl group mapped with hydrophobic feature correspoding to Van der Waal interaction with hydrophobic part of MET98 , hydrogen bond donor corresponding to interaction of OH groups of compound **1** with corresponding NH groups of ASN51 through bridging water HOH521, oxygen of phosphate group with NH group

of PHE138 and LYS112 in addition to bridging water HOH384, oxygen of amide group with NH group of LYS58, as shown in figure 3 (A,B,C).

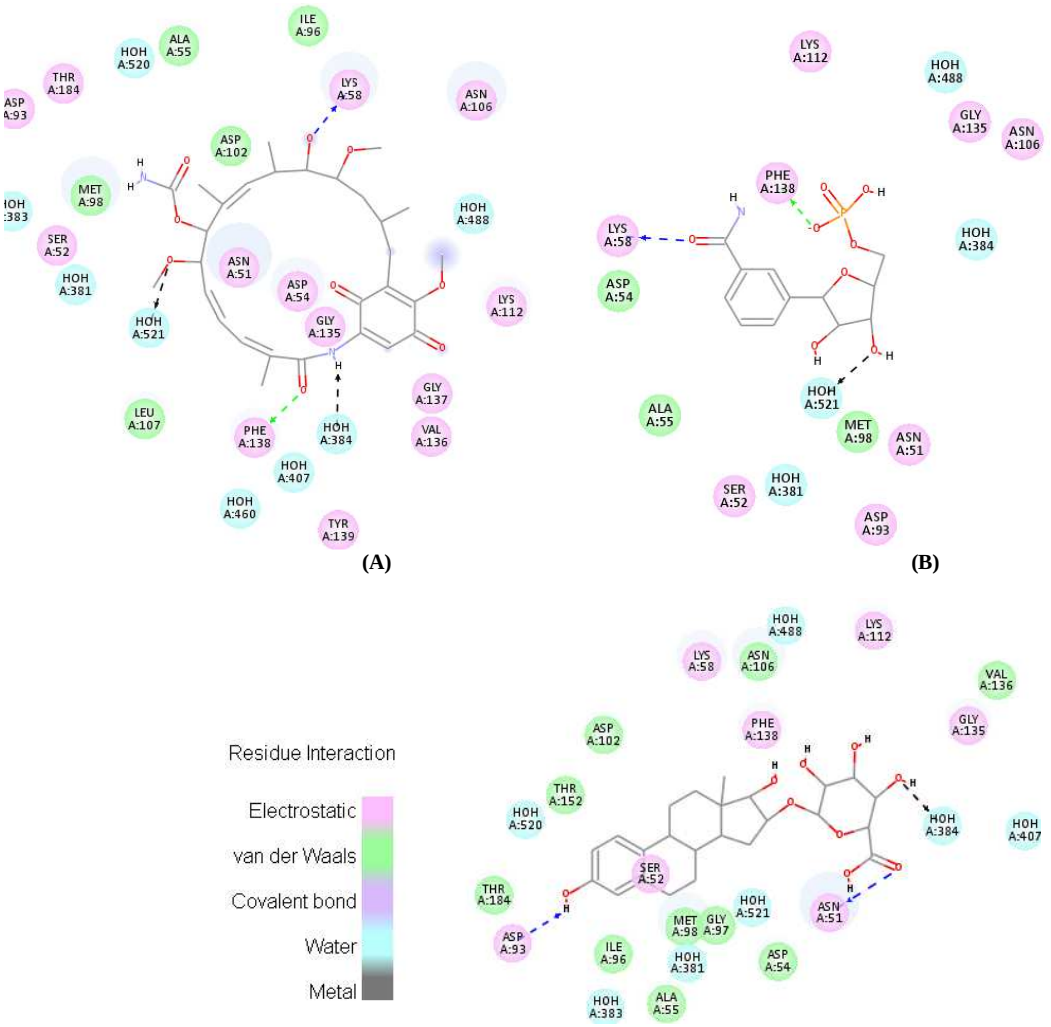
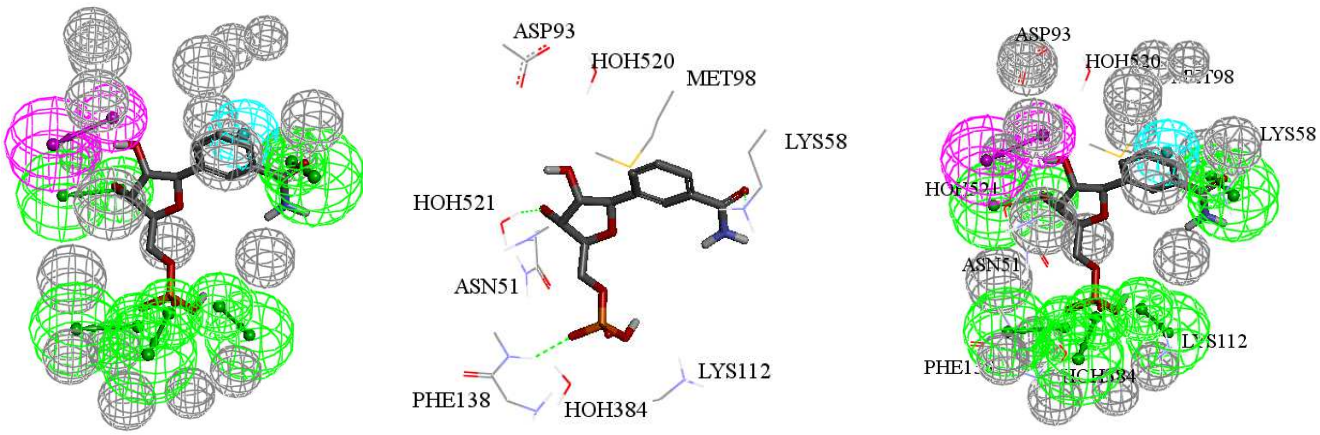
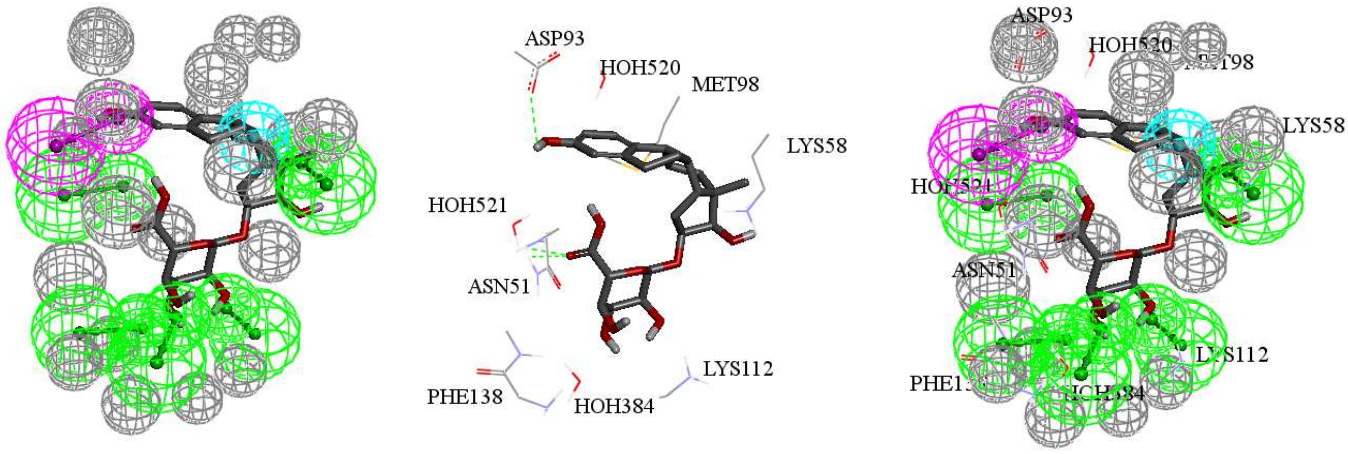


Figure 2: Two dimensional analysis of (A) the co-crystallized geldanamycin in the ATPase binding site of Hsp90 (1YET, resolution 1.9A) (B) compound 1, (C) compound 2 , using DS 3.5 visualizer.





(D) **(E)** **(F)**

Figure 3 : (A) Mapping compound (1) with Hypo1; co-crystallized pharmacophore generated by DS studio 4.0, HBA as green vectored two spheres, HBD as pink vectored two spheres, Hbic feature as blue sphere, Exclusion volume as gray spheres, (B)Docking compound (1) in ATPase binding pocket of Hsp90 (1YET, resolution 1.9A), (C) compound (1) mapped and docked (D) Mapping compound (2) with cocrystalized pharmacophore generated by DS studio 4.0, HBA as green vectored two spheres, HBD as pink vectored two spheres, Hbic feature as blue sphere, Exclusion volume as gray spheres, (E)Docking compound (2) in ATPase binding pocket of Hsp90 (1YET, resolution 1.9A), (F) compound (1) mapped and docked

Table 4: NCI compounds with Hsp90 inhibitory values

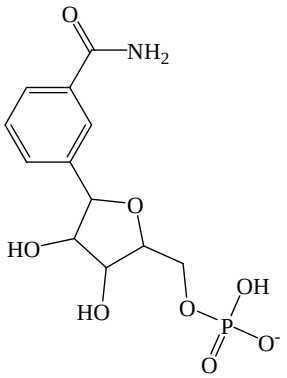
No	NCI[a]	Hypo1[b]	%Inhibition[c]
1	645648	1.94	41.6%
2	93823	1.1	36.3%
3	Coromolyn	1.32	22.7%
4	602892	1.04	20.12%
5	Riboflavin	0.01	19.93%
6	Cefapirin	1.22	14,6%
7	70544	1.06	13.2%
8	281311	1.44	11.77%
9	70532	1.98	11.53%
10	170988	1.44	11.29%
11	289523	1.23	11.0%
12	289643	1.24	10%
13	72234	1.80	9.4%
14	609428	2.60	9.2%
15	32977	1.57	8.4%
16	*AAG	---	75.73%

**AAG standard inhibitor for Hsp90*

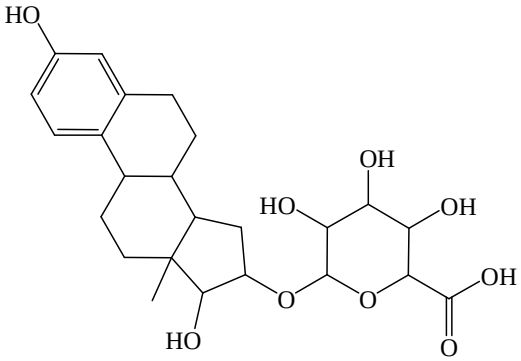
[a] *NCI screened and bioassay hits*

[b] *Hsp90 pharmacophoric model generated from co-crystallized structure.*

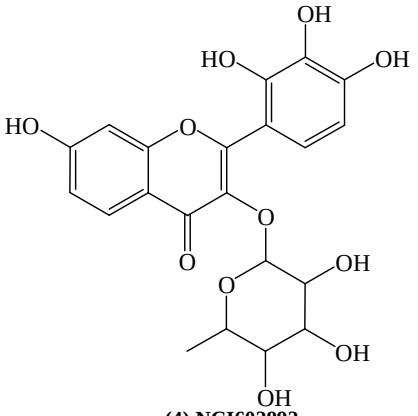
[c] *Percentage of inhibition at 100micM concentration of inhibitor.*



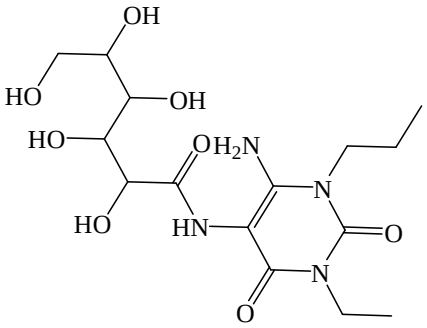
(1) NCI645648



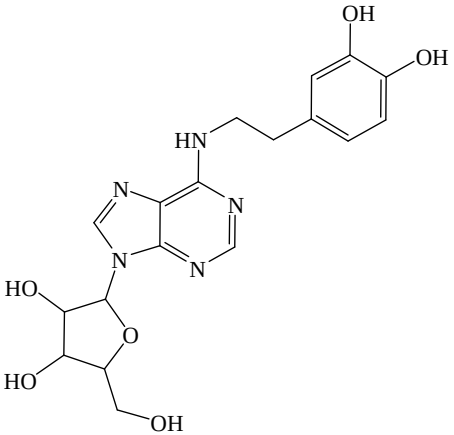
(2) NCI093823



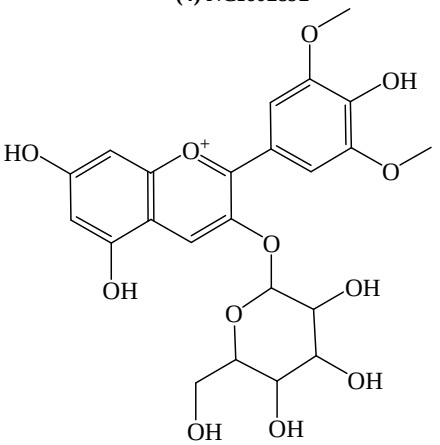
(4) NCI602892



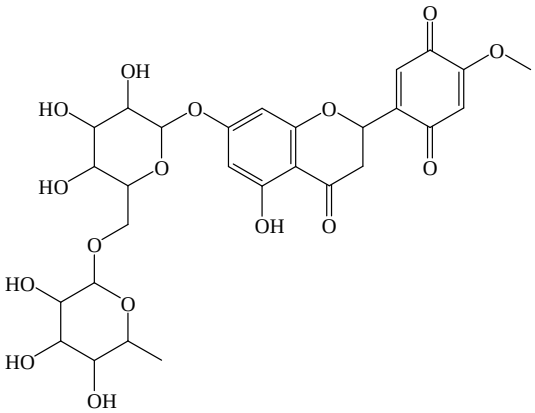
(7) NCI070544



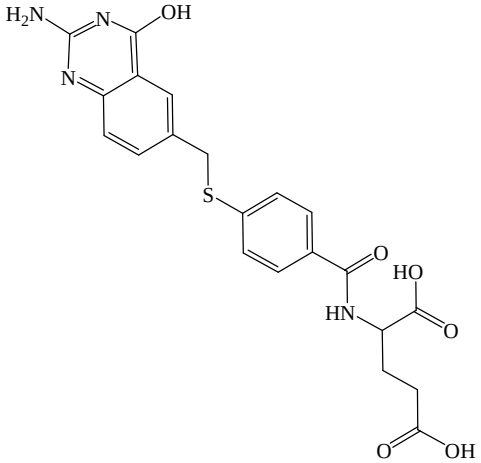
(8) NCI281311



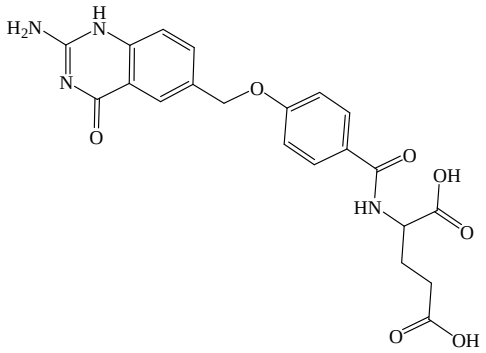
(9) NCI070532



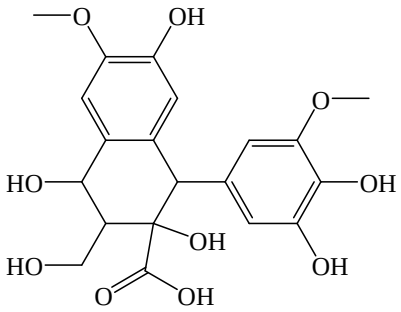
(10) NCI170988



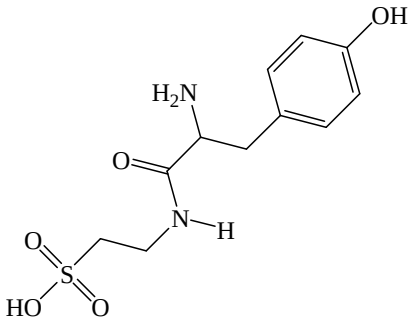
(11) NCI289523



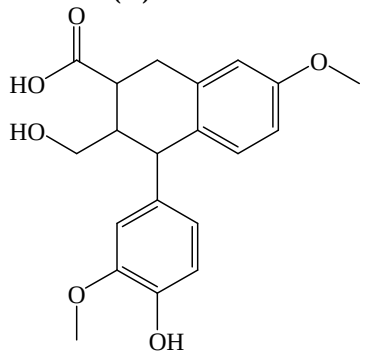
(12) NCI289643



(13) NCI072234

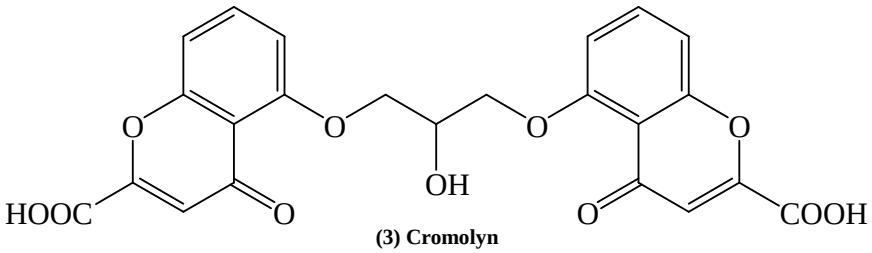


(14) NCI609428

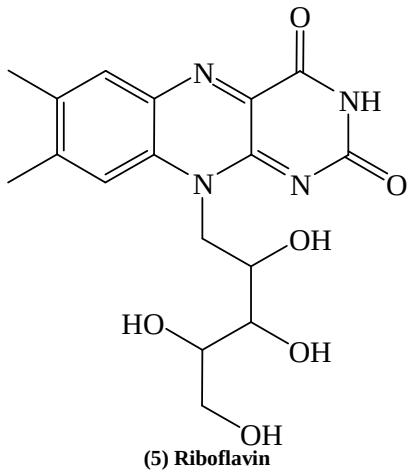


(15) NCI032977

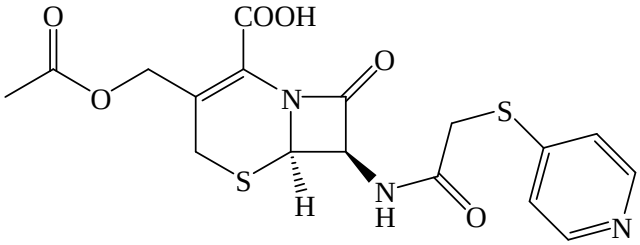
Figure 4: Chemical structure of tested NCI compounds



(3) Cromolyn



(5) Riboflavin



(6) Cefapirin

Figure 5: Chemical structure of drugs tested for Hsp90 inhibition

Moreover, compound **2** shows similar interactions as shown in figure 3 (D, E, F). Two dimensional analysis of co-crystallized ligand (GMD) of Hsp90 (pdb: 1YET, resolution 1.9A) using the function (Analyze single complex) in Discovery Studio DS 3.5 was used as shown in figure 2A, further analysis of docked poses of compound **1** and **2** as shown in figure 2 (B, C). It is clear that several types of interactions with corresponding amino acids in the binding pocket. However, figure 1 (B), figure 3 (B, E) show the most important amino acids that correlated with the mapped features in Hypo1.

CONCLUSION

Despite that the virtual co-crystallized structure lead to a pharmacophore model that captured moderately active compounds, it is still a good tool for new lead discovery with novel scaffolds. Further optimization of pharmacophore generation from virtual co-crystallized structure that consider the interaction with the most important amino acids inside the dynamic binding pocket of Hsp90 is needed for optimal model.

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