Identification Of Novel Inhibitors Against NRAS Target In Melanoma Using *In Silico* Approach

A project submitted to the

Bioinformatics Centre,

Savitribai Phule Pune University, Pune-07

For the degree of

M. Sc. in Bioinformatics

By

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Under the guidance of

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Submitted on May,2019

CERTIFICATE

This is to certify that the project entitled **"Identification of novel inhibitors against NRAS target in Melanoma using *in silico* approach.",** submitted by **Ms.Das Nayanika** in partial fulfillment of the requirements for the degree of Master of Science in Bioinformatics, has been carried out satisfactorily by her/him at the Bioinformatics Centre, Savitribai Phule Pune University.

Date: 20 /05 /2019
Place: Pune

Dr. Sangeeta V Sawant
Director
Bioinformatics Centre
Savitribai Phule Pune University,
Pune 411007

CERTIFICATE

This is to certify that the project entitled **"Identification of novel inhibitors against NRAS target in Melanoma using *in silico* approach."**, submitted by **Ms./Das Nayanika** in partial fulfillment of the requirements for the degree of Master of Science in Bioinformatics, has been carried out satisfactorily by her/him at the Bioinformatics Centre, Savitribai Phule Pune University, under my/our guidance and supervision.

Dr.Vijay B. Baladhye
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Date: 20 /05 /2019
Place: Pune

DECLARATION & UNDERTAKING

I hereby declare that the project entitled, "**Identification of novel inhibitors against NRAS target in Melanoma using *in silico* approach** ", submitted in partial fulfillment of the requirements of the degree of Master of Science in Bioinformatics, has been carried out by me at Bioinformatics Centre, Savitribai Phule Pune University under the guidance of **Dr.Vijay B.Baladhye** (Guide) and **Dr.Rohan Meshram** (Co-guide). I further declare that the project work or any part there of has not been previously submitted for any degree or diploma of any University.

I also declare that to the best of my ability, I have ensured that the submission made herein, including the main text, supplementary data, deposited data, database entries, software code, figures, does not contain any plagiarized material, content or ideas, and that all necessary attributions have been appropriately made and all copyright permissions obtained, cited and acknowledged.

I also declare that any further extension, continuation, publication, patenting or any other use of this project (either in full or in part), if any, shall be undertaken with prior written consent from the Director, Bioinformatics Centre, Savitribai Phule Pune University and the Project Supervisor/s.

I further state that I shall explicitly mention, "Bioinformatics Centre, Savitribai Phule Pune University" as "place of work" and acknowledge "the M.Sc. Bioinformatics training programme at Savitribai Phule Pune University for infrastructure and facilities" in the publication (print and online)/patent based on this work. I shall also acknowledge source of M.Sc. studentship (DBT or IGIB-GNR), if availed.

Date: 20 /05 /2019 Ms. Nayanika Das

Place: Pune BIM_2017_07

ACKNOWLEDGEMENTS

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Nayanika Das

BIM_2017_07

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ABSTRACT

Melanoma is a type of skin cancer which is uncured till date. It is most frequently seen in people with fairer skin and lesser melanin production. Melanoma is wide spread in the western parts of the world. The most important targets of melanoma are BRAF and NRAS.These targets are found to be highly aggressive and responsible for cell growth and proliferation. There are few MEK (Mitogen Kinase) inhibitors developed to control the progression of BRAF and NRAS. Binimetinib, a MEK inhibitor was found to be highly effective towards mutant NRAS and could inhibit it's activity upto 20-30%.Immune based therapies are widely in practice to target NRAS, clinical trials are on going and now it is under third clinical trial. The wild type structure was of NRAS was already available in PDB, after doing a literature survey the amino acid residues that have been mutated was found and it was 12,13 and 61 residue of wild type. Therefore, the three residues were mutated according to the knowledge from literature for carrying out virtual screening of 5322 natural compounds present in ZINC database natural product repository. The best compounds were then considered for further analysis based on their binding free energy , the lesser the binding energy the better can it be a potential inhibitor. The interaction plots of the top eleven compounds was made to analyse the hydrophobic interaction and the residues that have the capability to form hydrogen bonds with the ligand. This gave an idea about how strongly the compounds can bind to NRAS and show inhibitory activity. The stability of the compounds was determined by these interactions.

INTRODUCTION

Melanoma is a type of skin cancer which has evolved with time is people who have lesser melanin production to fight against the disease.It is the 19th most common cancer all over the world. Melanin helps to fight against UV radiations which are harmful and is one of the environmental reason causing melanoma. Up till date there are no permanent cure of melanoma .NRAS and BRAF are the two important targets to stop melanoma. The first stages of melanoma is associated with The MAPK signaling pathway with includes NRAS as second most important target in drug designing and drug repurposing. Noonan syndrome, a developmental disorder characterized by heart defects, reduced growth, facial dysmorphism and variable changes in the body, is caused by dysfunctioning of the RAS-MAPK signaling pathway. These findings provides us with proper idea as to how NRAS function is related to human development and growth.(Ion C Cirstea) Melanoma in the US has grown to a very high leve,about 96000 will be diagnosed in 2019.It can be diagnosed at younger 30 and above 60.Vaccines might help to stop the growth of the melanoma cells.No results are found even at the third stage of melanoma trials.But researchers try to find different types of vaccines including talimogene laherparepvec (T-VEC) which uses a form of the cold sore virus.(https://www.cancerresearchuk.org/about-cancer/melanoma/research-clinical-trials/research). There are few reports in the literature on cutaneous malignant melanoma (CMM) in the Asian population. India is one of the regions of the world were melanoma is found in lesser patients.While 25.2% is reported in cutaneous melanomas, almost one-third of the patients from Trivandrum. (http://medind.nic.in/maa/t09/i3/maat09i3p292.pdf). World's highest melanoma risk is accounted in Australia and New Zealand followed by North America. Melanoma is the third most frequent cancer among women age 20-39 and in men age 20-39. 10% of all people with melanoma have a genetic triat. About 4,740 males and 2,490 females are seen to be dying in the U.S. of melanoma during 2018.Therapeutic vaccination with dendritic cells for melanoma was first found in the 1990s, knowledge has grown with regard to their development. Trametinib, a MEK inhibitor that can be taken orally has shown to decrease cell proliferation and induce apoptosis.(https://www.ajmc.com/journals/evidence-based-oncology/2013/2013-1-vol19-sp3/the-future-of-melanoma-treatment?p=2)

Signs Of Melanoma.

Asymmetry: Irregular shape, Border: The edge is not smooth, Color: The mole has dark spots, Diameter:The mole changing in size, shape or texture.(https://www.cancercenter.com/cancer-types/melanoma/symptoms)

Types Of Melanoma.

Superficial spreading melanoma- The most common type.Mostly found on the arms, legs, chest and back. The melanoma cells spread out across the surface of the skin.(https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html)

Nodular melanoma-The second most common type. It can grow more quickly than other melanomas. It is also more likely to loose its colour when growing, becomes red. It is more found on the chest, back, head or neck.(https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html)

Lentigo maligna melanoma- It is usually found in older people, in parts of skin with sun exposure for prolong periods. It is often found on the face and neck and is slow growing type.(https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html)

Acral lentiginous melanoma- It is the rarest type found on the palms of the hands, soles of the feet, or under fingernails or toenails. It is not related to sun exposure.(https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html)

A B C D

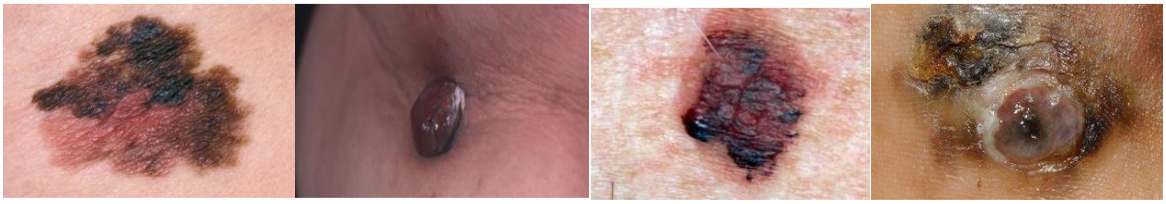

Fig 1.Images have been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

A- Superficial spreading melanoma

B- Nodular melanoma

C- Lentigo maligna melanoma

D- Acral lentiginous melanoma

Important Pathways Involved In Melanoma.

PATHWAY	COMPONENTS MUTATED/ACTIVATED	TYPE OF ALTERATION
RAS/RAF/MEK/ERK	NRAS BRAF MEK1	Mutation Mutation Mutation
RAS/PI3K/PTEN/AKT/mTOR	PIK3CA PTEN AKT1,AKT2 AKT3	Mutation Mutation Rare mutation Amplification

CDK	CDK4 CCND1	Mutation/Amplification Mutation
MITF	MITF	Mutation/Amplification

Table I. The contents of this table has been borrowed from Shtivelman E, Davies MQ, Hwu P, et al. Pathways and therapeutic targets in melanoma. Oncotarget. 2014;5(7):1701-52.

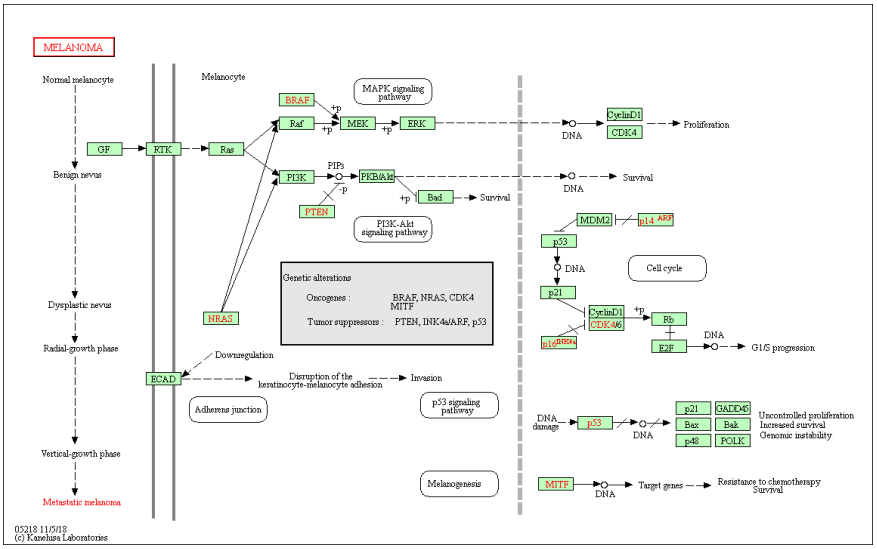

Fig.2 This image has been borrowed from https://www.genome.jp/kegg-bin/show_pathway?hsa05218

MAPK Pathway

Dysregulation of MAPK pathway is very common in many cancers due to mutations in RAS genes (Inamdar GS, Madhunapantula SV, Robertson GP 2010).The MAPK pathway is one of the major pathways involved in progress of melanoma. It harbors BRAF,NRAS,ERK and MEK as it's components. It is important because of the signaling, growth, proliferation of the oncogenic cells. About 28% of the mutations are seen in NRAS of this pathway(Wellbrock C, Arozarena I 2016) Studying this pathway for quite sometime MEK inhibitors were designed to target BRAF and

NRAS, but it did not show 100% inhibitory activity towards both.The crosstalks with MAPK and the other pathways is very important for further targeting NRAS in future.NRAS is second most aggressive target after BRAF.

PI3K/AKT/mTOR pathway

PI3K-Akt pathway has a role in RAF and MEK inhibitor combination resistance for a good combined target of drugs (Siroy A.E., Davies M.A., Lazar A.J. 2018) The mutations found in mTOR are in acral,mucosal form(Yan Kong,et.al 2016).PTEN(phosphatase and tensin) acts as a tumor suppressor for this pathway.

CDK4 Pathway

This pathway follows the cell cycle arrest and death of healthy cells leading to progression of melanocytes (Sheppard KE1, McArthur GA 2013). The dormant cells enter into G1 phase from G0 phase.(Lee B, McArthur GA 2015)BRAF has proved to be an essential biomarker while treating melanoma by CDK4 pathway.

MITF Pathway

MITF (microphthalmia-associated transcription factor) is a transcription factor found mostly in malignant melanoma(Hartman ML, Czyz M 2015).mRNA act as an unlikely change in MITF transcript(Hartman ML, Czyz M 2015).

NRAS (Neuroblastoma Ras viral oncogene)

NRAS(Neuroblastoma Ras viral oncogene)gene produces NRAS protein .It acts like a switch for GTP and GDP molecules which is turned On and Off.The N-Ras protein must be turned on by attaching to a molecule of GTP. The N-Ras protein is turned off when it converts the GTP to GDP(https://ghr.nlm.nih.gov/gene/NRAS).When switch is turned off no activation and no signaling is seen by NRAS.This gene belongs to oncogenic family which are cancer causing genes. The proteins produced from NRAS are GTPases. These proteins play important roles in cell division, cell differentiation,cell proliferation. In N-Ras the differences cluster at residues 94–95 (helix 3) and 131–132 (helix 4)(Johnson CW, Reid D, Parker JA, et al. 2017).

This is the wild type structure of NRAS which is curated in PDB.NRAS is most frequently mutated at 12 13 and 61 positions(Douglas B. Johnson, M.D. and Igor Puzanov, M.D 2016).

G-D = 12 (Glycine-Aspartic Acid)

G-D = 13 (Glycine-Aspartic Acid)

Q-R = 61 (Glutamine-Arginine)

A clear difference between isoforms of NRAS is the binding of (RBD) Ras Binding Domain. (Johnson CW, Reid D, Parker JA, et al. 2017) The G-domain, which catalyzes GTP hydrolysis and mediates downstream signaling, is 95% conserved between the Ras isoforms. (Johnson CW, Reid D, Parker JA, et al. 2017) The Wild Type NRAS has a total of 189 amino acids. The overall structure can be divided into a catalytic G domain, which is comprised (residues 1-86) and (residues 87-166). This is the first crystal structure of NRAS solved at 1.67Å resolution.(Johnson CW, Reid D, Parker JA, et al. 2017)

Fig 3.Wild Type Structure of NRAS [PDB ID-5UHV]

Data collection and refinement statistics for wild type N-Ras bound to GppNHp

Wavelength (Å)	1.54
Resolution range (Å)	28.5–1.67 (1.73–1.67)[a]
Space group	$P3_221$
Unit cell	
a, *b*, *c* (Å)	30.01, 39.01, 159.2
α, β, γ (°)	90, 90, 120
Total reflections	17,229 (1459)[a]
Unique reflections	195,722
Multiplicity	11.4
Completeness (%)	98.7 (87.7)[a]
Mean I/σ	24.0 (1.31)[a]
Wilson B factor	26.4
R_{merge}	0.079
R_{work} (%)	18.8 (26.8)[a]
R_{free} (%)	22.8 (30.6)[a]
No. of non-hydrogen atoms	1434
Macromolecules	1281
Ligands	33
Water	120
Protein residues	166
Root mean square (bonds)	0.007
Root mean square (°)	1.11
Ramachandran favored (%)	97
Ramachandran outliers (%)	0
Clash score	4.3
B-factor (average)	28.5
Macromolecules	27.7
Ligands	25.4
Solvent	36.7

Fig 4. The image has been borrowed from Christian W. Johnson, Derion Reid, Jillian A. Parker , Shores Salter , Ryan Knihtila , Petr Kuzmic , and Carla Mattos From the Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115 and BioKin Ltd., Watertown, Massachusetts 02472

NRAS As A Potential Target

NRAS is associated with immune based therapies.The MEK inhibitors particularly Binimetinib has shown good response towards NRAS.Computational methods to find NRAS have not yet been done or practiced.Clinical trials are still going on which show only 20-30% survival in patients.Hence,virtual screening and drug repurposing approach was performed to find the most effective inhibitors that can be further used as drug targets in inhibiting the activity of NRAS. MEK protein is a kinase protein with an adenosine triphosphate (ATP) binding pocket that fixes between catalytic active (phosphorylated) site and catalytic inactive (unphosphorylated) site.Several such MEK inhibitors are in clinical trials for the disease, the most important among those are based on early-stage clinical trial data.

Binimetinib

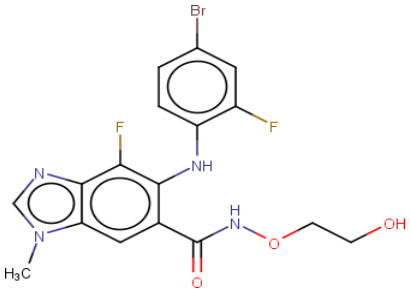

Fig 5.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

The MEK inhibitor that is particular to mutant NRAS.This is the only MEK inhibitor showing a strong activity towards NRAS mutant melanoma.Three trials were taken for binimetinib and the disease showed a stability of 3.7 months.Along with Binimetinib other inhibitors were combined and trails are still in process for it.

MATERIALS & METHODS

Stucture Validation Tools.

ProSA (https://prosa.services.came.sbg.ac.at/prosa.php**)**

ProSA is frequently in use for protein structure validation. ProSA calculates an overall quality score for a specific input structure. The z-score indicates overall model quality . Its value is displayed in a plot that contains the z-scores of all protein chains in current PDB. ProSA-web visualizes the 3D structure of the input protein using the molecule viewer Jmol. ProSA was used for validation of the structure of Mutant NRAS build in SPDBV.

PROCHECK(https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/**)**

PROCHECK is a model evaluation and validation tool.PROCHECK checks for the stereochemical quality of a protein structure, producing a number of PostScript plots analysing its residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR technique.The G-factor will give measure of how normal or how unusual the property in itself is.It gives the Ramachandran plot to understand if the residues are in allowed or disallowed regions.PROCHECK was used for validation of the structure of Mutant NRAS build in SPDBV.

Database used for obtaining natural compounds for screening.

Zinc DB (https://zinc.docking.org/**)**

Natural Products occupy an important part of small molecule space because they are recognized by at least two proteins: the end of their biosynthetic pathway and their evolutionary biological target.The natural compounds that were to be screened were obtained from Zinc Database natural product library.

Filtering of compounds.

FAFdrugs4

FAF-Drugs4 (Free ADME-Tox Filtering Tool) is a program for filtering large compound libraries prior to *in silico* screening experiments or related modeling studies.(http://fafdrugs4.mti.univ-paris-diderot.fr/)The tool can perform computational prediction of some ADME-Tox properties (Adsorption, Distribution, Metabolism, Excretion and Toxicity) in order to assist hit selection before chemical synthesis or ordering.(http://fafdrugs4.mti.univ-paris-diderot.fr/). This tool was used to filter out the natural compounds present in Zinc database in order to remove the interfering compounds and only use the lead compounds and the main aim is to have starting point molecules after screening that have the potential to be optimized with comparitively small log P and thus molecules that could be used further to increase affinity. PAINS moieties, standing for Pan Assay Interference Compounds, are compounds that are frequent occurers many biochemical high throughput screens.Therefore,the filters used were LEAD-like compounds and PAINS moieties.

Protein and Ligand Preparation.

PDB(Protein Data Bank)

The Wild Type NRAS structure with best resolution and curated in Uniprot was selected.The PDB ID is 5UHV.The uniport ID is P01111. N-Ras is a small GTPase structure found in human cancers and is one of the most important therapeutic target of Melanoma.It is responsible for cell survival,cell proliferation,cell differentiation.A clear difference between isoforms of N-Ras is the binding of (RBD) Ras Binding Domain.[Johnson CW, Reid D, Parker JA, et al.]NRAS has only A chain associated with it.Hence,docking was carried out on the A chain of NRAS on the active site.The G-domain, which catalyzes GTP hydrolysis and mediates downstream signaling, is 95% conserved between the Ras isoforms.[Johnson CW, Reid D, Parker JA, et al.]

Molecular Docking.

PyRx

PyRx is a virtual screening tool which was used for docking of natural compounds.This uses AutoDock 4.2 suite for docking.The algorithm used is Lamarckian GA.The tool was first evaluated

using NRAS mutant structure and the ligand phosphoaminophosphonic acid-guanylate ester which is a non-hydrolyzable analog of GTP.The nucleotide is a potent stimulator of adenylate cyclase was docked.The RMSD tolerance of 2.0 Å which indicated the good stability of the software.The Grid box was set to 50 x 50 x 50 with spacing of 0.3750 Å .All the ligands chosen were converted to pdbqt format in PyRx tool.The Autodock was set to run after which there were 10 poses for each compounds.Only the ones with the best binding score was chosen and tabulated for each compound in the sdf files.This generated the best protein ligand binding for further analysis.

Analysis of docking results.

LigPlot^{+} is an advanced version of LIGPLOT program for automatic generation of 2D ligand-protein interaction diagrams(https://www.ebi.ac.uk/thornton-srv/software/LigPlus/).The analysis of docked poses with best scores was done using this tool.The hydrophobic interactions and hydrogen bond interactions contribute to the stability of the protein-ligand complex. This helps us to determine the potential of each compound to be an inhibitor of mutant NRAS.

RESULTS & DISCUSSION

Structure

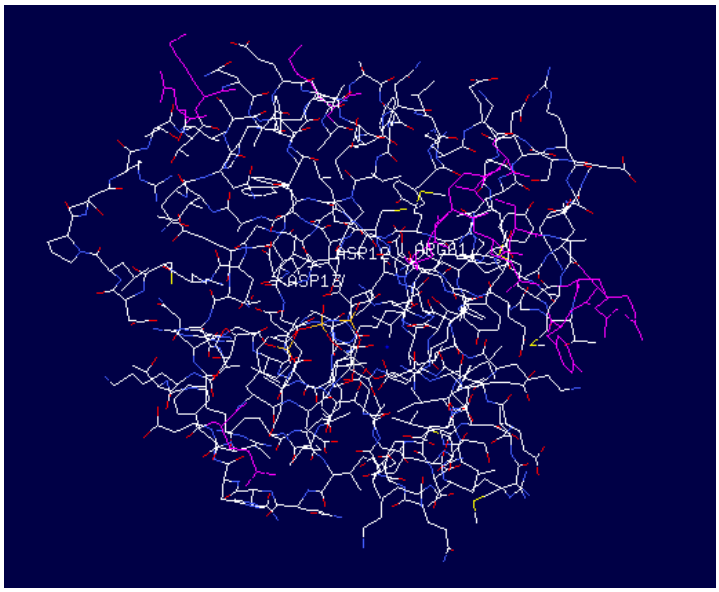

Fig 6.Mutant NRAS structure built in SPDBV

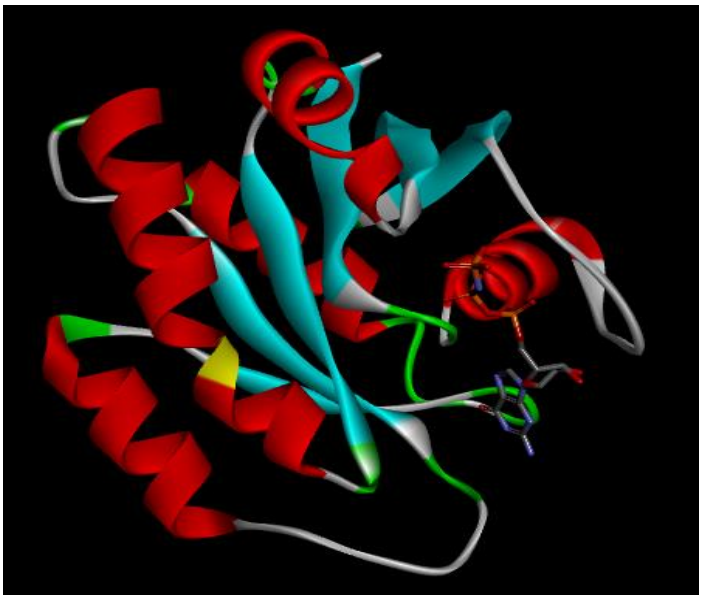

Fig 7.Mutant NRAS structure built in discovery studio

This is the mutant structure of NRAS which was built in SPDBV.The three amino acid residues at 12 13 and 61 positions G-D = 12 (Glycine-Aspartic Acid),G-D = 13 (Glycine-Aspartic Acid),

Q-R = 61 (Glutamine-Arginine) were mutated. The structure was further evaluated using PROSA, PROCHECK .

Structure Validation

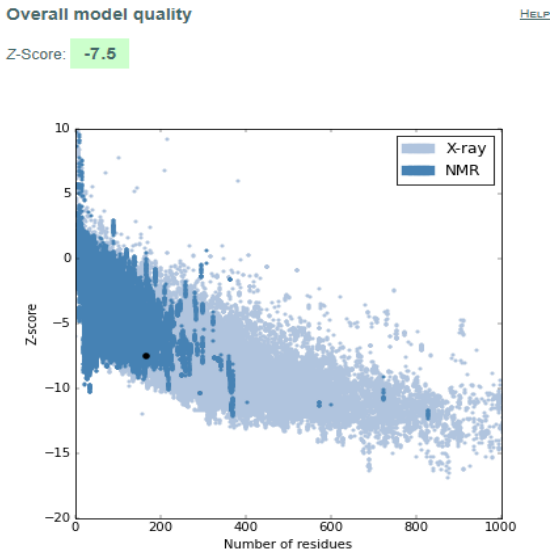

Fig.8 PROSA analysis of Mutant NRAS

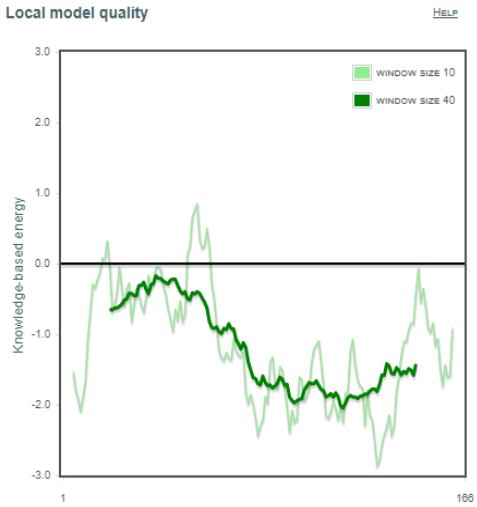

Fig.9 PROSA analysis of Mutant NRAS

According to PROSA results,the negative values indicate good results and positive values indicate erroneous points.Most of the part of the structure is found in the negative part and therefore,erroneous points are lesser.

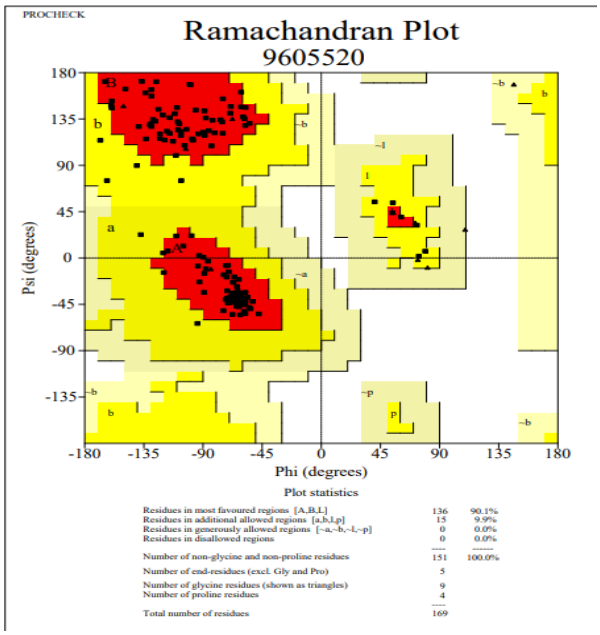

Fig.10 PROCHECK analysis of Mutant NRAS

Plot statistics

Residues in most favoured regions [A,B,L]	136	90.1%
Residues in additional allowed regions [a,b,l,p]	15	9.9%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	151	100.0%
Number of end-residues (excl. Gly and Pro)	5	
Number of glycine residues (shown as triangles)	9	
Number of proline residues	4	
Total number of residues	169	

Fig.11 PROCHECK analysis of Mutant NRAS

PROCHECK results indicate that most of the residues are in the allowed region. The black dots are the residues in allowed region.

Virtual Screening Results.

5322 natural compounds were screened from Zinc Database natural products Using Pyrx Autodock 4.2.The binding site was defined using AutoGrid in the Pyrx virtual screening tool.These were the best poses that were selected after screening 5322 compounds. This was because of their binding score criteria according to the tool.

ZINC ID	Binding free energy (kcal/mol)	Hydrogen bonding pairs HD::HA	Residues in Hydrophobic Interactions or van der Waals contact
ZINC02107288	-10.62	Lys-16(N):: Unk0(C)^{OXT} Lys-16(NZ):: Unk0(C)^{OXT} Gly-15(N):: Unk0(C)^{OXT} Ser-17(N)::Unk0(C)^{O} Asp-33(N)::Unk0(C_{11})$^{O}{}_{3}$	Val-14,Asp-13, Gly-60,Asp-12, Tyr-32,Glu-31,Ala-18, Asn-116,Lys-117, Asp-119,Lys-147, Phe-28,Pro-34
ZINC02096813	-10.61	Thr-35(N):: Unl1(C_{23})$^{O}{}_{5}$ Asp-12(OD1):: Unl1(N) Gly-60(N):: Unl1(OXT) Lys-16(NZ):: Unl1(OXT)	Ser-17,Gly-15, Asn-116,Lys-117, Phe-28,Lys-147, Ala-146,Asp-119, Ser-145,Ala-18,Tyr-32, Asp-13
ZINC02096815	-10.33	Lys-16(N):: Unl1(C_{23})$^{O}{}_{5}$ Lys-16(NZ):: Unl1(C_{23})OXT Gly15(N):: Unl1(C_{23})$^{O}{}_{5}$ Ser17(OG):: Unl1(C20)^{O4}	Asp-12,Thr-58, Asp-13,Asp-33,Try-32, Glu-31,Phe-28,Lys-147, Asp-119,Ala-146, Lys-117,Asn-116,Thr-35
ZINC02114326	-10.23	Asn-116(ND2):: Unl1(CL) Lys-147(NZ):: Unl1(OXT)	Phe-28,Asp-119, Leu-120,Gly-15,

		Asp-33(N):: Unl1 (C_{12})$^{O}_{4}$	Lys-117,Asp-30, Lys-16,Ser-17, Ala-18,Tyr-32,Glu-31
ZINC01020381	-10.16	Gly-15(N):: Unl1(C_{24})$^{O}_{2}$ Lys-16(N):: Unl1(C_{24})$^{O}_{2}$ Lys-16(NZ):: Unl1(C_{24})$^{O}_{2}$	Asp-119,Lys-147, Asn-116,Ala-146, Ser-145,Glu-31, Phe-28,Asp-30,Asp-13, Thr-58,Asp-33
ZINC06167356	-10.16	Thr-58(O)::Unl1(C_{6})$^{O}_{6}$ Gly-60(N):: Unl1(C_{6})$^{O}_{6}$ Lys-117(NZ):: Unl1(C_{18})$^{O}_{5}$ Asp-30(N):: Unl1(C_{16})$^{O}_{4}$ Glu-31(N):: Unl1(C_{16})$^{O}_{4}$ Ala-18(N):: Unl1(C_{9})$^{O}_{2}$ Ser-17(N):: Unl1(O1)	Asp-33,Asp-12, Lys-16,Thr-35, Gly-15,Pro-34, Ala-59,Asp-13,Val-29
ZINC02120343	-10.14	Asp-33(N):: Unl1(C_{9})$^{O}_{2}$ Ser-17(N):: Unl1(C_{22})$^{O}_{5}$ Lys-16(N):: Unl1(C_{22})$^{O}_{4}$ Lys-16(NZ):: Unl1(C_{22})$^{O}_{4}$ Asp-13(N):: Unl1(C_{22})$^{O}_{4}$ Gly-15(N):: Unl1(C_{22})$^{O}_{4}$	Asp-119,Ser-145, Lys-147,Ala-146, Phe-28,Ala-18,Asn-116, Lys-117,Glu-31,Tyr-32, Pro-34,Asp-12,Val-14
ZINC02102162	-10.14	Lys-16(N):: Unl1(C_{22})$^{O}_{6}$ Lys-16(N)::Unl1(C_{22})$^{O}_{5}$ Val-14(N):: Unl1(C_{22})$^{O}_{5}$	Pro-34,Asp-12, Asp-13,Asp-33, Glu-31,Tyr-32,

		Gly-15(N):: Unl1(C_{22})$^{O}_{5}$ Ser-17(N):: Unl1(C_{22})$^{O}_{6}$	Phe-28,Lys-117, Asn-116,Ala-18, Ala-146,Lys-147, Asp-119
ZINC02114643	-10.1	Asp-33(N)::Unl1(C_{16})$^{O}_{4}$ Lys-16(N)::Unl1(C_{24})$^{O}_{5}$ Lys-16(NZ)::Unl1(C_{24})$^{O}_{6}$ Gly-15(N)::Unl1(C_{24})$^{O}_{5}$	Asp-19,Phe-28, Lys-147,Lys-147, Leu-120,Tyr-32, Ala-18,Ala-146, Lys-117,Thr-35, Glu-31,Pro-34,Asp-13
ZINC00518885	-10.06	Lys-117(N):: Unk0(C_{9})$^{O}_{2}$ Ala-146(N):: Unk0(C_{9})$^{O}_{2}$ Asn-116(ND2)::Unk0(O3) Asp-13(N):: Unk0(OXT) Lys-16(N)::Unk0(O) Lys-16(NZ)::Unk0(O) Gly-15(N)::Unk0(O) Val-14(N)::Unk0(O)	Asp-119,Phe-28, Asp-33,Pro-34,Asp-12, Ala-18,Ser-17,Lys-147, Ser-145
ZINC03882094	-10.04	Lys-16(NZ)::Unl1(C_{21})$^{O}_{3}$ Gly-15(N):: Unl1(C_{21})$^{O}_{3}$ Ser-17(N)::Unl1(C_{22})$^{O}_{4}$	Thr-35,Asp-13, Val-14,Asp-12, Asp-30,Glu-13, Asp-119,Lys-117, Phe-28,Asp-57, Ala-18,Thr-58

Table II : Summary of docking results

The above are the top eleven compounds with the best binding energy score. The binding free energy gives the nature of the protein and the ligand that has bound to it. Also the conserved residues that take part in the hydrophobic interaction around the protein which are an indicative of stability to the structure and hydrogen bond formation between donor and acceptor atoms and residues of the protein are tabulated in the same table. The conserved hydrophobic residues like Asp-119, Phe-28 etc. along with hydrogen bond forming residues like Gly-15, Asp-33 and so on are reported in the table.

Role of conserved residues in inhibition of NRAS

1.Thr-35

This residue shows hydrophobic interactions which are responsible for the stability of the structure. Thr-35 residues are important for Mg2 coordination and interactions.(Johnson CW, Reid D, Parker JA, et al. 2017) In complex 2 with Zinc ID ZINC02096813_6040 Thr-35 forms a hydrogen bond between N of Thr-35 and O^5 of Unl1(ligand) at a distance of 3.25 Å .In complex nine and eleven with Zinc IDs- ZINC02114643_7056 and ZINC03882094_17080 respectively it shows hydrophobic interactions.

2.Tyr-32

The crystal structure of NRAS-GppNHp has switch I ordered in the active site, with Tyr-32 interacting with the γ-phosphate of a neighbouring symmetry-related molecule of NRAS.(Johnson CW, Reid D, Parker JA, et al. 2017).When the switch 1 is in state 2 then Tyr-32 is turned away from active site in state 1.In complex one, three, seven, eight and nine with Zinc IDs- ZINC02107288_19972,ZINC02096815_6057, ZINC02120343_9364, ZINC02102162_14892 and ZINC02114643_7056 respectively Tyr-32 shows hydrophobic interaction stabilizing the complex.

3.Phe-28

Phe-28 is a residue showing hydrophobic interactions in complex three, four, seven, eight, nine, ten, eleven with Zinc ID- ZINC02107288_19972, ZINC02114326_14245, ZINC02120343_9364, ZINC02102162_14892,ZINC02114643_7056, ZINC00518885_18475, ZINC03882094_17080

respectively. Aided by effector lobe residue Phe-28, helps to stabilize nucleotide binding(Johnson CW, Reid D, Parker JA, et al. 2017).

4.Asp-119

Asp-119 makes nucleotide-specific interactions with the guanine base and is critical for nucleotide binding.(Johnson CW, Reid D, Parker JA, et al. 2017).It does not show hydrophobic interactions or Van der Waals contact in complex six and nine with Zinc IDs- ZINC06167356_6831, ZINC02114643_7056 respectively. It is found to be showing stability in rest of the nine complexes.

2D interaction diagrams of the selected ligands after docking.

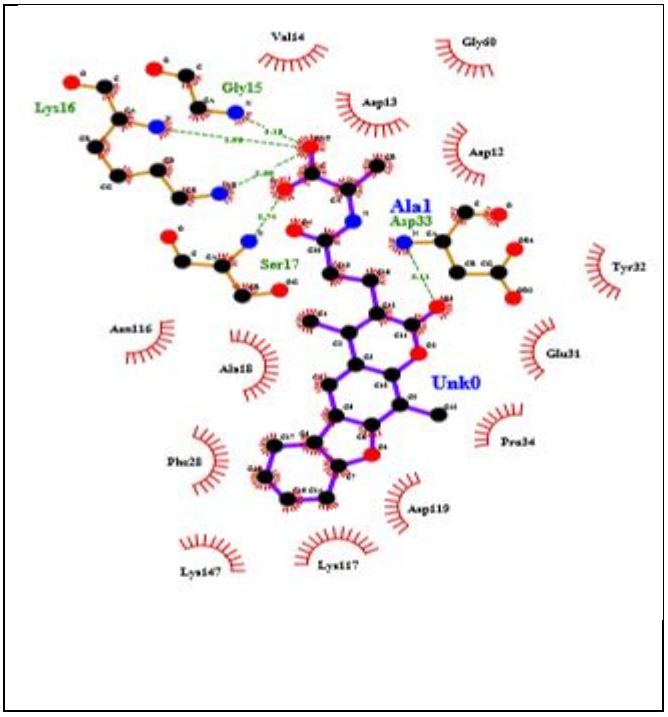

Fig.12 Molecular Docking Interaction of ZINC02107288

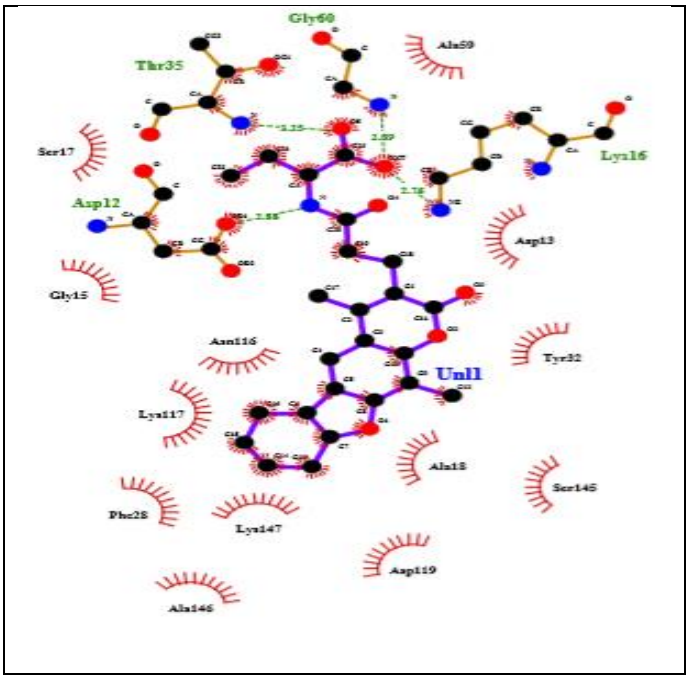

Fig.13 Molecular Docking Interaction of ZINC02096813

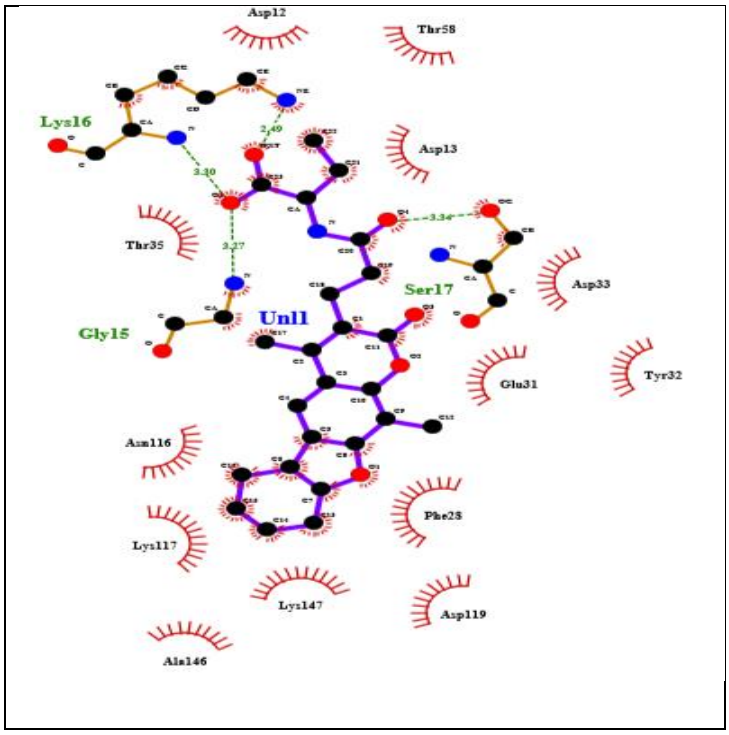

Fig.14 Molecular Docking Interaction of ZINC02096815

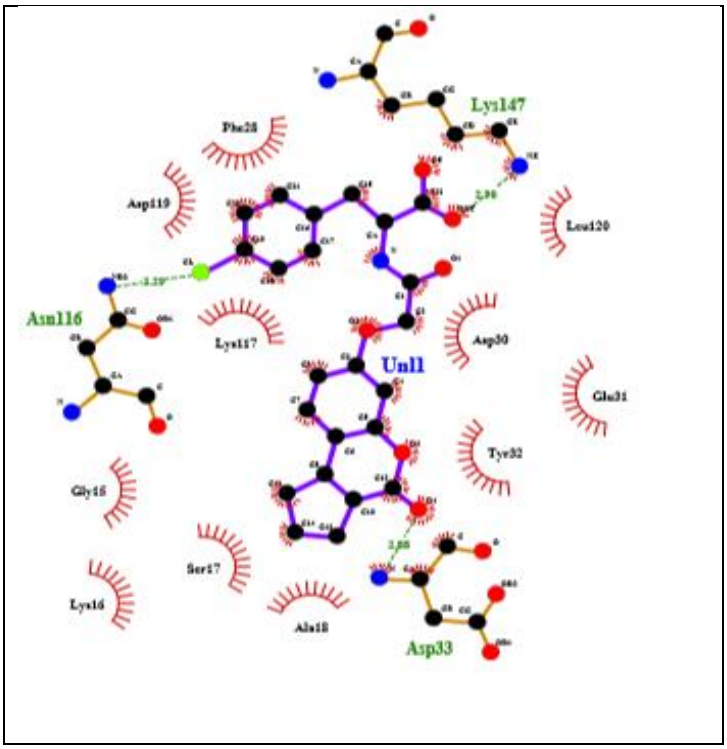

Fig.15 Molecular Docking Interaction of ZINC02114326

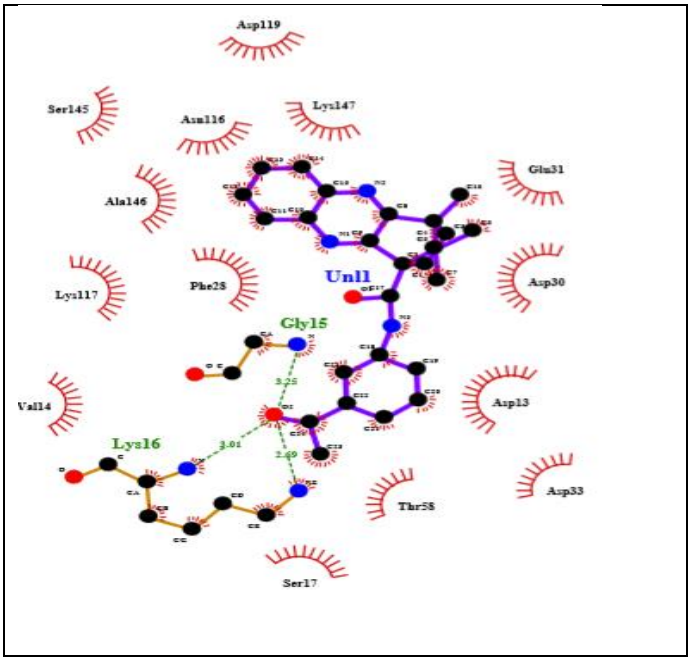

Fig.16 Molecular Docking Interaction of ZINC01020381

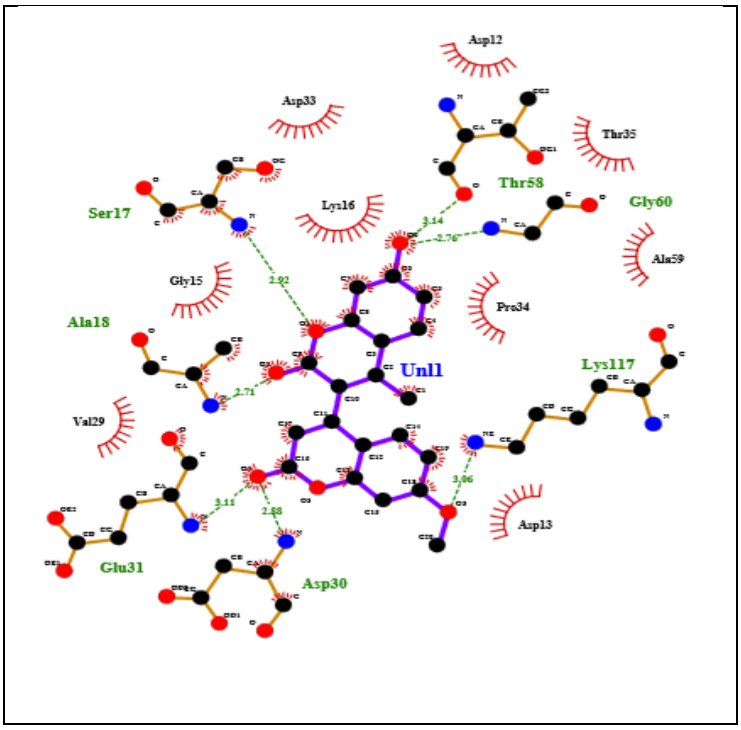

Fig.17 Molecular Docking Interaction of ZINC06167356

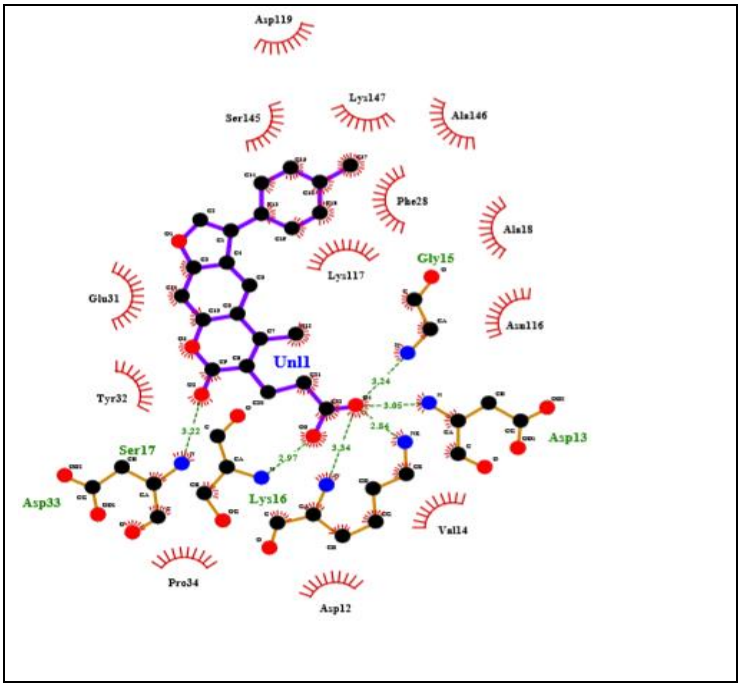

Fig.18 Molecular Docking Interaction of ZINC02120343

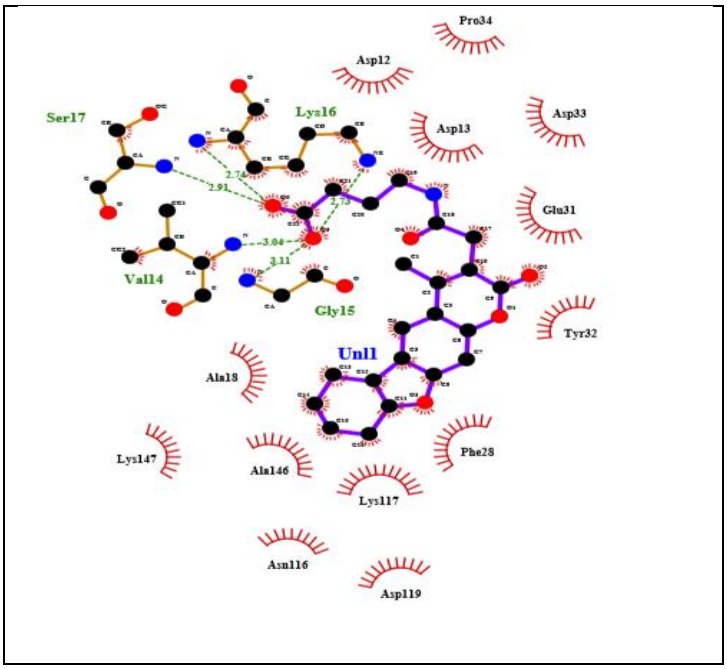

Fig.19 Molecular Docking Interaction of ZINC02102162

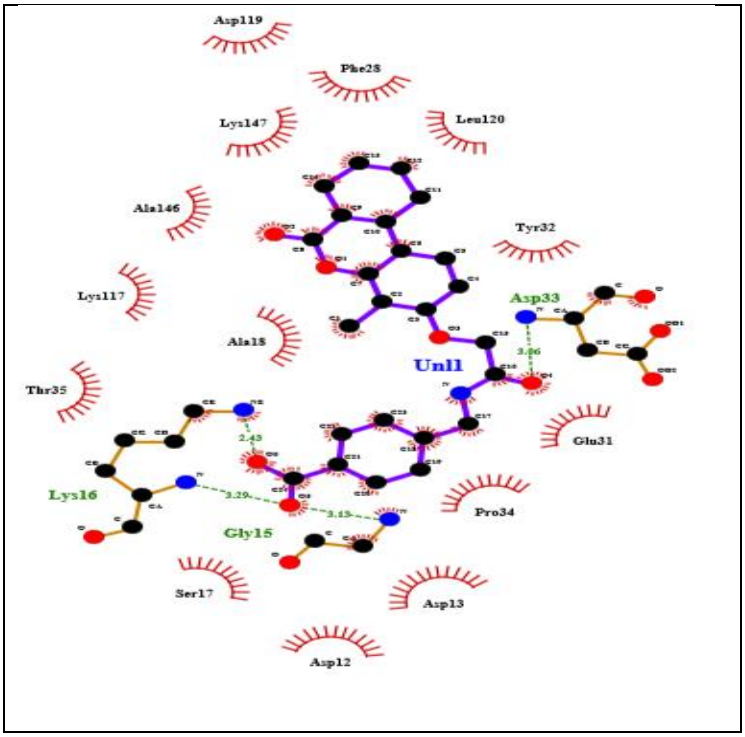

Fig.20 Molecular Docking Interaction of ZINC02114643

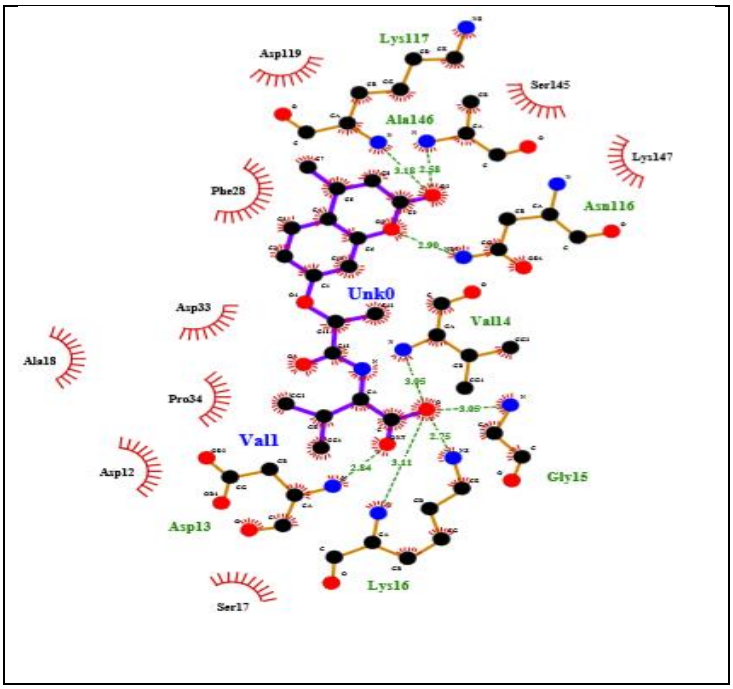

Fig.21 Molecular Docking Interaction of ZINC00518885

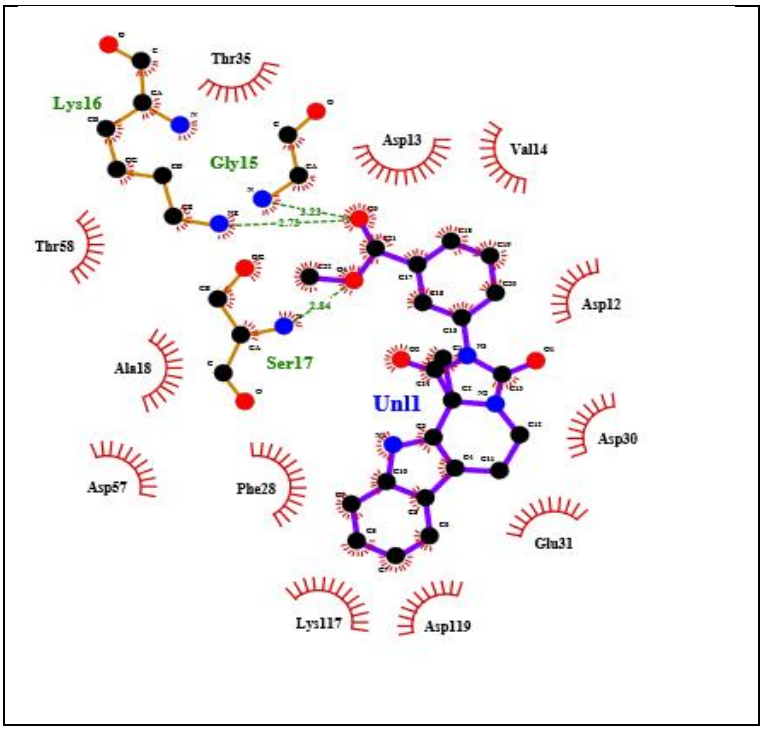

Fig.22 Molecular Docking Interaction of ZINC03882094

The hydrogen bonding pattern is not same in all the complexes. In compound two with Zinc ID- ZINC02096813,the glycine residue at position 12 in wild type NRAS being mutated to Asp-12 in mutant type NRAS shows formation of hydrogen bond with the ligand at a distance of 2.88 and , and Asp-13 forms hydrophobic interaction around the compound. Similarly in compound seven and ten with Zinc ID- ZINC02120343 and ZINC00518885 respectively Asp-13 which is also a mutated residue of Gly in mutant NRAS shows formation of hydrogen bond at a distance of 3.05 and 2.84 respectively and Asp-12 forms hydrophobic interaction around the compound.Phe-28 shows hydrophobic interaction in compound seven and ten which is very important in stabilizing nucleotide binding. And Asp-119,a critical residue in nucleotide biding stability, is present in all the complexes except six and nine with Zinc IDs- ZINC06167356_6831, ZINC02114643_7056 respectively.

CONCLUSIONS

NRAS is a potential target for melanoma. Clinical trials have not shown much of positive response towards ceasing its function and therefore, a potential inhibitor to stop the growth of cancer cells due to functioning of NRAS was need of the hour. In this report we have validated the structure of mutant NRAS after mutating three distinct residues in wild type NRAS and further screened natural compounds over it's active site to check the binding affinity of the protein and ligand. This helped to understand and select only those compounds with the best binding free energy score for future analysis.A drug repurposing approach enabled to widely spread through the different and diverse compounds in nature which could act as a novel inhibitor for mutant NRAS in future. The analysis of interactions after molecular docking gave a point towards understanding the interactions of mutant residues with the active site of the protein. Three out of eleven compounds with the best scores have hydrogen bonding between the mutant residues and the ligand and also the distance is below 3 for two of them which means these two compounds are quite stable. Also to increase the stability of the compound, Phe-28 and Asp-119 which are critical stabilizers are also present in all the three compounds showing hydrophobic interactions. Based on molecular docking studies, these three compounds with Zinc ID-ZINC02096813, ZINC02120343 and ZINC00518885 can act as a potential inhibitors of mutant NRAS. These inhibitors can be used to stop spreading of melanoma at the first stage when detected.

FUTURE WORK

Immune based therapies are highly significant for NRAS. But there are no specific inhibitors particular to NRAS. Hence, the inhibitory activity shown by some specific compounds after screening and further analysis, can be computationally validated by molecular dynamics simulations to check for the stability of the system during its inhibition and by experimental techniques on the selected compounds so as to further justify the action of the inhibitors and prove them as potent drugs against the activity of NRAS in melanoma.

References

1.Johnson CW, Reid D, Parker JA, et al. The small GTPases K-Ras, N-Ras, and H-Ras have distinct biochemical properties determined by allosteric effects. *J Biol Chem*. 2017;292(31):12981–12993. doi:10.1074/jbc.M117.778886

2. Wellbrock C, Arozarena I. The Complexity of the ERK/MAP-Kinase Pathway and the Treatment of Melanoma Skin Cancer. *Front Cell Dev Biol*. 2016;4:33. Published 2016 Apr 27. doi:10.3389/fcell.2016.00033

3. Inamdar GS, Madhunapantula SV, Robertson GP. Targeting the MAPK pathway in melanoma: why some approaches succeed and other fail. *Biochem Pharmacol*. 2010;80(5):624–637. doi:10.1016/j.bcp.2010.04.029

4. Douglas B. Johnson, M.D. and Igor Puzanov, M.D. Department of Medicine, Division of Hematology/Oncology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 777 Preston Research Building, 2220 Pierce Avenue, Nashville, TN 37232, USA. Treatment of NRAS-Mutant Melanoma. Curr Treat Options Oncol. 2015 April ; 16(4): 15. doi:10.1007/s11864-015-0330-z

5. Yan Kong, Lu Si, Yiqian Li, Xiaowen Wu, Xiaowei Xu, Jie Dai, Huan Tang, Meng Ma, Zhihong Chi, Xinan Sheng, Chuanliang Cui and Jun Guo.Analysis of mTOR Gene Aberrations in Melanoma Patients and Evaluation of Their Sensitivity to PI3K–AKT–mTOR Pathway Inhibitors.Clin Cancer Res February 15 2016 (22) (4) 1018-1027; DOI: 10.1158/1078-0432.CCR-15-1110

6. Siroy A.E., Davies M.A., Lazar A.J. (2016) The PI3K-AKT Pathway in Melanoma. In: Torres-Cabala C., Curry J. (eds) Genetics of Melanoma. Cancer Genetics. Springer, New York, NY

7. Sheppard KE1, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma.Clin Cancer Res. 2013 Oct 1;19(19):5320-8. doi: 10.1158/1078- 0432.CCR-13-0259.

8. Lee B, McArthur GA. CDK4 inhibitors an emerging strategy for the treatment of melanoma. Melanoma Manag. 2015;2(3):255-266

9.Hartman ML, Czyz M. MITF in melanoma: mechanisms behind its expression and activity. Cell Mol Life Sci. 2014;72(7):1249-60.

10. Shtivelman E, Davies MQ, Hwu P, et al. Pathways and therapeutic targets in melanoma. Oncotarget. ;5(7):1701–1752. doi:10.18632/oncotarget.1892

URLs

1.https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html

2.https://www.nhs.uk/conditions/melanoma-skin-cancer/

3.https://ghr.nlm.nih.gov/gene/NRAS

4. https://www.cancerresearchuk.org/about-cancer/melanoma/research-clinical-trials/research

5. http://medind.nic.in/maa/t09/i3/maat09i3p292.pdf

6.https://www.ajmc.com/journals/evidence-based-oncology/2013/2013-1-vol19-sp3/the-future-of-melanoma-treatment?p=2

7. https://www.cancercenter.com/cancer-types/melanoma/symptoms

8.https://www.macmillan.org.uk/information-and-support/melanoma/understanding-cancer/melanoma-types.html

9. https://www.nhs.uk/conditions/melanoma-skin-cancer/

10. https://www.genome.jp/kegg- bin/show_pathway?hsa05218

11. https://www.ebi.ac.uk/thornton-srv/software/LigPlus/

12. http://fafdrugs4.mti.univ-paris-diderot.fr/

13. https://prosa.services.came.sbg.ac.at/prosa.php

14. https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/

15. https://zinc.docking.org/

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