

In-silico Analysis to Determine the Efficient Drug for Malignant Melanoma using Molecular Dynamics

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To assess an effective medication against BRAF(V600E), the gene which causes melanoma skin cancer, a literature survey was performed and three drugs were identified that are effective melanoma cell inhibitors that induce skin cancer and help patients cure skin cancer. In this analysis we analyzed these three drugs namely Aknadycin as (Drug1) and 16beta-hydroxy-vendolinine-n-oxide as (Drug2) and PID (215) pronounced (1Z)-5-(2-(4-(dimethylamino)ethoxy)phenyl)-5-pyridin-4-yl-1H-imidazol-ylindian-1-oneoxime as (Drug3) using various methodologies and simulation techniques to find out how highly successful these drugs are among them. In this research, we developed the 3D structure of BRAF(V600E) through homology modeling and validated it through Ramachandran plot and verify3D and implemented the method of docking through virtual screening of protein with drug and simulation of molecular dynamics to further suggest the powerful potent novel inhibitors for malignant melanoma management. Obtained a wild form BRAF(V600E) 3D structure and docked protein structure with each drug and continued molecular dynamic simulation by measuring the stability and energy interaction between the protein and these drugs. An RMSD graph displays the drug's efficacy. Analysis of three different drugs using computational techniques by performing different methodologies of docking, molecular simulation and interaction energy and results of each drug in these methodology, reveals the efficiency of drugs. Based on the results we identify that the Drug3 is a highly effective inhibitor of melanoma among other drugs.

Keywords: BRAF inhibitor, homology modelling, structure-based, virtual screening, docking, charge calculation, molecular dynamic simulation, interaction energy.

Melanoma is the skin-cancer that arises in the cells through gene mutation and grows uncontrolled or advanced metastatic cells. BRAF(V600E) is the gene that causes skin cancer of melanoma when amino acid valine(V) is substituted by glutamic acid(E) at the 600th position of the amino acid sequence, whereas the protein found in BRAF transforms it into wild

BRAF that causes skin cancer¹. This transition has led to the constitutive activation of RAS mutation on the MAPK pathway². In patients with malignant melanoma who have a single mutation, use of BRAF inhibitors has become an exciting therapeutic option. One of the first options to treat metastatic melanoma was that of BRAF inhibitors¹. Treatment choices for patients with metastatic

melanoma have been through in recent years but the overall survival rate has so far failed to increase. So, this affects innovative and successful methods to discover and design³.

Various medications have been proposed for melanoma such as ipilimumab and vemurafinab², aknadacin, 16-beta-hydroxy-vendolinine-n-oxide¹ and 215 (Drug3)³, yet none of these have been evaluated. Based on the molecular behavioral research, the computational methods have been proposed for in-silico study of cancer dynamics to help us compute model and compare these drugs⁴. In this study, we analysed and compared the three potential inhibitors of melanoma cells where two novel inhibitors Drug1 and Drug2 are considered¹ as they were found efficient drugs against melanoma cells and another drug inhibitor present in the protein complex with protein id 2FB8 namely 215(Drug3)³ are compared with the computational methodologies as explained in the later stages. The protocols used are as same as approaches used by Chen et.al. 2015.

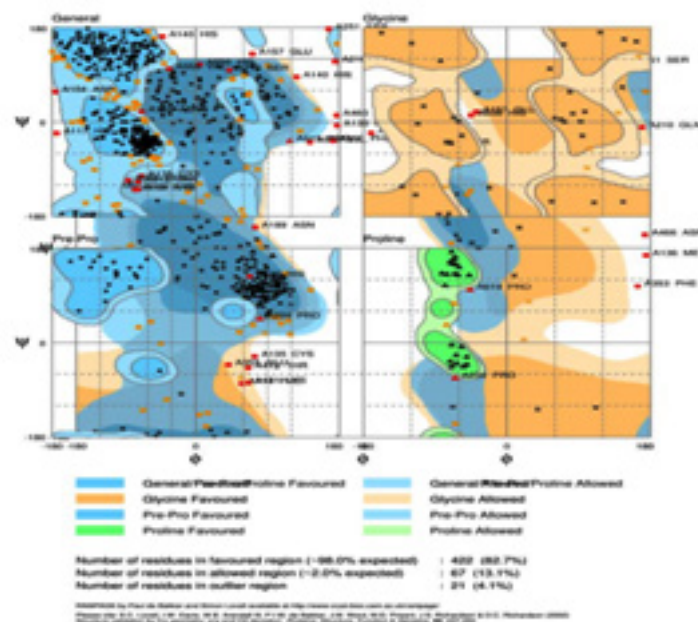
In this pipeline, we mainly follow three methodologies: firstly homology modelling of the protein structure to obtain 3D- structure of

the protein, further evaluating the 3D-structure with the construction of Ramachandran plot with rampage <http://mordred.bioc.cam.ac.uk/~rapper/rampage.php> and verify3D⁵. Secondly, docking was performed for the predicted protein structure along with the three drug components [mentioned above] to check their interaction with the protein by analysing binding affinity. Thirdly, molecular dynamics (MD) simulation was performed for analysing the physical movements of atoms and molecules. MD method is frequently applied to study the motions of macromolecules such as proteins and nucleic acids, which can be useful for analysing modelling interactions with other molecules as in ligand docking. Total interaction energy, root mean square deviation, root mean square fluctuation were also calculated in this method.

MATERIALS AND METHODS

Retrieval of Drugs

We have considered three drugs in this study for comparison, to find the optimal drug for wild type BRAF. The drug aknadicine(drug1)

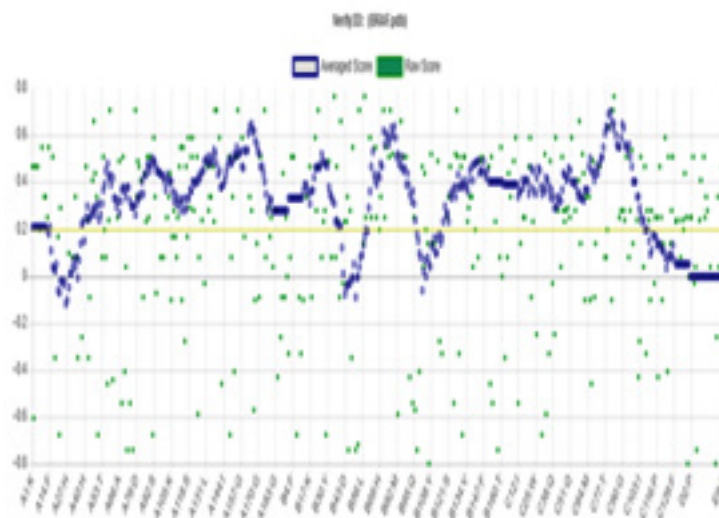


(a)

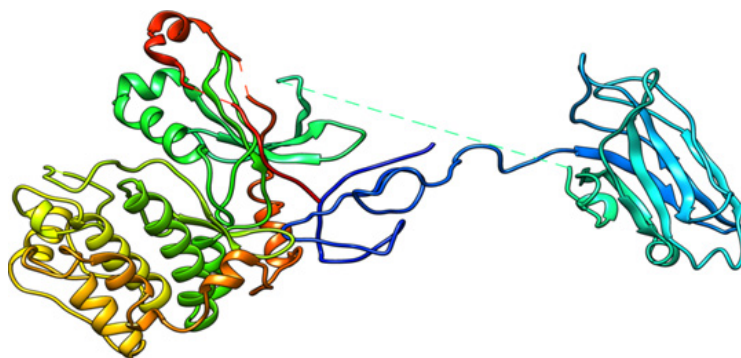
Number of residues in favored region (~98.0% expected): 422 (82.7%).

Number of residues in allowed region (~2.0% expected): 67 (13.1%).

Number of residues in outlier region: 21 (4.1%).



(b) Positive scores mean accurate structure of amino acids.



(c)

Fig. 1. (a) Ramachandran plot of BRAF protein structure. (b) Verify3D score of BRAF protein. (c) BRAF(v600e) 3D protein structure

pubchem ID (442156)⁴ was downloaded in the form of SDF format, later converted into PDB format from Obabel⁶. The drug 16beta-hydroxy-19s-vindoline N-oxide(drug2) with external ID (5318348) as 3D structure was not available, first we have obtained 2D structure with its ID by drawing in mobile molecular model application from google play store and visualised in discovery studio⁷ to obtain 3D structure we retrieve the structure in PDB format. The third Drug3 was retrieved from RCSB⁸ where protein name 2FB8 with the PID 215³.

Homology modelling

A human BRAF obtained the UniProt Knowledgebase (P15056, human), the sequence

of it with its residues 1–766 and 3D structure residues 445–723 from RCSB Protein ID 2FB8⁸. The homology modeling system for BRAF(V600E) with wild-type crystal structure BRAF (2FB8) on Modeller Software^{9,10,8,11} was created. When modeling homology we first replace the amino acid valine(V) with glutamic acid (E) at position 600 in the BRAF protein sequence and create the BRAF(V600E)-modelled structure based on the wild-type BRAF (2FB8, human) prototype. Also the structure was constructed through Phyre software⁹ and was compared through chimera UCSF¹⁰. Further, the obtained protein structure from modeller software was validated using Ramachandran plot using Rampage and verify3D⁵.

Molecular Docking and Charge Calculation

Molecular Docking was performed using mgl tools¹¹ and AutoDock Vina¹² using Lamarckian genetic algorithm [LGA] with 25,000,000 steps. The grid parameters were set to size 60, 64, 54 with centers 16.904, -0.229 and 56.704 for all three drugs. The resultant docked structure was

visualized through Chimera and polar-coordinates of the docked images were visualised through Pymol¹³. The charge for the drug was calculated using Resp Esp charge Derive Server (REDS)¹⁴ which is used to generate force field parameters using AMBER.

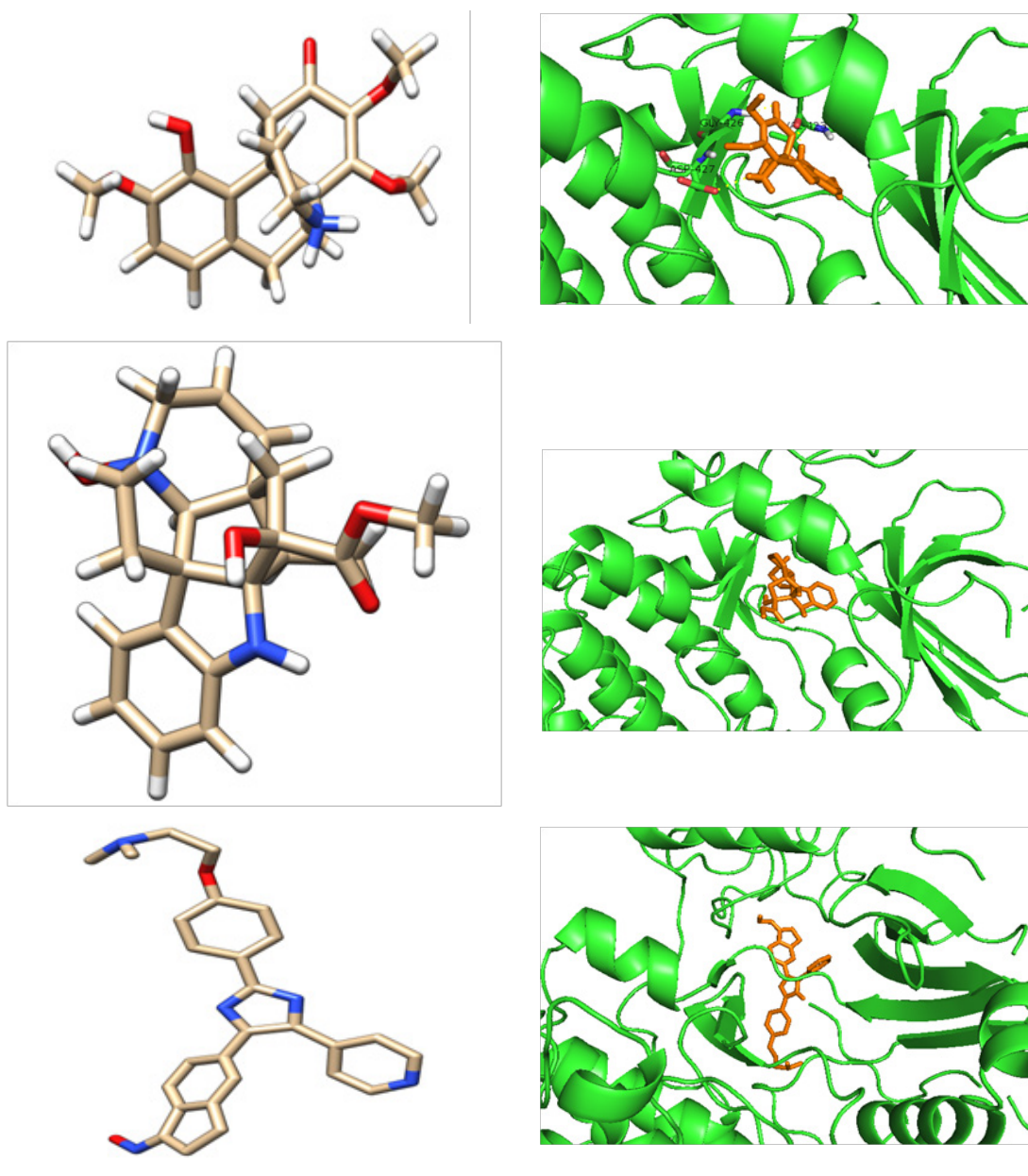


Fig. 2. (a) 3D structure of aknadycin drug as (Drug1). (b) BRAF protein docked structure of protein with Drug1. (c) 3D structure of 16beta-hydroxy-vendolinine-n-oxide drug as (Drug2) (d) BRAF protein docked structure of protein with Drug2. (e) 3D structure of (215) (Drug3) (f) BRAF protein docked structure of protein with Drug3

Molecular Dynamics and Binding Energy Estimation

Molecular dynamics (MD) simulation was performed using Gromacs 5.1.4^{15,16}. Amber99 force field was applied to generate the gromacs topology for protein, AMBER16 [17] for used to check the parameters of the drug and acpype¹⁸ was used to generate gromacs topology for drug. A dodecahedron box was used with extended simple point charge water molecule¹⁹ Energy minimization was performed for 50,000 steps using the steepest descent minimization process. Simulation of the system's position-restrained dynamics (equilibration phase) (NVT and NPT) at 300 K for 200 ps followed by MD production run for 10 ns. The plot for the RMSD was produced with Xmgrace²⁰. The 3D structures were generated using Chimera and visualized using VMD²¹.

RESULTS

Constructing 3D structure of protein using homology modelling

The BRAF(V600E) protein 3D structure was modeled using Modular Software as shown in Figure 1(c). The structure was validated using the Ramachandran plot Figure 1(a), the model structure showed that 82.7% of residues were in the preferred region, 13.1% were in the permissible area, and just 4.1% were in the outer area. Verify3D score indicated that the BRAF(V600E)-modelled structure is an ideal conformation model. Verify3D score of BRAF(V600E) protein showed that almost all the residues had positive values Figure 1(b).

Docking or Virtual Screening

A structural docking of all the drugs was performed with BRAF(V600E) protein. The Drug1, Drug2 and Drug3 binding affinity score was -8.1, -8.4 and -8.8 kcal/mol respectively as shown in Table 1. The docked 3D structure of the

drugs with protein is shown in Figure 2 (b, d and f) of the three drugs represented in Figure 2 (a, c and e) docked with the BRAF(V600E), respectively. Binding affinity scores shows that Drug3 has a higher binding affinity with the protein. We further proceeded to MD simulation to find the stability of these drugs with the protein.

Molecular Dynamic(MD) Simulation

The 10ns MD simulation of BRAF protein with each drug complex is carried out using AMBER16 forcefield, the gromacs topology is applied from protein, the REDS(red server) is used to calculate drug topology and is validated using antechamber. The energy minimization protocol is used in the SPC water model. The stability of the BRAF- drug complex is computed using RMSD as shown in Figure 3(a). From the result, it is revealed that the BRAF- Drug3 complex was more stable than the BRAF- Drug1 and BRAF-Drug2 complex.

To know the fluctuation of the protein with its drug, RMSF was computed as shown in Figure 3(c). Drug3 indicates less fluctuation. To know the measure of compactness of the protein, the radius of gyration is calculated as represented in Figure 3(b). With Figure 3(b), we can know that protein with Drug3 is more compact than other two complexes.

Further, the interaction energy of all the three complexes, BRAF-Drug1 is -210.0979 +/- 4.8 kJ/mol, BRAF-Drug2 is -176.838 +/- 2.5 kJ/mol and BRAF-Drug3 is -226.9318 +/- 8.2 kJ/mol which is as represented in Table 2. The interaction energy also confirms that BRAF-Drug3 is having low interaction energy than other two complexes.

DISCUSSION

Through literature survey, we have identified top three drugs namely aknadacin and 16beta-hydroxy-vendolinine-n-oxide referred as

Table 1. Binding affinity score of all three drugs with BRAF protein

| Drug Name | Docking Score |
|------------------------------------|---------------|
| Aknadacin | -8.1 kcal/mol |
| 16beta-hydroxy-vendolinine-n-oxide | -8.4 kcal/mol |
| (215) Drug3 | -8.8 kcal/mol |

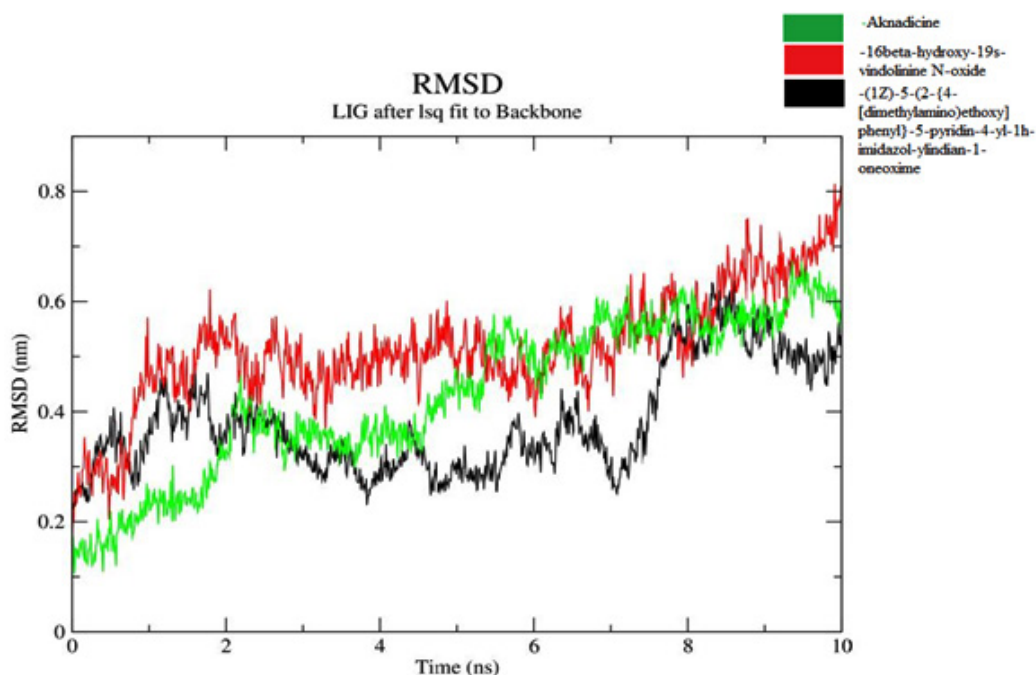
Table 2. Interaction energy of all three drugs with its BRAF protein

| Drug Name | Interaction energy |
|------------------------------------|--------------------------|
| Aknadacin | -210.0979 +/- 4.8 kJ/mol |
| 16beta-hydroxy-vendolinine-n-oxide | -176.838 +/- 2.5 kJ/mol |
| (215) Drug3 | -226.9318 +/- 8.2 kJ/mol |

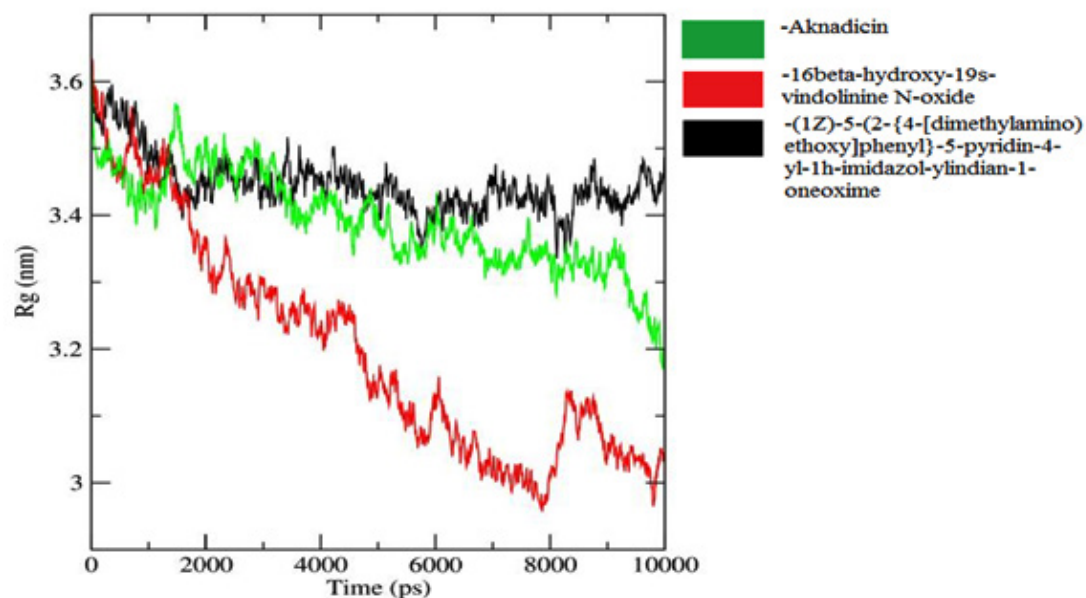
Drug1 and Drug2 respectively and third drug 215 (Drug3) was retrieved from RCSB where Protein ID 2FB8 with the Drug ID 215.

In this study, we are identifying the most efficient drug or inhibitor to melanoma

cells causing skin cancer by comparing these three drugs using computational analysis. Here, we constructed the 3D structure of a wild-type BRAF(V600E) protein using homology modelling and validated it through the Ramachandran plot and



Radius of gyration (total and around axes)



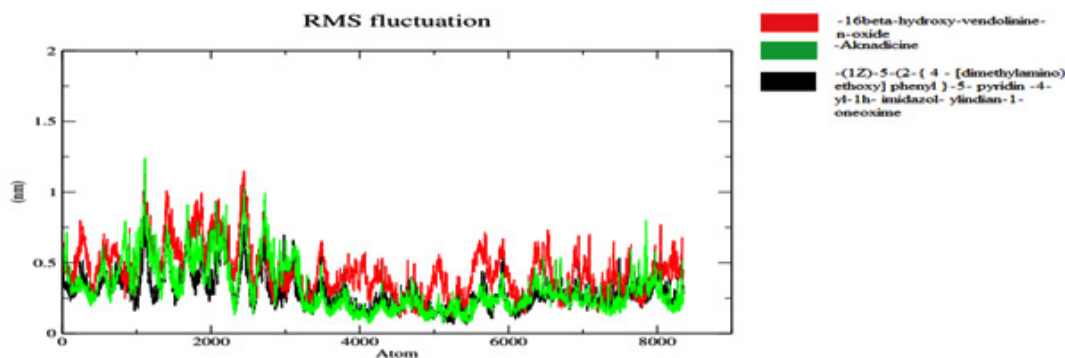


Fig. 3. (a) RMSD of BRAF protein with its three drugs (b) Radius of Gyration of BRAF protein with its three drugs (c) RMSF of BRAF protein with its three drugs

verify3D. Then we perform structural docking of protein with each drug and calculate the binding affinity score. Binding affinity score of Drug1, Drug2 and Drug3 was -8.1 kcal/mol, -8.4 kcal/mol, and -8.8 kcal/mol respectively. From the result, it is revealed that the BRAF-Drug3 complex has higher binding affinity than the BRAF-Drug1 and BRAF-Drug2 complex.

We further proceeded to MD simulation to analyze stability of these drugs with protein and to know the conformational changes of the drug with protein. Results of RMSD, RMSF and radius of gyration indicate that the BRAF-Drug3 complex is more stable than the BRAF-Drug and BRAF-Drug2 complex. The computed interaction energy of all the three complexes, BRAF-Drug1 is -210.0979 +/- 4.8 kJ/mol, BRAF-Drug2 is -176.838 +/- 2.5 kJ/mol and BRAF-Drug3 is -226.9318 +/- 8.2 kJ/mol also revealed that Drug3 is more efficient than other two drugs. Thus from the result of docking, MD simulation, interaction energy calculation it is concluded that Drug3(215) is the most efficient drug or potent inhibitor of melanoma cells among three drugs.

CONCLUSION

On the basis of results of homology modelling, structure-based virtual screening or docking, MD simulation and interaction energy calculation of protein and drug complex, we recommend Drug3 (215 pronounced as (1Z)-5-(2-(4-(dimethylamino)ethoxy)phenyl)-5-pyridin-4-yl-1h-imidazol-ylindian-1-one-oxime) as a

more efficient drug or potent inhibitor of melanoma cells causing skin cancer.

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