LIGAND-BASED VIRTUAL PREDICTIONOF CANCER STEM CELLS INHIBITORS VIA HEDGEHOG SIGNALLING PATHWAY USING PRELIMINARY MOLECULAR DOCKING ANALYSIS

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Abstract

Millionsofpeopleworldwidediefromcancereveryyear.Researchsuggeststhatcancerstemcellsareresponsible for recurrence and resistance. Drug resistance in cancer stem cells is associated withnumerouspathways.OneofthoseisHedgehog(Hh)pathway.Giventhis,forty-fivealreadymarketedanti-cancermoleculeswerechosen and screened for inhibition of the Hh pathway using Hedgehog protein (**PDB ID: 4C4M**) andSmoothened protein (**PDB ID: 4JKV**). Preliminary molecular docking analysis was performed to studydrug-receptor interactions using AutoDockVina. Upon conducting the docking analysis, it was observed that the formation of hydrogen bonds was primarily influenced by the presence of hetero atoms such as oxygen and nitrogen in diverse concentrations. Moreover, the benzene ring displayed advantageous pi-pi stacking interactions with several amino acids, resulting in an overall improvement of the docking score. Out of the forty-five molecules tested, Alectinib, Ibrutinib, BSM202, Palbociclib, and Pembrolizumab exhibited the strongest binding affinity with Smoothened protein, with dock scores of -8.5 kcal/mol, -7.9 kcal/mol,-7.5 kcal/mol, -8.1 kcal/mol, and -8.4 kcal/mol, respectively. Additionally, Ibrutinib and Palbociclib demonstrated the highest binding affinity with Hedgehog protein, with dock scores of -7.0 kcal/mol and -7.3 kcal/mol, respectively.In conclusion, the potent inhibition of both proteins by Ibrutinib and Palbociclib indicates a promising capacity to suppress cancer stem cells.

Keywords: Hedgehog protein, Smoothened protein, molecular docking, repurposing, dock score,cancerstemcells,4C4M,4JKV,AutodockVina

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1. Introduction

Worldwide, cancer ranks as one of the leading causes of death and a major barrier to improving lifeexpectancy. Approximately 19.3 million newly diagnosed cancer cases were reported in the latestGLOBOCANstatistics(Sung et al. 2021).

Alargeportionofthehighcancer-relateddeathratecanbeattributedtoresistancetochemotherapy.The cancer stem cells (CSCs) may be a primary factor behind this resistance (Walcher et al. 2020, Ayob et al. 2018). CSCs are cancer cellsthatdivideslowlyincancertissuesandappeartoresemblestemcellsinthebody.SinceCSCsareslow-growing, chemotherapy may not be effective, leading to the recurrence of cancer. As a result, CSCsare nowbelievedtobetheprimarycellsresponsibleforspreadingcancerthroughoutthebody (Deanet al. 2008).

StemcellactivityisregulatedlargelybytheHedgehogsignalingpathway,whichisresponsibleforproliferation, differentiation, and survival[5]. A deregulated pathway in cancer stem cells promotesabnormal growth and development of cancer, and the Hedgehog pathway is therefore considered atherapeutictargetto treatcancer (Ruch et al. 2013).

In the Hedgehog Pathway, the hedgehog protein binds to the Patched protein (Ptch) and renders itinactive. Ptch is a twelve trans-membrane glycoprotein molecular component that inhibits the activityof Smoothened (Smo) protein(Mahindroo et al. 2009). The inhibition of Ptch by Hh protein results in the activation of the Smoprotein, which increases the concentration of Gli. A transcription factor called Gli influences thetranscription of genes related to cancer and CSCs. It is, therefore, more effective to inhibit Hh-Smo-Gli- proteins together than to inhibit each protein individually, thereby decreasing the chances ofdevelopingdrugresistance (Jaitak et al. 2016,

Watkins et al. 2003).

It is advantageous to repurpose drugs approved by regulatory agencies through the drug repurposingapproach.Theycanbedevelopedasviabletherapiesthatmaybebeneficialforthose diseases that pose significant diagnostic or treatment challenges. Also, since the safety profile is often well-known,itsimplifiesandstreamlines futureapprovalprocesses (Jain et al. 2021, To et al. 2022, Zhang et al. 2020, Xue et al. 2010).

The current study aims to explore the binding interaction of some FDA-approved anticancer drugswith Smo protein and Hh protein of the hedgehog signaling pathway through preliminary dockingevaluation. The drugs exhibiting good dock scores will be the potential molecules to inhibit CSCs. Hence, the present work may be utilized to predict and develop anti-cancer stem cell inhibitors.

2. Materials andMethods

2.1 Ligand Molecules Preparation

Chemical structures of forty-five marketed anticancer drugs were drawn using ChemDraw software. All the structures were converted to .mol2 format using the Marvin view for ligand preparation.Optimizationofligands(.mol2)wascarriedoutbyAutoDocktoolsandsavedin.pdbqtformat.

2.2 TargetProtein Preparation

Crystal structures of Sonic Hedgehog (Hh) **(PBD ID: 4C4M)** and Smoothened (Smo) protein **(PBD ID:4JKV)** used were downloaded from the protein data bank. These proteins were prepared for dockingby deleting water molecules, heteroatoms, and ligand groups, using the script option of DiscoveryStudio 4.0. Themodifiedproteinwasthensavedin.pdbformat.

2.3 PreliminaryMolecular Docking

Molecular docking analysis was performed using AutoDockVina to evaluate the

hydrogenbondinteraction and binding affinities. After the minimization process, grid box resolution for Hh protein(**PDB ID:4JKV**) was set at -19.698, 14.1, and -12.523 along x, y, and z points respectively with aresolution of 4 Å, while grid dimensions were set at 126 x 126 x 104 Å. The grid box resolution for Smoprotein (**PDB ID: 4C4M**)was set at 4.283, -9.437, and -16.896 along x, y, and z points respectivelywitha resolutionof 4Å whilegriddimensionsweresetat126x126x126Å.

3. Results andDiscussion

To investigate potential drug candidates, a preliminary molecular docking analysis was conducted for forty-five anticancer agents against the binding pocket of Hh and Smo proteins, critical targets for cancer stem cell inhibition.Among the forty-five anticancer molecules assessed, only a subset of fifteen (**Table 1**) demonstrated successful docking with the Smo and Hh proteins.The dockinginteractionsofthesemoleculesaredepictedin**Figure1**.Allthedockedstructureswerevisualizedi nPyMOLWinandDiscoveryStudio2021.Anticancer compounds achieving a docking score of -7.0 or lower are deemed more potent in their ability to inhibit the hedgehog pathway.

Figure 1a illustrates the binding interactions of Alectinib, exhibiting the highest binding score (-8.5) with the Smo protein. The piperidine ring of Alectinib engages in an Alkyl bond formation with amino acids Ala181 and Val184. Additionally, a pi-pi stacking interaction is observed between the phenyl rings and pyrroline moiety of Alectinib with the amino acid Trp259.

Figure 1b depicts the binding interaction between Ibrutinib and the Smo receptor. The pyrazole ring of Ibrutinib establishes an H-bonding interaction with the amino acid Try677, while the ketone group forms another H-bonding interaction with Thr681. Furthermore, a pi-alkyl interaction is evident between the phenyl ring of Ibrutinib and the amino acid Lys684.

Figure 1c illustrates the docking interaction diagram of BMS202. In this instance, the pyridine moiety demonstrates pi-pi stacking and pi-sigma interactions with the amino acid Trp259, while

the benzene ring engages in a pi-sigma interaction with Val295. Moving on to **Figure 1d**, the acetyl group of Palbociclib forms H-bond interactions with amino acids His243 and Arg311, while the tetra hydro pyridine moiety exhibits pi-pi T-shaped interactions and H-bonding interactions with Phe308 and Tyr307.

In **Figure 1e** of Pembrolizumab, a noticeable pi-pi stacking interaction is observed between the pyridine ring and the amino acid Phe263. Additionally, the two benzene rings display distinct interactions with amino acids Trp259, Val184, Ala229, and Cys182.Ibrutinib and Palbociclib demonstrated favorable binding interactions with the Hh protein, as evident in **Figures 1f** and **1g**, respectively. With dock scores of -7.0 and -7.3 (**Table 1**), these compounds exhibited notable molecular interactions. **Figure 1f** illustrates the H-bonding interaction between the phenoxy benzamine oxygen and the amino pyrimidine 'NH2' group of Ibrutinib with the amino acid Glu15. In **Figure 1g**, hydrogen bond interactions are observed between the acetyl oxygen group of Palbociclib and the amino acid Lys7, while the nitrogen of the pyrimidine ring interacts with the amino acid Ser94. Additionally, Palbociclib displays hydrogen bond interactions at its amide linkage between the pyrimidine and pyridine moiety with the amino acid Glu15.

4. Conclusion

The docking analysis revealed diverse binding interactions between the anti-cancer drugs and the Smo and Hh proteins. Alectinib, Ibrutinib, BMS202, Palbociclib, and Pembrolizumab displayed strong affinity towards the Smo protein. Notably, Palbociclib and Ibrutinib exhibited the highest binding interactions with both the Smo and Hh receptors, indicating their potential to effectively inhibit cancer stem cells. Since these drugs have already received FDA approval, conducting further toxicity studies might be unnecessary, saving valuable screening time for assessing their anti-CSC (cancer stem cell) activity. Therefore, it can be concluded that Ibrutinib and Palbociclib possess the ability to inhibit cancer stem cell lines and potentially control cancer recurrence.

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Conflicts of Interest

Allauthorsdeclarethatthereisno conflictofinterestinthiswork.

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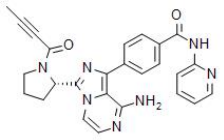
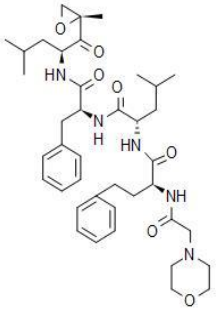
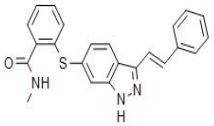
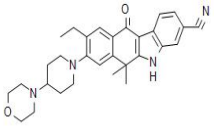
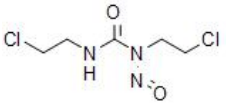
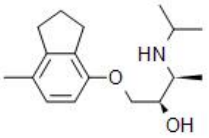
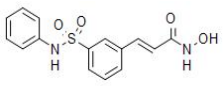
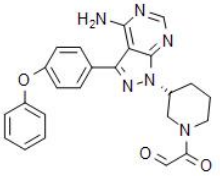
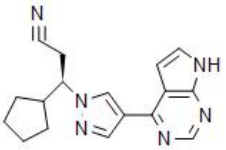
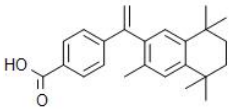
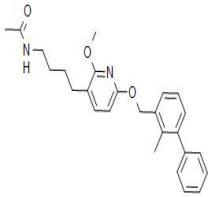
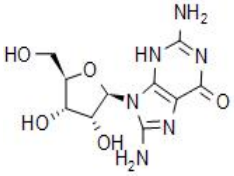
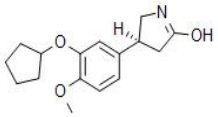
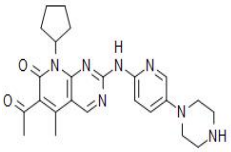
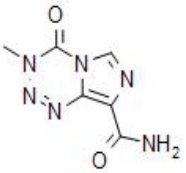
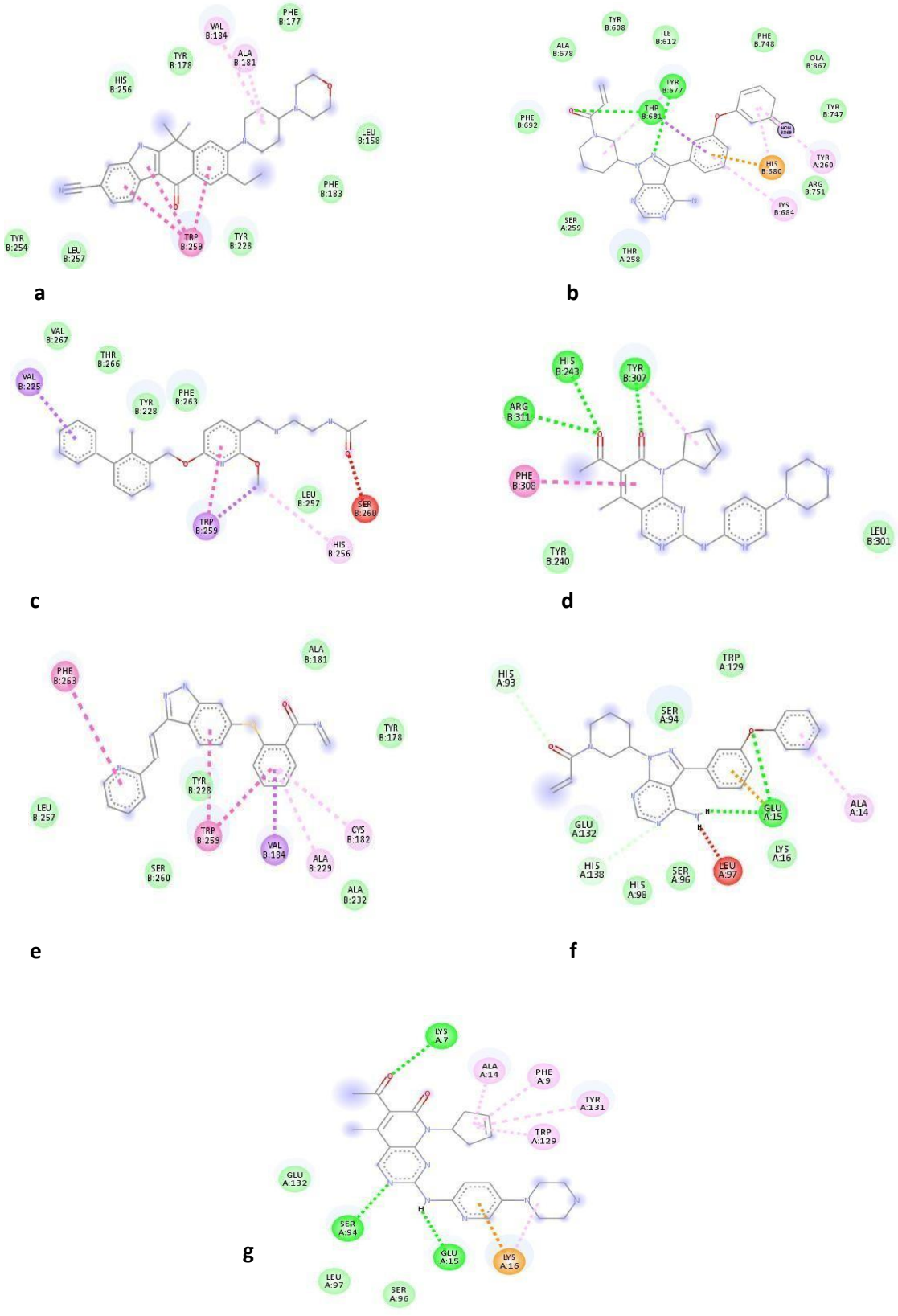
Structure and Name of the compound	Dock Score	Structure and Name of the compound	Dock Score	Structure and Name of the compound	Dock Score

Table1: Dock Score of FDA-approved anti-cancer drugs

 Acalabrutinib	4JKV -6.8 4C4M -5.3	 Carfilzomib	4JKV **-6.3** 4C4M **-5.9**	 Pembrolizumab	4JKV **-8.4** 4C4M -5.8
 Alectinib	4JKV **-8.5** 4C4M -6.5	 Carmustine	4JKV -4.5 4C4M -3.6	 Pertuzumab	4JKV -6.7 4C4M -4.4
 Belinostat	4JKV -6.3 4C4M -6.9	 Ibrutinib	4JKV **-7.9** 4C4M **-7.0**	 Ruxolitinib	4JKV -5.2 4C4M -5.5
 Bexarotene	4JKV **-8.1** 4C4M -6.5	 BMS202	4JKV **-7.5** 4C4M -6.3	 Trastuzumabmab	4JKV -5.9 4C4M -5.8

	4JKV -6.5 4C4M -6.0		4JKV -**8.1** 4C4M -**7.3**		4JKV -5.4 4C4M -4.7
Bevacizumab		Palbociclib		Temozolomide	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TRP B:259
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a
TYR B:608
ILE B:612
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OLA B:867
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TYR B:747
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HOH B:881
TYR A:260
HIS B:680
ARG B:751
LYS B:684
SER A:259
THR A:258
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VAL B:267
THR B:266
VAL B:295
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GLU A:15
LYS A:16
LEU A:97
SER A:96
g

Figure 1: Docking interactions of the representative anticancer drugs with a score less than -7.0 on Smo and Hh receptors. (a) interaction of Alectinib with Smo receptor; (b) interaction of Ibrutinib with Smo receptor; (c) interaction of Nivolumab with Smo receptor; (d) interaction of Palbociclib with Smo receptor; (e) interaction of Pembrolizumab with Smo receptor; (f) interaction of Ibrutinib with Hh receptor; (g) interaction of Palbociclib with Hh receptor.