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# Simvastatin and Alendronate sodium repurposing for cancer as HER2, EGFR kinase and AR potential inhibitors: *In silico* approach

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**ABSTRACT**

The aim of this work was to test repurposing of Simvastatin and Alendronate sodium against three targets HER2, EGFR kinase and AR involved in breast cancer, lung cancer and prostate cancer respectively using molecular docking. *In silico* screening was carried out by grip-based docking methodology. The molecular coupling analysis was performed with PyRx version 0.8 and the Biovia visualization study. *In silico* investigation resulted promising BE score with all HER2, EGFR kinase and AR targets. Docking study resulted hydrogen bonding interaction with amino acids like ASP863, LYS753, LYS745, THR854, THR790, LYS808, ARG752, GLN711 and GLY683. The molecular docking study resulted detail valuable insights on the new therapeutic indication to cancer treatment. Conclusively, this study provides a suitable platform for drug repurposing for cancer management.

**Keywords**
Alendronate sodium; Cancer; Molecular docking; Repurposing; Simvastatin

## Introduction

Drug repurposing also called as drug repositioning or drug re-profiling showed potential future that allows large number of methods in the discovery of novel treatments for diseases which are systematic and substantially less expensive while compared to traditional drug development[1-2]. It is a constructive strategy in drug molecule which is extremely efficient, time saving, low-cost and minimum risk of failure[3,4]. Thus, drug repositioning is an effective option to traditional drug discovery process[5].

Simvastatin is a lipid-lowering agent derived from a fermentation product of the fungus Aspergillus terreus which is associated with mild, asymptomatic and self-limited serum amino transferase elevations throughout therapy and infrequently with clinically apparent acute liver injury[6-7]. Alendronate Sodium is the sodium salt of alendronate, a second-generation bisphosphonate and synthetic analog of pyrophosphate with bone anti-resorption activity[8].
Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family having tyrosine kinase activity[9]. Dimerization of the receptor results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and initiates a variety of signaling pathways leading to cell proliferation and tumorigenesis. Amplification or overexpression of HER2 occurs in approximately 15–30% of breast cancers[10].

Epidermal growth factor receptor kinase (EGFR kinase) is a trans-membrane glycoprotein with an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain that regulates signaling pathways to control cellular proliferation. Epidermal growth factor receptor binding to its ligand results in autophosphorylation by intrinsic tyrosine/kinase activity, triggering several signal transduction cascades[11]. Constitutive or sustained activation of these sequences of downstream targets is thought to yield more aggressive tumor phenotypes. Mutations in epidermal growth factor receptor have been discovered in association with some lung cancers. Lung adenocarcinomas with mutated epidermal growth factor receptor have significant responses to tyrosine kinase inhibitors[12].
Androgen receptor (AR) is a steroid receptor transcriptional factor for testosterone and dihydrotestosterone consisting of four main domains, the N-terminal domain, DNA-binding domain, hinge region, and ligand-binding domain. AR plays pivotal roles in prostate cancer, especially castration-resistant prostate cancer (CRPC). Androgen deprivation therapy can suppress hormone-naive prostate cancer, but prostate cancer changes AR and adapts to

survive under castration levels of androgen[13]. These mechanisms include AR point mutations, AR overexpression, changes of androgen biosynthesis, constitutively active AR splice variants without ligand binding, and changes of androgen cofactors[14]. Recently, the studies have confirmed that the simvastatin is implicated in various pathways that increase the survival time of patients with cancer in combination with antineoplastic agents against the treatment[15]. In several types of cancers, it has been observed that there is a dysregulation of the lipid and the mevalonate pathway[16]. A number of studies have shown a strong correlation with the use of statins and the cancer[17]. Remarkable potential study showed that, the statins were able to improve the outcome in cancer[18-19]. The molecular docking study used to model the interaction amongst a small molecule and a protein at the atomic level that allow us to illustrate the performance of small molecules in the binding site of target proteins along with elucidating fundamental biochemical processes[20-21]. *In silico* pharmacology also called as computational therapeutics or computational pharmacology[22,23] which is a budding area that shelter the development of techniques with the help of software for capturing, analyzing and integrating biological and medical data from several diverse sources[24-25].

The potential of Simvastatin and Alendronate Sodium in providing safe and effective response have been accepted by researchers but it has not been screened computationally to its suitability for cancer target[26]. Moreover, repurposing for new indications has less tried till date. This demands the need for repurposing of Simvastatin and Alendronate sodium to prostate, lung and breast cancer targets which is a global need.

Considering these facts; the present study was attempted to focus on exploration of Simvastatin and Alendronate sodium for cancer targets by *in silico* methodology. The objective of the present study was to screen Simvastatin and Alendronate sodium on HER2, EGFR kinase and AR targets for breast cancer, lung cancer and prostate cancer respectively by molecular docking study.

## Materials and Methods

Simvastatin was gifted by Tocris Bio-Techne Mumbai, India. Alendronate Sodium was purchased from Sigma-Aldrich, Mumbai. India.

### Molecular docking study

The crystal structures of Human Epidermal Growth Factor Receptor 2 (HER2), Epidermal Growth Factor Receptor Kinase (EGFR kinase), and Androgen receptor (AR) were retrieved from RCSB protein data bank (http://www.rcsb.org) with protein data bank (PDB) 3RCD, 2ITY, and 1E3G respectively. Crystal structure with good resolution by X-ray diffraction method was the selection criteria for 3RCD, 2ITY, and 1E3G. 3RCD is structures of HER2 Kinase Domain Complexed with TAK-285, wherein TAK-285 is reported HER2 inhibitor compound. 2ITY is structures of EGFR kinase in complex with Iressa as inhibitor. 1E3G is structures of Human Androgen Receptor in complex with the ligand Metribolone (R1881) as an inhibitor. The files were saved as .pdb. To facilitate the docking studies with molecules in question, the ligands TAK-285, Iressa, and Metribolone (R1881) bound to active sites were removed from the crystal structure. Water molecules were removed and polar hydrogen atoms were added to the 3D structures for protein refinement using PyRx version 0.8 software. The macromolecule files were taken as a .mol2 file format for further analysis. The 2D molecular structures of Simvastatin and Alendronate Sodium were retrieved from the chemical database namely PubChem (https://pubchem.ncbi.nlm.nih.gov) in .sdf format. These structures were converted to 3D .mol2 format and then minimized using the Merck Molecular Force Field (MMFF).

Flexible and accurate protein-ligand docking methodology was performed in which rotation angle was kept at 10°, a number of placements were 30, the ligand was kept nonflexible and ligand wise 10 results (poses) were selected. Molecular docking analysis was carried out in the PyRx version 0.8 and Biovia visualization studio software. *In silico* screening was performed for drug repurposing to find the new therapeutic uses like anticancer effect of existing safe molecules, Simvastatin and Alendronate sodium as HER2, EGFR kinase and AR inhibitor respectively.

### Results and Discussion

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The Molecular docking was performed using PyRx (Version 0.8) docking tool which utilizes Autodock Vina as docking program. In order to test repurposing of Simvastatin and Alendronate sodium we have screened these small molecules against three targets involved in Breast Cancer, Lung cancer and Prostate cancer by using molecular docking and virtual screening. For breast cancer, binding affinity of Simvastatin and Alendronate Sodium was checked into HER2 protein which is known to be involved in development of breast cancer. Simvastatin showed hydrogen bonding interaction with amino acids like ASP863, LYS753 and hydrophobic interaction with LEU852, CYS805, ALA751 and VAL734. Alendronate Sodium showed hydrogen bonding interaction MET774, LEU785, GLU1021, LEU786 and ARG784. SVS binding interactions with HER 2 have been shown in **Figure 1** and **2**. ADS binding interactions with HER 2 have been shown in **Figure 3** and **4**.

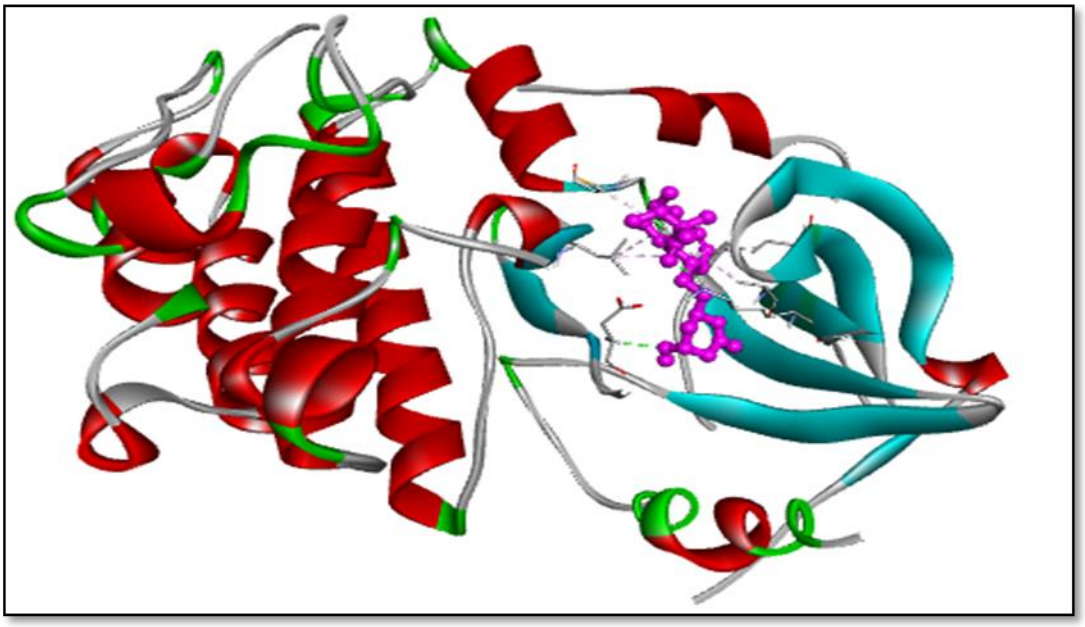

**Figure 1.** 3D SVS Binding interaction with HER 2

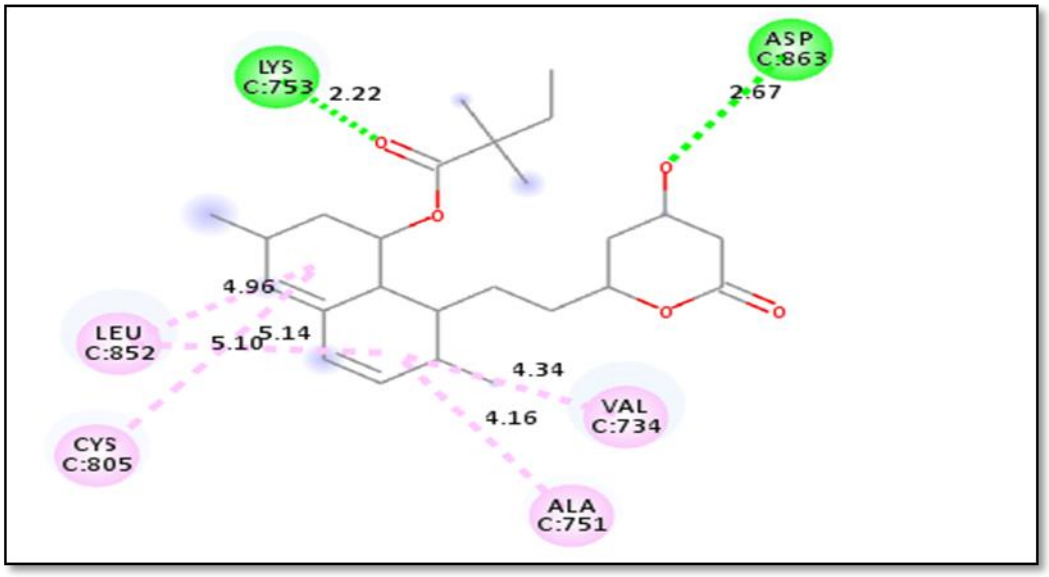


**Figure 2.**Binding interaction of SVS with HER 2

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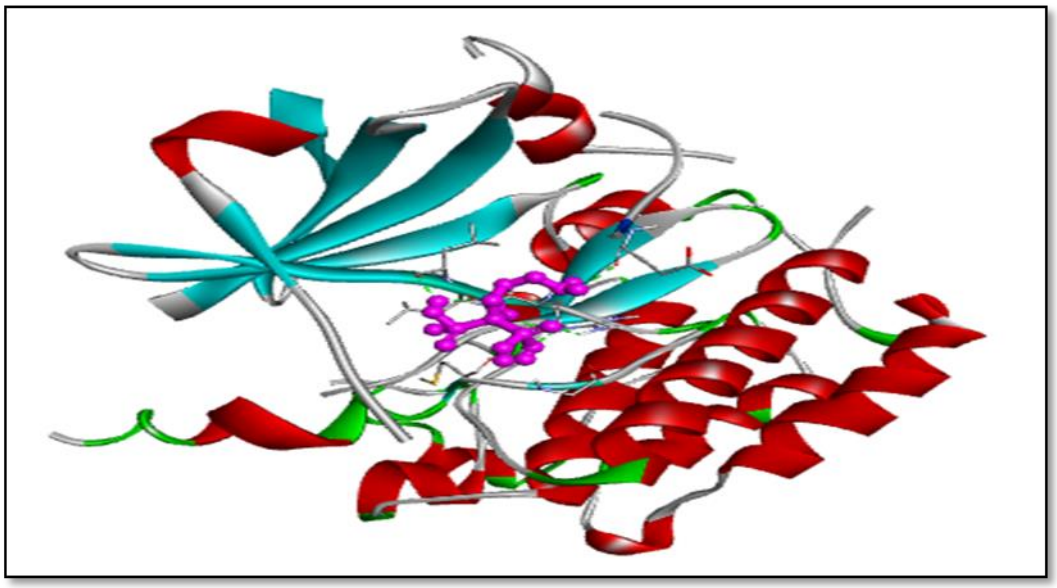

**Figure 3.**3D ADS Binding interaction with HER 2

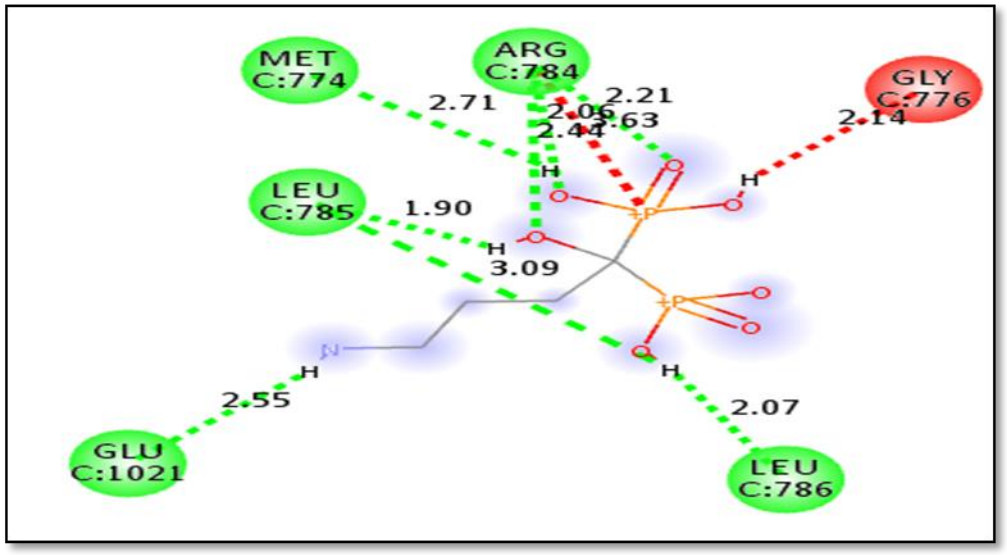


**Figure 4**.Binding interaction of ADS with HER 2

For lung cancer, binding affinity of Simvastatin and Alendronate Sodium was checked into EGFR kinasewhich is known to be involved in development of lung cancer. Simvastatin shows hydrogen bonding interaction with amino acids like LYS745, THR854 and THR790 while hydrophobic interaction with LEU718, VAL726, ALA751 and CYS797. Alendronate Sodium shows hydrogen bonding interaction LYS745 and ASP837. SVS binding interactions with EGFR kinase have been shown in **Figure 5** and **6**. ADS binding interactions with EGFR kinase have been shown in **Figure7** and **8**.

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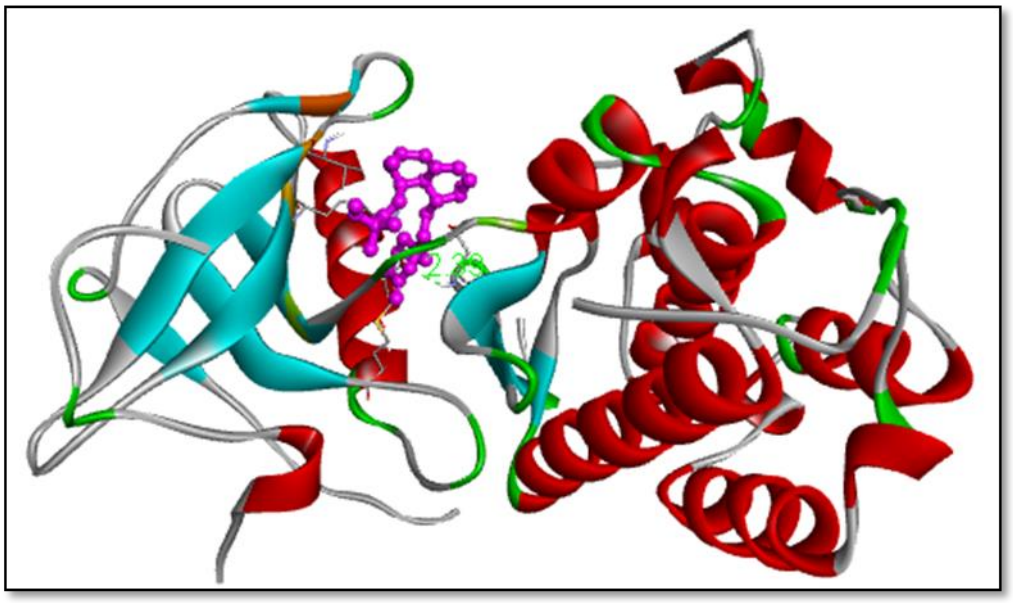

**Figure 5.**3D SVS Binding interaction with EGFR kinase

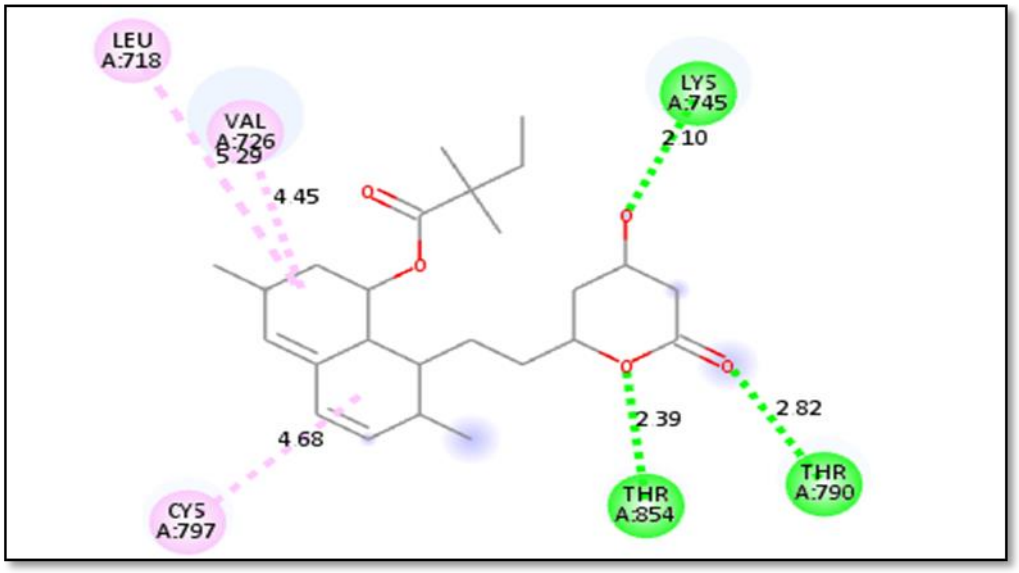


**Figure 6.**Binding interaction of SVS with EGFR kinase

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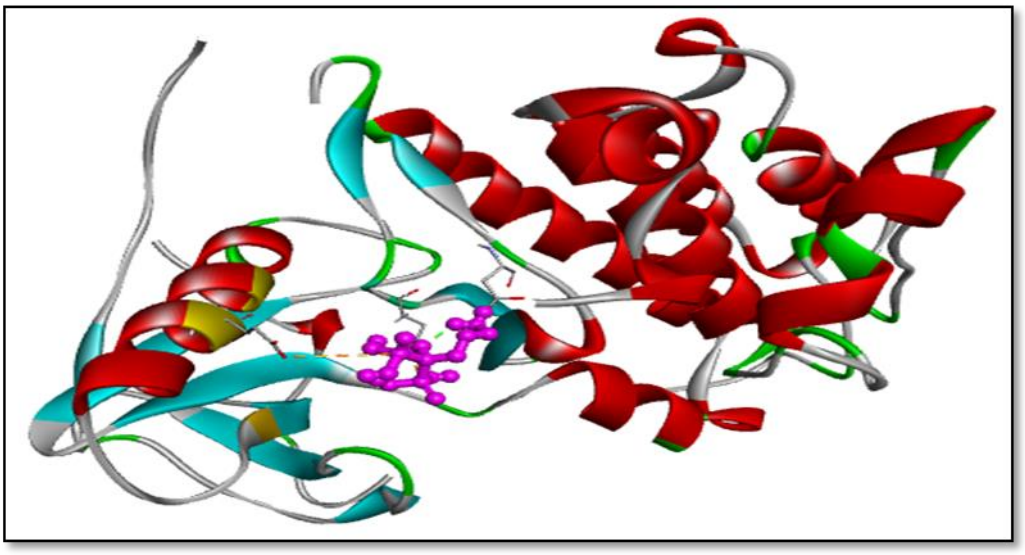

**Figure 7.**3D ADS Binding interaction with EGFR kinase

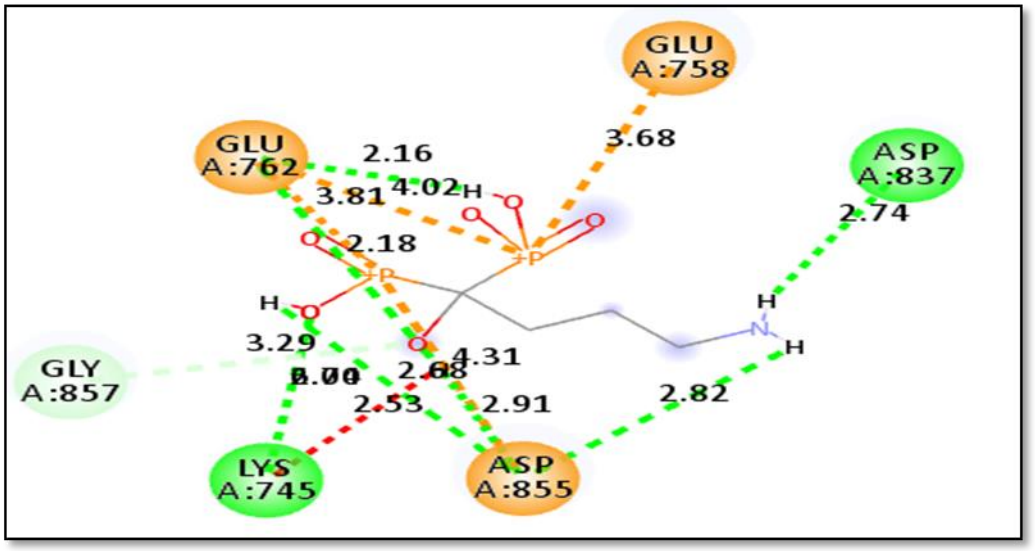


**Figure 8.**Binding interaction of ADS with EGFR kinase

For prostate cancer, binding affinity of Simvastatin and Alendronate Sodium was checked into AR which is known to be involved in development of prostate cancer. Simvastatin showed hydrogen bonding interaction with amino acids like LYS808, ARG752, GLN711 and GLY683 while hydrophobic interaction with GLU681. Alendronate Sodium showed hydrogen bonding interaction THR877 and ASN705 while hydrophobic interaction with LEU873, VAL746, MET742, MET787 and MET745. Results of docking score and binding affinity have been depicted in **Table 1** and **Table 2** respectively. SVS binding interactions with AR have been shown in **Figure 9** and **10**. ADS binding interactions with AR have been shown in **Figure 11** and **12**.

**Table 1.** Docking Score of SVS and ADS

| Target | Name of molecule | Docking Score |
|---|---|---|
| HER-2 | SVS | -8.4 |

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| | | |
|---|---|---|
| | ADS | -5.1 |
| EGFR kinase | SVS | -8.4 |
| | ADS | -4.8 |
| AR | SVS | -5.0 |
| | ADS | -5.4 |

**Table 2.** Binding interactions of SVS and ADS

| Target | Name of molecule | Interactions with amino acids | |
|---|---|---|---|
| | | **Hydrogen Bonding** | **Hydrophobic** |
| HER-2 | SVS | ASP863, LYS753 | LEU852, CYS805, ALA751, VAL734 |
| | ADS | MET774, LEU785, GLU1021, LEU786, ARG784 | -------- |
| EGFR kinase | SVS | LYS745, THR854, THR790 | LEU718, VAL726, CYS797 |
| | ADS | LYS745, ASP837 | -------- |
| AR | SVS | LYS808, ARG752, GLN711, GLY683 | GLU681 |
| | ADS | THR877, ASN705 | LEU873, VAL746, MET742, MET787, MET745 |

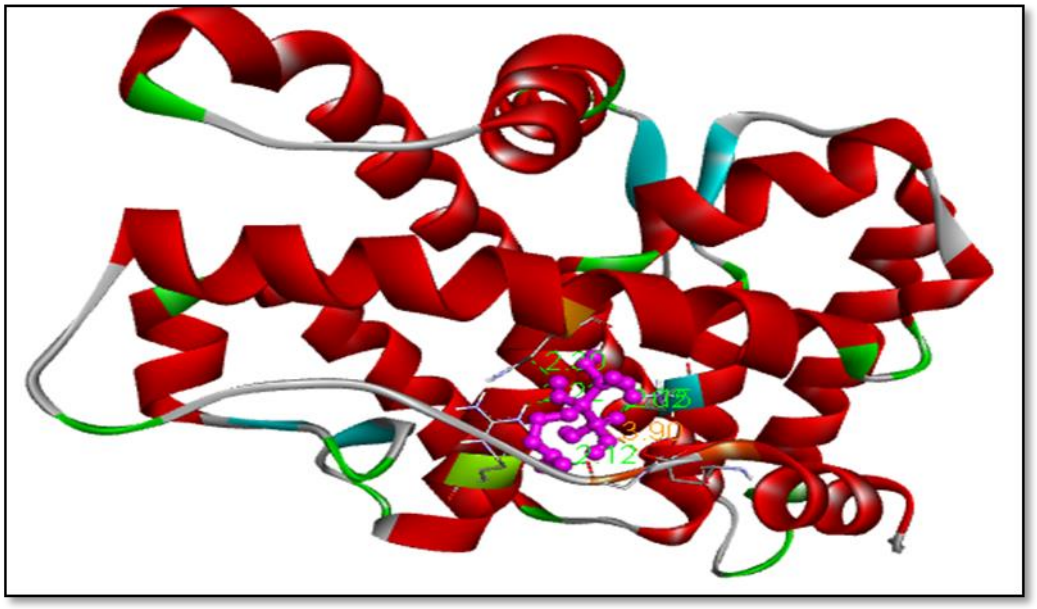

**Figure 9.**3D SVS Binding interaction with AR

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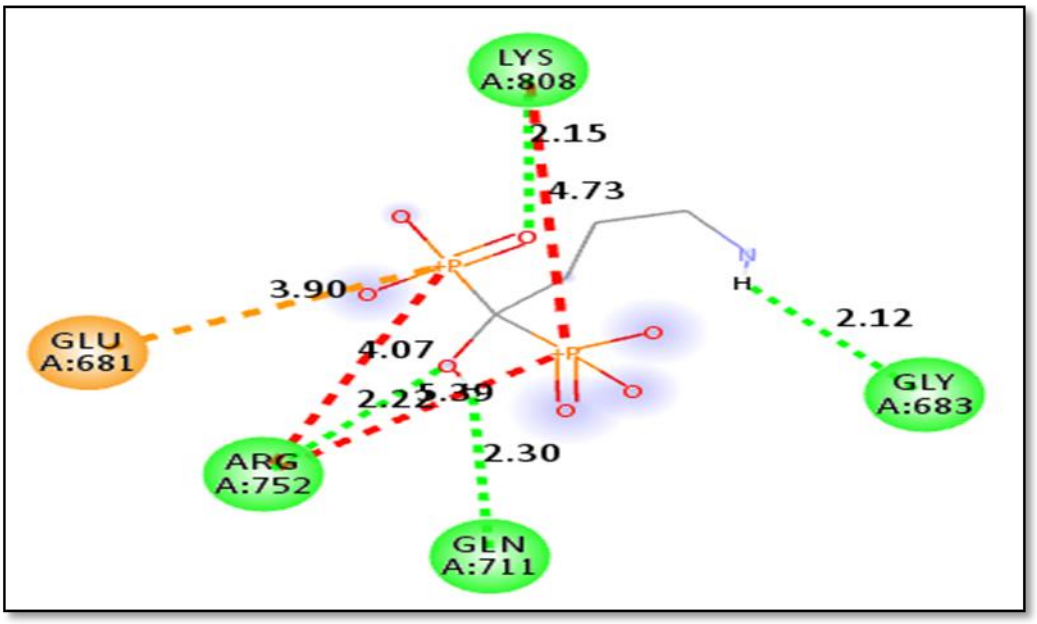


**Figure 10.**Binding interaction of SVS with AR

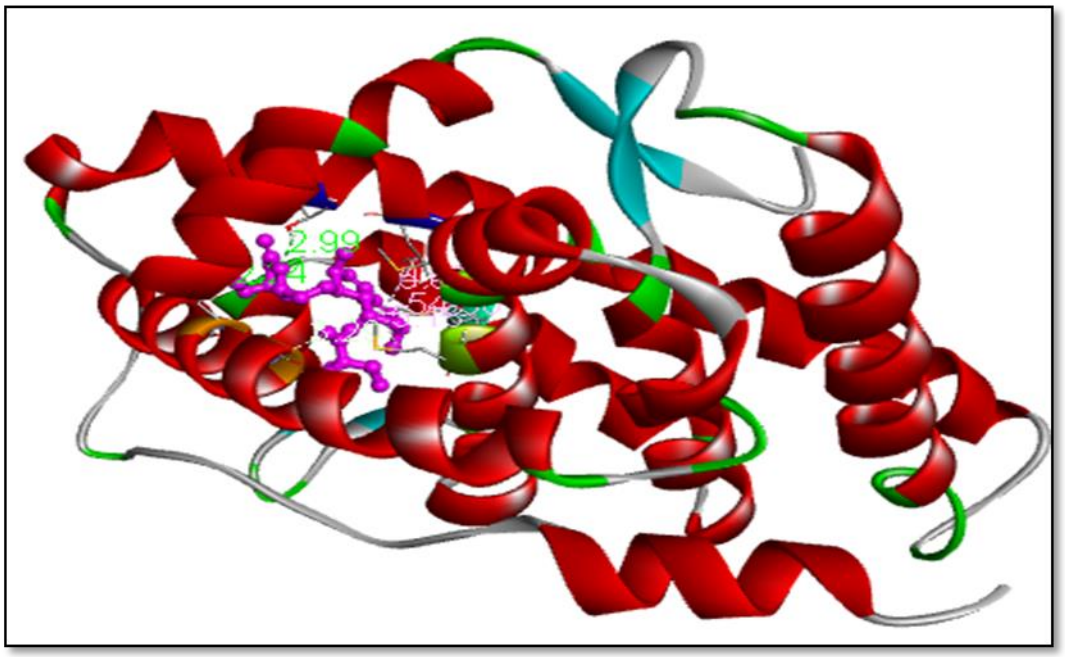


**Figure 11.**3D ADS Binding interaction with AR

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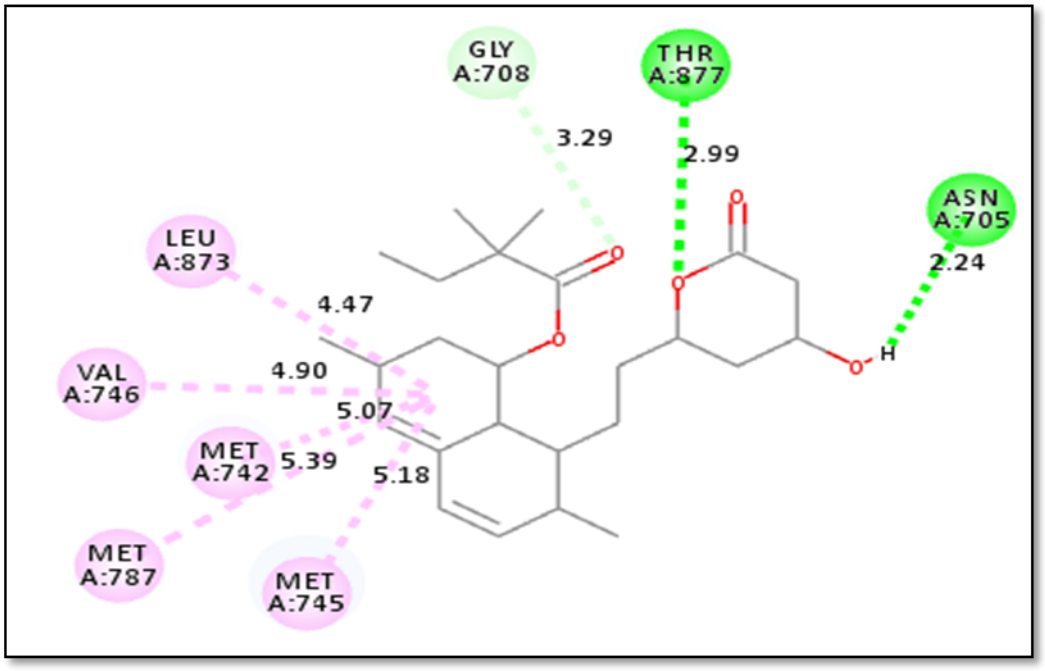


**Figure 12.**Binding interaction of ADS with AR

## Conclusion

In this study, we have explored computational approach for the drug repurposing on HER2, EGFR kinase and AR cancer targets. The binding mode and affinity of Simvastatin and Alendronate Sodium were confirmed from Autodock Vina docking program. Our present study suggested that the GRIP target-based approach can be used for identification of new therapeutic indication, which in turn can be used as repurposing of drugs. This study provides platform for drug repurposing research especially in cancer management.

## Limitations and Future Studies

This study provides platform for drug repurposing research especially in cancer management.

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