Natural compounds as potential Hsp90 inhibitors for breast cancer-Pharmacophore guided molecular modelling studies

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ABSTRACT

Breast cancer is one of the major impediments affecting women globally. The ATP-dependant heat shock protein 90 (Hsp90) forms the central component of molecular chaperone machinery that predominantly governs the folding of newly synthesized peptides and their conformational maturation. It regulates the stability and function of numerous client proteins that are frequently upregulated and/or mutated in cancer cells, therefore, making Hsp90 inhibition a promising therapeutic strategy for the development of new efficacious drugs to treat breast cancer. In the present in silico investigation, a structure-based pharmacophore model was generated with hydrogen bond donor, hydrogen bond acceptor and hydrophobic features complementary to crucial residues Ala55, Lys58, Asp93, Ile96, Met98 and Thr184 directed at inhibiting the ATP-binding activity of Hsp90. Subsequently, the phytochemical dataset of 3210 natural compounds was screened to retrieve the prospective inhibitors after rigorous validation of the model pharmacophore. The retrieved 135 phytocompounds were further filtered by drug-likeness parameters including Lipinski’s rule of five and ADMET properties, then investigated via molecular docking-based scoring. Molecular interactions were assessed using Genetic Optimisation for Ligand Docking program for 95 drug-like natural compounds against Hsp90 along with two clinical drugs as reference compounds – Geldanamycin and Radicicol. Docking studies revealed three phytochemicals are better than the investigated clinical drugs. The reference and hit compounds with dock scores of 48.27 (Geldanamycin), 40.90 (Radicicol), 73.04 (Hit1), 72.92 (Hit2) and 68.12 (Hit3) were further validated for their binding stability through molecular dynamics simulations. We propose that the non-macrocyclic scaffolds of three identified phytochemicals might aid in the development of novel therapeutic candidates against Hsp90-driven cancers.

1. Introduction

Breast cancer is one of the leading causes of cancer related mortalities noticed in women globally (Ghoncheh et al., 2016) with 39,620 recorded deaths in USA (Zagouri et al., 2013). Predominantly, breast cancer demonstrates metastasis to bone, lung, liver and brain (López de Victoria and Koculi, 2015). Though, Hsp90 is in increasing levels in many of the human cancers, its overexpression in breast cancer is aligned with poor progression (Bagatell and Whitesell, 2004). This chaperone communicates with the proteins promoting breast cancer such as estrogen receptor (ER), antiapoptotic kinase Akt, tumor suppressor p53 protein, Raf-1 MAP kinase, angiogenesis transcription factor HIF-1alpha, and receptor tyrosine kinases from erbB family (Miyata et al., 2013; Zagouri et al., 2013, 2012). Therefore, targeting Hsp90 would undermine the breast cancer cases altogether.

In recent years, molecular chaperones emerged as attractive drug targets for therapeutic research due to their distinct cellular distribution (Ahmad and Muzaffar, 2016). They participate in ensuring the proper folding of proteins (Hoter et al., 2018), thereby maintaining intricate balance between protein synthesis and degradation (Franke et al., 2013). The misfolded proteins may alter normal proliferation and physiological functioning of cells leading to the six hallmarks of cancer (Amolins and Blagg, 2009; Blagg and Kerr, 2006; Hanahan and Weinberg, 2011). Heat shock proteins comprise a group of molecular...
chaperones, of which, the highly conserved 90 kDa Hsp90 is the core component of oligomeric chaperone machine that collaborates with a cluster of co-chaperones to attain the folding, activation and stabilization of numerous client proteins, thus maintaining cellular homeostasis (Hoter et al., 2018; Verma et al., 2016). These client proteins involved in multiple signalling pathways are frequently upregulated in cancer cells, thus making inhibition of Hsp90 as a suitable strategy to develop new effective anti-cancer drugs (Ahmad and Muzaaron, 2016; Butler et al., 2016; Franke et al., 2013).

Hsp90 is a member of the Gyrase-Hsp90-histidine Kinase-MutL (GHKL) superfamily of homodimeric ATPases (Butler et al., 2016) with Bergerat fold geometry (Sidera and Patsavoudi, 2014). It prevails in four isoforms: cytosolic Hsp90α and Hsp90β, mitochondrial tumor necrosis factor (TNF) receptor-associated protein-1 (Trap-1) and

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Table 1

| S. No. | Parameters                          | In-house database | DUD database |
|-------|-------------------------------------|-------------------|--------------|
| 1     | Total number of molecules in database (D) | 160               | 83           |
| 2     | Total number of actives in database (A)   | 22                | 22           |
| 3     | Total number of hits retrieved from the database (Ht) | 25               | 18           |
| 4     | Total active molecules in the hit list (Ha) | 17               | 17           |
| 5     | % Yield of active [(Ha/Ht) × 100]         | 68                | 94.44        |
| 6     | % Ratio of actives [(Ha/A) × 100]         | 77.27             | 77.27        |
| 7     | Enrichment Factor (EF)                 | 4.9               | 3.5          |
| 8     | False negatives (A-Ha)                 | 5                 | 5            |
| 9     | False positives (Ht-Ha)                | 8                 | 1            |
| 10    | Goodness of fit score (GF)             | 0.668             | 0.88         |

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Fig. 1. Generation of receptor-based pharmacophore model. A) Pharmacophore model mapped against the co-crystallized ligand, 2-(1H-pyrrol-1-ylcarbonyl) benzene-1, 3, 5-triol (PYU) within the ATP-binding pocket of NTD of Hsp90. B) Each key residue has demonstrated with the required pharmacophore features C) Interfeature distances within the pharmacophore features.

Fig. 2. Retrieval of potential hit compounds from the natural compound phytochemical dataset using the pharmacophore model.

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new effective anti-cancer drugs (Ahmad and Muzaaron, 2016; Butler et al., 2016; Franke et al., 2013).

Hsp90 is a member of the Gyrase-Hsp90-histidine Kinase-MutL (GHKL) superfamily of homodimeric ATPases (Butler et al., 2016) with Bergerat fold geometry (Sidera and Patsavoudi, 2014). It prevails in four isoforms: cytosolic Hsp90α and Hsp90β, mitochondrial tumor necrosis factor (TNF) receptor-associated protein-1 (Trap-1) and
Table 2

| Compound Name | GOLD Fitness Score | Interaction Generation | Residues involved in van der Waals interactions | Residues involved in H-bond interactions (distance in Å) |
|---------------|--------------------|------------------------|-----------------------------------------------|------------------------------------------------------|
| Reference1 (Geldanamycin) | 48.27              | Asn51:Asn55, Gln58:Glu86, Ala55:Val92, Gln93:Glu90, Ala56:Glu90, Val92:Glu102, Leu107:Glu138, Thr184:Thr184 | Asn51 : Asn55, Gln58:Glu86, Ala55:Val92, Gln93:Glu90, Ala56:Glu90, Val92:Glu102, Leu107:Glu138, Thr184:Thr184 | Asn51 : Asn55, Gln58:Glu86, Ala55:Val92, Gln93:Glu90, Ala56:Glu90, Val92:Glu102, Leu107:Glu138, Thr184:Thr184 |
| Reference2 (Radicicol) | 40.90              | Lys58:NZ-O2, Asp93:OD2-H41, Asn51, Ser52, Asp54, Ile91, Ile96, Asp102, Asn106, Leu107, Thr184:OG1-O42 | Lys58:Asp93:Asn51,Ser52,Asp54,Ile91,Ile96,Asp102,Asn106,Leu107,Thr184:OG1-O42 | Lys58:Asp93:Asn51,Ser52,Asp54,Ile91,Ile96,Asp102,Asn106,Leu107,Thr184:OG1-O42 |
| Hit1 | 73.04              | Lys58:NZ-O44, Asp93:OD1-H80, Asn106:O-H74, Ile91:Asp54, Gly95, Ile96, Gly97, Asp102, Leu103, Thr184:OG1-O42 | Lys58:Asp93:Asn51,Ser52,Asp54,Ile91,Ile96,Asp102,Asn106,Leu107,Thr184:OG1-O42 | Lys58:Asp93:Asn51,Ser52,Asp54,Ile91,Ile96,Asp102,Asn106,Leu107,Thr184:OG1-O42 |
| Hit2 | 72.92              | Asn51:O-H59, Val92:Asp93, Phe138:N-O5, Ile26, Asp54, Ala55, Val92, Asp93, Ile96, Gly97, Asn106, Leu107, Ile26, Asp54, Ala55, Val92, Asp93, Ile96, Gly97, Asn106, Leu107 | Asn51:O-H59, Val92:Asp93, Phe138:N-O5, Ile26, Asp54, Ala55, Val92, Asp93, Ile96, Gly97, Asn106, Leu107 | Asn51:O-H59, Val92:Asp93, Phe138:N-O5, Ile26, Asp54, Ala55, Val92, Asp93, Ile96, Gly97, Asn106, Leu107 |
| Hit3 | 68.12              | Thr184:OG1-O7, Ile26, Glu47, Lys58:Asp93, Ile91:Asp54, Gly95, Ile96, Asn106, Ile110, Ala111, Gly132, Gly135, Gly137, Tyr139 | Thr184:OG1-O7, Ile26, Glu47, Lys58:Asp93, Ile91:Asp54, Gly95, Ile96, Asn106, Ile110, Ala111, Gly132, Gly135, Gly137, Tyr139 | Thr184:OG1-O7, Ile26, Glu47, Lys58:Asp93, Ile91:Asp54, Gly95, Ile96, Asn106, Ile110, Ala111, Gly132, Gly135, Gly137, Tyr139 |

2. Materials and methods

2.1. Generation of the structure-based pharmacophore model

Receptor-based pharmacophore model utilizes the known active site of a protein to identify effective competitive inhibitors and hence the structure of Hsp90 substrate binding domain with its bound inhibitor was retrieved from RCSB (PDB ID: 3EKO, chain A) (Kung et al., 2008). Binding site was defined within 10 Å around the bound co-crystallized ligand elucidating the key complementary features as evaluated by the Interaction Generation module available with Discovery Studio (DS) v18.1.0. Subsequently, the Receptor-Ligand Pharmacophore Generation module within DS was employed for the generation of pharmacophore models.
2.2. Validation of the pharmacophore models

The validation of selected pharmacophore is an important criterion to ensure that the model retrieves active compounds from a given dataset. Accordingly, the chosen model was escalated to map two different datasets such as the decoy set (an in-house dataset), also called as Güner-Henry scoring method (Jones and Willet, 2000) and the directory of useful decoys (DUD). Accordingly, the model was computed on the basis of goodness of fit (GF) score and enrichment factor (EF) (Sakkiah et al., 2010) for evaluating the robustness of pharmacophore, where the GF score functions as a pivotal factor determining the quality of pharmacophore ranging between 0 (null model) and 1 (ideal model).
Table 3  
Comparison of pivotal amino acids required for Hsp90 inhibition analysed from 27 co-crystallized ligands of X-ray structures with better resolution than PDB ID: 3EKO.

| PDB ID | Resolution (Å) | 2D-structure of co-crystallized ligand | Asn51 | Ala55 | Lys58 | Asp93 | Met98 | Gly97 | Phe138 | Thr184 |
|--------|----------------|----------------------------------------|-------|-------|-------|-------|-------|-------|--------|--------|
| 1BYQ   | 1.50           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |
| 2YEF   | 1.55           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |
| 2YI7   | 1.40           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |
| 2YK9   | 1.32           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |        |
| 2YKE   | 1.43           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |        |
| 2YKJ   | 1.46           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |
| 3B27   | 1.50           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3B28   | 1.35           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔      |         | ✔      | ✔      |
| 3EKO   | 1.55           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      |
| 300I   | 1.47           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3T1K   | 1.50           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3T2S   | 1.50           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3T10   | 1.24           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3VHA   | 1.39           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      | ✔      |
| 3VHC   | 1.41           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3VHD   | 1.52           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |
| 3WHA   | 1.30           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      | ✔      |
| 4LWE   | 1.50           | ✔                                      | ✔     | ✔     | ✔     | ✔     | ✔     | ✔      | ✔      | ✔      |

(continued on next page)
where $D$ signifies the total molecules and $A$ represents the total active molecules in the data set, $Ht$ indicates the total retrieved hits from the database, while $Ha$ refers to actives present within the retrieved hits.

2.3. Virtual screening of the natural compounds dataset

The generated pharmacophore model was utilized to screen the natural compounds dataset of 3210 compounds employing the Ligand Pharmacophore Mapping module with rigid fitting method to search for the novel scaffolds with an ability to inhibit Hsp90. The compounds were chosen from datasets with known anticancer properties, such as NPACT (Mangal et al., 2013) and unknown anticancer properties. The ADMET and Ro5 was executed for the compounds with unknown anticancer properties. Natural compounds are exceptional to rule of 5 (Ming-Qiang Zhang and Barrie Wilkinson, 2007). The compounds mapped were monitored for their drug-likeness and pharmacokinetics by Lipinski’s rule of 5 (Ro5) (Lipinski, 2004) and absorption, distribution, metabolism, excretion and toxicity (ADMET) properties (Tareq Hassan Khan, 2010). Accordingly, the ADMET Descriptors module within the DS was employed for evaluation of ADMET properties of the mapped compounds. The resultant compounds were further scrutinized by Ro5 which determines molecular weight ($< 500$ Da), hydrogen bond donors ($< 5$), hydrogen bond acceptors ($< 10$), rotatable bonds ($< 10$) and lipophilicity ($\log P < 5$). The obtained drug-like compounds were subjected to molecular docking along with 2 reference compounds – GA (Reference 1) and RD (Reference 2).

2.4. Molecular docking studies

The compounds that conformed the above-mentioned criteria were further evaluated by molecular docking with Genetic Optimisation for Ligand Docking (GOLD) program v5.2.2 (Verdonk et al., 2003). Molecular docking guides in screening the compounds that accommodate well within the protein active site elucidating the ideal binding mode of small molecules for which GOLD uses genetic algorithm revealing partial flexibility of protein accompanied by ligand flexibility exploring...
the conformational space within the active site (Kitchen et al., 2004). The inhibitor binding positions and accuracy of GOLD docking is ensured by two scoring functions—GoldScore and ChemScore (Verdonk et al., 2003). The default GoldScore operates by scoring the sum of protein-ligand van der Waals energy and H-bonding energy, ligand torsional strain and internal van der Waals energy, whereas ChemScore assesses the total free energy associated with ligand binding along with metal-binding and hydrogen-bonding interactions.

The 3D structure of Hsp90 complexed with compound PYU (2-(1H-pyrrol-1-ylcarbonyl) benzene-1, 3, 5-triol) was retrieved from PDB (ID: 3EKO). Prior to docking, the protein was prepared by utilizing the Clean Protein module present in DS followed by supplementing the missing residues and hydrogen atoms after removing water molecules and the bound ligand PYU. To gauge on the docking parameters, the ligand in crystal structure was redocked into the active site that resulted in the binding of docked pose at the site of inbound ligand, (Supplementary 1). This finding validates the docking methodology as well as ensures the molecular docking parameters and the same were applied further. The active site was predicted that comprises of all atoms within 10 Å range around the co-crystallized ligand. The obtained drug-like molecules were subsequently docked along with reference compounds, into the defined active site of Hsp90, allowing 50 conformers to be generated for each ligand while keeping all other parameters as default. This was followed by clustering, in which the best binding mode was retrieved from the largest cluster after examining compounds with higher dock scores than reference molecules and key interactions at the binding pocket. Correspondingly, the selected poses were further evaluated by molecular dynamics simulations using GROningen MACHine for Chemical Simulations (GROMACS) v5.0.6 (Abraham et al., 2015).

2.5. Molecular dynamics simulation studies

To further decipher the dynamic behavior of filtered hits at the active site of Hsp90 for affirming the obtained binding modes retrieved from docking results, molecular dynamics (MD) simulations were executed. GROMACS was employed for assessing the best docked poses utilizing CHARMM27 force field (Van Der Spoel et al., 2005; Rampogu et al., 2018a) and ligand topologies generated by SwissParam (Zoete et al., 2011; Rampogu et al., 2018c). Simulations were undertaken in a dodecahedral water box solvating with TIP3P water model and system was neutralized with counter ions. The steepest descent energy minimization algorithm was executed to circumvent bad contacts from the initial structures, further subjecting them to NVT and NPT equilibration, independently. The NVT ensemble (constant number of particles, volume and temperature) was orchestrated at 300 K for 1 ns with a V-rescale thermostat complemented with NPT ensemble (constant number of particles, pressure and temperature) at 1 bar pressure for 1 ns with a Parrinello-Rahman barostat (Parrinello and Rahman, 1981). The geometry of water molecules and bond constrains were monitored by SETTLE (Miyamoto and Kollman, 1992) and LINear Constraint Solver (LINCS) (Hess et al., 1997) algorithms. This was followed by employing Particle Mesh Ewald (PME) (Darden et al., 1993) for computing long-range electrostatic interactions with a cut-off of 1.2 nm, while calculating short-range non-bonded interactions within a cut-off of 1.2 nm. The equilibrated NPT ensembles of each system were subjected to MD simulations for 10 ns. The obtained results were
investigated using visual molecular dynamics (VMD) (Humphrey et al., 1996) on the basis of root mean square deviation (RMSD). Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) methodology was employed for each of the five systems, extracting 25 snapshots from stable MD trajectories by energy_MM.xvg and the results were evaluated on the basis of protein-ligand total energy (Baker et al., 2001; Kumari et al., 2014).

3. Results

3.1. Structure-based pharmacophore model reveals features required for effective inhibition of Hsp90

A structure-based pharmacophore model generated from human Hsp90α domain complexed with PYU, comprises of a hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) and hydrophobic (HyP) features (Fig. 1A and B). The three-featured model with interfeature distance constraints between the obtained features (Fig. 1C) is consistent with the key residues, Asp93 and Thr184 that are required for the inhibition of Hsp90 along with residues Ala55, Lys58, Ile96, and Met98.

3.2. Decoy set validation of the pharmacophore model

The pharmacophore model was subsequently validated by goodness of fit score (GF) for evaluating its ability in distinguishing active and inactive compounds, according to Güner-Henry (GH) scoring method. As expected, the pharmacophore model had the capability of discriminating known actives from the inactives. This validation was instigated by screening an in-house database (D) of 160 molecules and Directory of Useful Decoys (DUD) database (D) of 83 molecules, keeping the 22 active molecules (A) in both the databases. The GF of 0.668 (in-house database) and 0.88 (DUD database) confirmed the reliability of the pharmacophore model. Furthermore, the percentage ratio of actives was found to be 77.27, thus approving that the selected pharmacophore exhibited an excellent quality (Table 1).

3.3. Identification of drug-like natural compounds by virtual screening

The validated pharmacophore model retrieved 135 phytochemicals that satisfied the pharmacophore features, which were subsequently filtered on basis of their ADMET properties and Lipinski’s rule of five (Ro5), which resulted in 95 natural compounds as potential candidates to inhibit Hsp90 (Fig. 2). These 95 phytochemicals along with reference molecules were taken forward to scrutinize their inter-molecular interactions with key residues Asp93 and Thr184 as well as other residues including Asn51, Ala55, Lys58, Gly97, Met98 and Phe138 by docking them at the ATP-binding site of Hsp90.

3.4. Molecular docking studies to discover potential hits for Hsp90 inhibition

Virtual screening was followed by molecular docking to discover potential hits based on their binding affinity with Hsp90. The 95 phytochemicals were subjected to molecular docking along with two clinical drugs – GA and RD as reference molecules. Ten phytochemicals demonstrating a higher dock score than the reference compounds were investigated in detail for suitable molecular interactions needed for the inhibition of Hsp90. The top three compounds herein referred to as hits conferred a dock score of 73.04 (Hit1), 72.92 (Hit2) and 68.12 (Hit3), while reference compounds displayed a lower dock score of 48.27 for GA (Reference 1) and 40.90 for RD (Reference 2) (Table 2). The three hits and two reference molecules were manually probed for their interactions with the key residues and analyzed through MD simulations for their stability in the Hsp90 active site.
Table 4: Intermolecular interactions and docking scores of reference and hit compounds.

| Compound Name | GOLD Fitness | Hydrogen bond interactions | van der Waals interactions |
|---------------|--------------|----------------------------|----------------------------|
| Reference     | 67.77        | Ser16, Thr19, Thr184       | Met98, Tyr139, Leu107, Val86 |
| Hit1          | 72.71        | Ala21, Thr19, Thr184, Ser16, Ile104, Leu107, Val86, Tyr139, Met98, Val186, Gly95, Ala55, Gly135, Ile104, Gly108, Ile110, Gly135, Val136, Ala55, Gly58, Met98, Gly135, Val186 |
| Hit2          | 72.25        | Ala21, Thr19, Thr184, Ser16, Gly95, Ala55, Gly135, Ile104, Leu107, Val86, Tyr139, Met98, Val186, Gly95, Ala55, Gly58, Met98, Gly135, Val186 |
| Hit3          | 72.25        | Ala21, Thr19, Thr184, Ser16, Gly95, Ala55, Gly135, Ile104, Leu107, Val86, Tyr139, Met98, Val186, Gly95, Ala55, Gly58, Met98, Gly135, Val186 |

Further, upon outlining the intermolecular interactions of hits with Hsp90, it was observed that reference compound, GA formed seven hydrogen bonds with residues Asn51, Lys58, Asp93, Ile96, Gly97, Asn106, and Gly135, while Leu48, Ser52, Gly95, Asp102, Val1136, Gly137, Thr152, Gly183, Thr184 and Val186 held GA via van der Waals interactions. The ND2 atom of residue Asn51 formed a hydrogen bond of length 3.2 Å with the O4 atom of the benzoquinone ring of the ligand. The NZ atom of Lys58 interacted with the O3 atom of the ansa ring of ligand by hydrogen bond length of 3.0 Å. Another hydrogen bond was formed between the OD1 atom of Asp93 and the H67 atom of the ansa ring of ligand with an acceptable bond length of 2.1 Å. The N atom of both Ile96 and Gly97 have interacted by hydrogen bonding with bond lengths of 3.1 Å and 2.5 Å, respectively with the O9 atom of ansa ring of the ligand. The O8 atom and the H56 atom of ansa ring functioned as hydrogen bonds with atoms ND2 and O of Asn106 and Gly135 with bond length of 2.7 Å, respectively. Additionally, Asn51 and Ala55 have formed π-π stacked and π-alkyl hydrophobic interactions with the benzoquinone ring of the ligand with bond distance of 4.0 Å and 4.7 Å, respectively. Met98 interacted with the ansa ring via two alkyl hydrophobic bonds of lengths 4.9 Å and 3.0 Å. Furthermore, Leu107 and the benzene ring of Phe138 formed alkyl and π-alkyl interactions with C18 atom of the ansa ring with a distance of 4.3 Å and 4.1 Å, respectively. In addition, Asn106 and Gly135 hold GA via carbon-hydrogen bonds (Fig. 4A and Table 2, Supplementary 2).

Reference compound, RD has formed two hydrogen bonds with residues Lys58 and Asp93 of Hsp90. The NZ atom of Lys58 has interacted with O2 atom of the epoxide moiety of RD with a bond length of 3.0 Å. Another hydrogen bond was observed near the resorcinol ring of the ligand with OD2 atom of Asp93 interacting with H41 atom by a bond length of 2.6 Å. The residue Ala55 formed a hydrophobic alkyl bond with C13 of the ansa ring with a bond distance of 3.5 Å. The ansa ring of RD interacted with Met98 via alkyl bond of length 4.0 Å. The residues Leu48 and Val186 held RD firmly through alkyl hydrophobic interactions with a bond distance of 4.2 Å and 4.4 Å, respectively, while benzene ring of Phe138 formed a π-alkyl bond of 4.1 Å at the C1 atom of resorcinol ring. Furthermore, the residues Asn51, Ser52, Asp54, Ile91, Ile96, Asp102, Asn106, Leu107, and Thr184 have assisted the binding of RD via van der Waals interactions and Gly97 via carbon-hydrogen bond (Fig. 4B and Table 2, Supplementary 2).

The hit compound, Hit1 formed four hydrogen bonds with residues Lys58, Asp93, Asn106 and Thr184 with bond lengths < 3 Å. The OD1 atom of Asp93 interacted with H80 atom of Hit1 with a length of 2.1 Å. Another hydrogen bond was observed between the OG1 atom of Thr184 and O42 atom of the ligand with bond distance 2.1 Å. The O44 atom of Hit1 bonded with NZ atom of Lys58 at an acceptable bond length of 2.7 Å. The O atom of Asn106 and H74 atom of ligand formed hydrogen bond of length 2.1 Å. Furthermore, the ring A has interacted with Ala55

3.5. Molecular dynamics simulations

MD simulations discern the dynamic behavior of hit compounds at the active site of Hsp90 and were thoroughly analyzed with their root mean square deviation (RMSD) profiles, binding modes and hydrogen bond counts. The inferred RMSD profiles demonstrated that all the compounds recorded an average RMSD of 0.30 nm. While Reference 1 and Reference 2 rendered a RMSD of 0.20 nm and 0.19 nm, the Hit1, Hit2 and Hit3 documented values of 0.20 nm, 0.20 nm and 0.18 nm respectively (Fig. 3A). Additionally, the conformations from last 2 ns were extracted and superimposed for subsequent binding mode analysis of the respective hit compounds and it was observed that the three hit compounds and the two reference compounds positioned in the binding pocket in a similar manner as the co-crystallized ligand PYU anchored by hydrogen bonding, van der Waals and hydrophobic interactions, (Fig. 3B). Delin-eating on the hydrogen bond interactions between the hits and key residues of Hsp90 revealed that the hits have rendered comparatively higher number of hydrogen bonds as compared to reference compounds (Fig. 3C).

Further, upon outlining the intermolecular interactions of hits with Hsp90, it was observed that reference compound, GA formed seven hydrogen bonds with residues Asn51, Lys58, Asp93, Ile96, Gly97, Asn106, and Gly135, while Leu48, Ser52, Gly95, Asp102, Val1136, Gly137, Thr152, Gly183, Thr184 and Val186 held GA via van der Waals interactions. The ND2 atom of residue Asn51 formed a hydrogen bond of length 3.2 Å with the O4 atom of the benzoquinone ring of the ligand. The NZ atom of Lys58 interacted with the O3 atom of the ansa ring of ligand by hydrogen bond length of 3.0 Å. Another hydrogen bond was formed between the OD1 atom of Asp93 and the H67 atom of the ansa ring of ligand with an acceptable bond length of 2.1 Å.

The N atom of both Ile96 and Gly97 have interacted by hydrogen bonding with bond lengths of 3.1 Å and 2.5 Å, respectively with the O9 atom of ansa ring of the ligand. The O8 atom and the H56 atom of ansa ring functioned as hydrogen bonds with atoms ND2 and O of Asn106 and Gly135 with bond length of 2.7 Å, respectively. Additionally, Asn51 and Ala55 have formed π-π stacked and π-alkyl hydrophobic interactions with the benzoquinone ring of the ligand with bond distance of 4.0 Å and 4.7 Å, respectively. Met98 interacted with the ansa ring via two alkyl hydrophobic bonds of lengths 4.9 Å and 3.0 Å. Furthermore, Leu107 and the benzene ring of Phe138 formed alkyl and π-alkyl interactions with C18 atom of the ansa ring with a distance of 4.3 Å and 4.1 Å, respectively. In addition, Asn106 and Gly135 hold GA via carbon-hydrogen bonds (Fig. 4A and Table 2, Supplementary 2).

Reference compound, RD has formed two hydrogen bonds with residues Lys58 and Asp93 of Hsp90. The NZ atom of Lys58 has interacted with O2 atom of the epoxide moiety of RD with a bond length of 3.0 Å. Another hydrogen bond was observed near the resorcinol ring of the ligand with OD2 atom of Asp93 interacting with H41 atom by a bond length of 2.6 Å. The residue Ala55 formed a hydrophobic alkyl bond with C13 of the ansa ring with a bond distance of 3.5 Å. The ansa ring of RD interacted with Met98 via alkyl bond of length 4.0 Å. The residues Leu48 and Val186 held RD firmly through alkyl hydrophobic interactions with a bond distance of 4.2 Å and 4.4 Å, respectively, while benzene ring of Phe138 formed a π-alkyl bond of 4.1 Å at the C1 atom of resorcinol ring. Furthermore, the residues Asn51, Ser52, Asp54, Ile91, Ile96, Asp102, Asn106, Leu107, and Thr184 have assisted the binding of RD via van der Waals interactions and Gly97 via carbon-hydrogen bond (Fig. 4B and Table 2, Supplementary 2).

The hit compound, Hit1 formed four hydrogen bonds with residues Lys58, Asp93, Asn106 and Thr184 with bond lengths < 3 Å. The OD1 atom of Asp93 interacted with H80 atom of Hit1 with a length of 2.1 Å. Another hydrogen bond was observed between the OG1 atom of Thr184 and O42 atom of the ligand with bond distance 2.1 Å. The O44 atom of Hit1 bonded with NZ atom of Lys58 at an acceptable bond length of 2.7 Å. The O atom of Asn106 and H74 atom of ligand formed hydrogen bond of length 2.1 Å. Furthermore, the ring A has interacted with Ala55
(π-σ, bond length 3.0 Å) and Met98 (π-alkyl, bond length 5.3 Å), ring B has interacted with Met98 (π-alkyl, bond length 4.5 Å), ring C has interacted with Val186 (π-alkyl, bond length 5.4 Å, Met98 (π-sulfur, bond length 5.3 Å) and ring D has joined with Lys112 (π-alkyl, bond length 4.5 Å). Additionally, Hit1 is held firmly by the residues Ile26, Leu48, Ser52, Asp54, Gly95, Ile96, Gly97, Asp102, Leu103, Asn105, Leu107, Ile110, Ala111, Val136, Gly137, Phe138, Tyr139 and Gly183 via van der Waals interactions and residues Asn51 and Gly135 via carbon hydrogen bonds (Fig. 4C and Table 2, Supplementary 2).

The hit compound, Hit2 formed three hydrogen bonds with residues Asn51, Ile91 and Phe138 with bond lengths < 3 Å, one carbon hydrogen bond with Asn51 and one π-donor hydrogen bond with Ser52. The O atom of residues Asn51 and Ile91 have interacted via hydrogen bonding with H59 and H74 of ligand at bond lengths of 2.7 Å and 2.4 Å, respectively. Another hydrogen bond was observed between N atom of Phe138 and O5 of ligand at 2.7 Å. Additionally, the ring A has interacted with Lys112 (π-alkyl, bond length 4.0 Å) and Val136 (π-alkyl, bond length 5.4 Å). The ring B has interacted with Leu48 (π-alkyl, bond length 5.2 Å), Asn51 (π-π stacked, bond length 4.7 Å) and Val186 (π-σ, bond length 3.2 Å) as well as ring C has interacted with Lys58 (π-cation, bond length 4.2 Å, Met98 (π-sulfur, bond length 5.9 Å) and Asp102 (π-anion, bond length 4.5 Å), respectively. Furthermore, the residues Ile26, Asp54, Ala55, Val92, Asp93, Ile96, Gly97, Asn106, Leu107, Ile110, Ala111, Gly132, Gly135, Gly137, Tyr139, His154, Thr184 and Lys185 have positioned the ligand firmly at its site via van der Waals interactions (Fig. 4D and Table 2, Supplementary 2).

The hit compound, Hit3 formed one hydrogen bond with residue Thr184, where OG1 atom of the residue interacted with O7 atom of Hit3 with bond length of 2.5 Å. Moreover, the ring A has interacted with residues Val136 and Lys112 (π-alkyl, bond length 4.9 Å). The ring B has formed a bond with Leu107 via alkyl hydrophobic interaction of length 5.2 Å. The residues Ala55 (π-alkyl, bond length 4.7 Å) and Met98 (π-alkyl, bond length 5.1 Å) have both interacted with the ring C of Hit3. Ring D has formed hydrophobic interactions with Leu48 (π-alkyl, bond length 4.7 Å), Asn51 (π-π stacked, bond length 5.0 Å), Ile91 (π-alkyl, bond length 5.0 Å) and Val186 (π-alkyl, bond length 4.9 Å). In addition, van der Waals interactions with residues Ile26, Glu47, Lys58, Val92, Asp93, Ile96, Asn106, Gly108, Ile110, Ala111, Thr115, Gly132, Gly135, Gly137, Phe138, Tyr138, Tyr139, and Lys185, carbon hydrogen bond with Gly97 and π-donor hydrogen bond with Ser52 has held the ligand firmly at the ATP-binding pocket of Hsp90 (Fig. 4E and Table 2, Supplementary 2).

4. Discussion

The heat shock protein, Hsp90 comprises of about 1–2% of the total cytosolic proteins that are found to communicate with around 200 client proteins including the co-chaperones, thereby demonstrating a significant role in protein-folding and thus gained importance as a drug target for various diseases (Toft, 1998; Amolins and Blagg, 2009). The inhibition of Hsp90 has a great potential in breast cancer therapeutics due to their amplitude in hindering a host of signalling pathways responsible for oncogenesis (Zagouri et al., 2013). It is well documented that the Hsp90 inhibitors degrade the HER2 and regulates the signalling of estrogen and progesterone receptor signals as they are the Hsp90 client proteins (Bagatell et al., 2001; Münster et al., 2001; Zagouri et al., 2012). These scientific evidences provide glimpses about the role of Hsp90 inhibitors across the major breast cancer subtypes (Caldas-Lopes et al., 2009; Song et al., 2010; Zagouri et al., 2012). Encouraged from
the scientific reports, we pursued our research towards identifying the phytochemical compounds as inhibitors for Hsp90. Earlier, our group has investigated for the Hsp90 inhibitors employing 3D-QSAR approach (Sakkiah et al., 2010) and that motivated to proceed with the structure-based pharmacophore modelling. Structure-based pharmacophore modelling works by employing the key interactions existing between the protein residues and the co-crystallized ligand.

The top three natural NPACT compounds (hits) from the resulting ten compounds were further evaluated on basis of their binding mode within the ATP-binding pocket of Hsp90 protein and the intermolecular interactions between hit compounds and active site residues of Hsp90. Importantly, the emphasis was given on critical amino acids – Asp93 and Thr184 that are required for Hsp90 inhibition. MD simulations of the three hits revealed that the natural compounds retain their intermolecular interactions and position in the binding pocket as observed with the reference compounds.

Although there are many Hsp90 experimentally determined structures reported till date, Hsp90 co-crystallized with PYU (PDB ID: 3EKO) was considered based on the atomic resolution (1.55 Å) and owing to the small size of co-crystallized ligand. Additionally, we analysed the critical amino acids for Hsp90 inhibition from 27 X-ray structures with better resolution than above mentioned structure, as reported in the previous study (Table 3) (Sakkiah et al., 2010). We intended at discovering phytochemicals larger than the co-crystallized ligand considering the ligand as a ‘fragment’ around which the obtained hits can be structured, with the perspective of identifying inhibitors having less adverse effects. We also analysed whether the ligands positioned at the ATP-binding pocket of Hsp90 by superimposing the 28 protein- ligand complexes as displayed (Fig. 5). It was observed that the ligands reflect similar binding modes as witnessed in chosen crystal structure which demonstrates its aptness.

The validated pharmacophore model and the molecular dynamics studies have retrieved three hit compounds displaying a higher dock score than the reference compounds, Geldanamycin and Radicicol. The identified phytochemicals have additionally demonstrated the key residue interactions with an RMSD below 3 Å throughout the simulations.

The intermolecular interactions put forth that the identified hits have nested in the active site clamped by several key residues. Upon scrupulous analysis of the hits, it was revealed that the key residue Asp93 has demonstrated a hydrogen bond interaction with Hit1, while in Hit2 and Hit3, it represented a van der Waals interaction whereby the significant interaction was preserved (Table 2). Furthermore, the MD simulation studies have demonstrated the presence of Ala55 rendered by hydrophobic π-π/π-alkyl interactions, in both the reference and in the hits. On the contrary, in the Hit2, Ala55 residue has prompted a van der Waals interactions, (Table 2). Such interactions were also previously reported and illuminates that the identified inhibitors might be effective Hsp90 inhibitors (Abassi et al., 2017). The intermolecular hydrogen bond interaction analysis have implied that the identified hits have demonstrated a higher number of hydrogen bonds than the reference compounds guiding to contemplate on the therapeutic usability of the hit compounds and have portrayed with the required pharmacophore features (Supplementary 3).

The MM/PBSA of Hsp90-ligand complexes with the reference ligands and three hits for 25 snapshots was computed to quantify the protein-ligand total energy (Fig. 6). Total energy of hit compounds, Hit 1 (−367.57 kcal/mol) and Hit 2 (−329.38 kcal/mol) was observed to be comparatively lower as compared to reference compounds GA (−277.67 kcal/mol) and RD (−239.58 kcal/mol), while Hit 3 (−262.95 kcal/mol) demonstrated higher total energy than GA and lower than RD.

Owing to the small size of our co-crystallized ligand (PDB ID: 3EKO) and large size of obtained hits, the crystal structure (PDB ID: 5LRL) with a relatively larger ligand size and better resolution was considered for comparing its interaction and docking score (Table 3), with that of our hits. Docking parameters were optimized by redocking the co-crystallized ligand (2-azanyl-5-chloranyl-1H-imidazo[4,5-c]pyridin-2-yl)-9H-fluoren-9-yl) pyrimidine-4-carboxamide, 73S) into the active site, resulting in a structural overlap of the docked pose with 73S. The three hit compounds were subsequently docked into the active site and evaluated on the basis of their GoldScore, binding mode and intermolecular interactions. The hit compounds positioned in the binding pocket in a similar fashion as the co-crystallized ligand, 73S (PDB ID: 5LRL) (Fig. 7). The hit compounds Hit1, Hit2 and Hit3 rendered better docking scores than 73S, retaining vital intermolecular interactions at the binding site (Table 4) and (Fig. 8). Manual scrutiny of the hits and compound, 73S revealed that the key residue Met98 formed hydrogen bond interaction with reference and Hit2, while in Hit1 and Hit3 it formed a π-π lone pair. Furthermore, Leu103 was observed to hydrogen bond with reference, whereas it formed a van der Waals interaction with hit compounds.

Hydrogen bond with Tyr139 was observed with all compounds, except Hit3, where it formed a van der Waals interaction.

5. Conclusion

Receptor-based pharmacophore model employing the 3D structure of Hsp90 protein bound with co-crystallized PYU revealed the pharmacophore features required for Hsp90 effective inhibition. The model targeting the ATP-binding site of Hsp90 comprises three features- hydrogen bond donor, hydrogen bond acceptor and hydrophobic features, which was subsequently used for virtual screening against a phytochemical dataset. The acquired 95 drug-like natural compounds after filtering by ADMET properties and Lipinski’s rule of five were taken forward to discover potential inhibitors against Hsp90. The three phytochemicals showed higher dock scores than the reference compounds (Geldanamycin and Radicicol) and significant binding interaction with Asp93 via hydrogen bond and van der Waals interactions. The identified hits demonstrated effective binding with Hsp90 throughout the MD simulations. Finally, the three identified hits can serve as novel scaffolds for developing efficient N-terminal domain ATP-binding site inhibitors against Hsp90 for breast cancer therapeutics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.compbiochem.2019.107113.

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