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RESEARCH ARTICLE

Lung Cancer: A Better Rational Drug Designing, Docking and Predicting the Efficacy of Drugs

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Abstract

Understanding the role of bio molecular dynamics in cellular processes leading to human diseases and the ability to rationally manipulate these processes is of fundamental importance in scientific research. Lung cancer occurs when a malignant (cancerous) tumor grows inside the lungs, in structures such as the bronchi (small tubes that connect the windpipe to the inner surfaces of the lungs where gas transfer takes place). GNB2L2 is identified as the potential target and modeled using Swiss prot and Modeler. The 3D structure of protein is taken for predicting active site, cavities and flexibility. The de novo drugs such as Melatonin + caffine, Benzamide + aspirin, Camptothecin + sodium, Cardamonin +aspirin are designed and validated based on ADME properties, drug likeness score and drug toxicity. Then the designed ligands are docked with target using patch dock server, which shows that the modified ligands have better binding drug target than the existing ligands with low energies. More generally, the protocol described in this project work can be included in a drug discovery pipeline in an effort to discover novel drug leads with desired safety profiles, and therefore accelerate the development of new drugs.

INTRODUCTION

Worldwide, lung cancer is the most common cancer in terms of both incidence and mortality. In 2008, there were 1.61 million new cases, and 1.38 million deaths due to lung cancer. The highest rates are in Europe and North America.[1] The population segment most likely to develop lung cancer is over-fifties who have a history of smoking. In contrast to the mortality rate in men, which began declining more than 20 years ago, women's lung cancer mortality rates have been rising over the last decades, and are just recently beginning to stabilize.[2] In the USA, the lifetime risk of developing lung cancer is 8% in men and 6% in women.[3]

For every 3–4 million cigarettes smoked, one lung cancer death occurs.[4] The influence of "Big Tobacco" plays a significant role in the smoking culture.[5] Young non-smokers who see tobacco advertisements are more likely to take up smoking.[6]

The role of passive smoking is increasingly being recognized as a risk factor for lung cancer,[7] leading to policy interventions to decrease undesired exposure of nonsmokers to others' tobacco smoke.[8] Emissions from automobiles, factories, and power plants also pose potential risks.[9]

Eastern Europe has the highest lung cancer mortality among men, while northern Europe and the U.S. have the highest mortality among women. In the United States, black men and women have a higher incidence.[10] Lung cancer incidence is currently less common in developing countries.[11] With increased smoking in developing countries, the incidence is expected to increase in the next few years, notably in China[12] and India.[13]

Objectives:

To find out the potential protein in the LUNG CANCER and predict the structure using homology modeling - GNB2L1 Gene To design the drugs for LUNG CANCER and validate based on ADME properties and drug toxicity. To dock it to the target protein causative for the disease.

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MATERIALS AND METHODS

The gene responsible for the cause of the disease, GNB2L1 was selected from NCBI. The Protein sequences of the target were retrieved in FASTA format from NCBI. The analysis of the Gene (GNB2L1) were done using CCDS database. It showed the introns, exons and splice regions of sequence. Sequence comparison studies was done using BLAST P program to find out the similarities to the target. The three dimensional structure prediction of GNB2L1 gene was performed using an automated Fold recognition modeling server called SWISS MODEL and MODELLER to model the 3D structure of the target protein. The following ligands Melatonin,Benzamide, Camptothecin, Cardamonin ligands were selected using NCBI-Pubchem chemical databases. The Denevo ligands were developed , Melatonin + caffine, Benzamide + aspirin, Camptothecin + sodium, Cardamonin +aspirin, were developed using XEMISTRY WEB SKETCHER DEMONSTRATION. Tertiary structure of denevo ligands were viewed in MOLECULAR NETWORKS server . Toxicity prediction and pharmacophore features were performed using three tools –, Toxtree ,Toxmatch. The docking mechanisms of the target protein and designed ligand molecules were performed in Patch dock server Protein - Ligand interaction studies were done using Molegro molecular viewer.

RESULTS AND DISCUSSION

NCBI - GENE IDENTIFICATION:

gi|49456773|emb|CAG46707.1| GNB2L1 [Homo sapiens]
MTEQMTLRGTLKGHNGWVTQIATTPQFPDMILSASRDKTIIMWKLTRDETNYGIPQRALRGHSHFVSDVV
ISSDGQFALSGSWDGTLRLWDLTTGTTTRRFVGHTKDVLSVAFSSDNRQIVSGSRDKTIKLWNTLGVCKY
TVQDESHSEWVSCVRFSPNSSNPIIVSCGWDKLVKVWNLANCKLKTNHIGHTGYLNTVTVSPDGSLCASG
GKDGQAMLWDLNEGKHLYTLDGGDIINALCFSPNRYWLCAATGPSIKIWDLEGKIIVDELKQEVISTSSK
AEPPQCTSLAWSADGQTLFAGYTDNLVRVWQVTIGTR

The target gene sequence (GNB2L1) was retrieved from NCBI in FASTA fomat. It had 317 aminoacid residues and GeneID 10399.

Figure 1: Similarity Search ofGNB2L1.

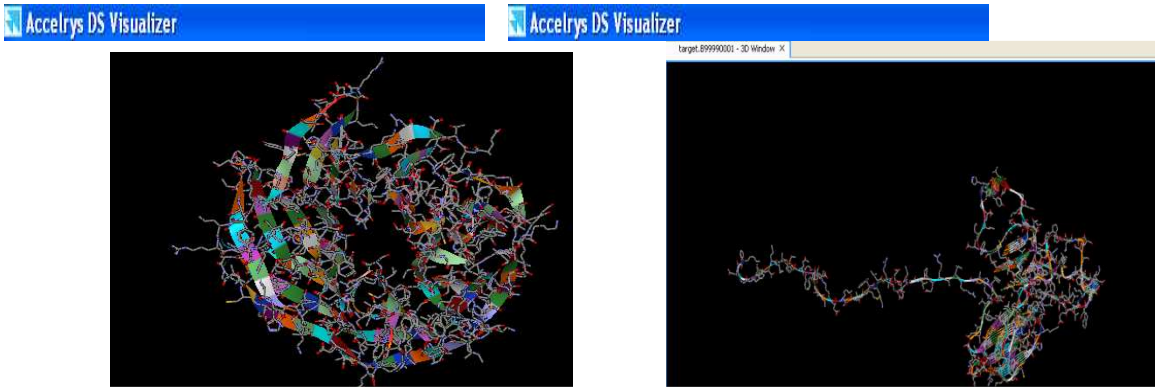
BLAST - SIMILARITY SEARCH:

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage
2ZKQ_AA	Chain a, Structure Of A Mammalian Ribosomal 40s Subunit Within An	653	653	100%
3DM0_A	Chain A, Maltose Binding Protein Fusion With Rack1 From A. Thaliana	436	494	98%
3IZB_AA	Chain a, Localization Of The Small Subunit Ribosomal Proteins Into A	347	389	97%
3JYV_R	Chain R, Structure Of The 40s Rrna And Proteins And PE TRNA FOR E	345	387	97%
1TRJ_A	Chain A, Homology Model Of Yeast Rack1 Protein Fitted Into 11.7a C	345	345	97%
3FRX_A	Chain A, Crystal Structure Of The Yeast Ortholoque Of Rack1, Asc1	339	339	97%
1VYH_C	Chain C, Paf-Ah Holoenzyme: Lis1ALFA2 >pdb|1VYH|D Chain D, Paf-	127	127	94%
2XL2_A	Chain A, Wdr5 In Complex With An Rbbp5 Peptide Recruited To Nove	113	388	97%
2GNQ_A	Chain A, Structure Of Wdr5	113	388	97%
3EMH_A	Chain A, Structural Basis Of Wdr5-Mll Interaction	113	387	99%
2G99_A	Chain A, Structural Basis For The Specific Recognition Of Methylatec	112	388	99%
2H9M_A	Chain A, Wdr5 In Complex With Unmodified H3k4 Peptide >pdb|2H9M	112	387	98%
2H68_A	Chain A, Histone H3 Recognition And Presentation By The Wdr5 Mod	112	388	98%
2H9L_A	Chain A, Wdr5delta23 >pdb|2H9P|A Chain A, Wdr5 In Complex With	112	387	98%
3PSL_A	Chain A, Fine-Tuning The Stimulation Of Mll1 Methyltransferase Acti	112	387	98%
2H13_A	Chain A, Crystal Structure Of Wdr5HISTONE H3 COMPLEX >pdb|2H1	112	387	98%

The Similarity Search was done using Blast. The Sequences with low E-value - 0.0 represent similarity to target sequence. Thus it may have similar structure and function.

Figure: 2 TERTIARY STRUCTURE PREDICTION
DISCOVERY STUDIO - PROTEIN STRUCTURE VISUALISATION:
SWISS MODEL MODELLER



The above 3D structureof target were modeled using Swiss model and Modeller and visualized in Discovery Studio Visualizer 2.5. The display style of protein is solid ribbon – Secondary type. Here red color – alpha helix, blue color – beta sheet and green color – coils.

PROTEIN STRUCTURE VALIDATION - RAPPER SERVER

Evaluation of residues:

Table 1: Evaluation of Residues in Swiss Model and Modeller.

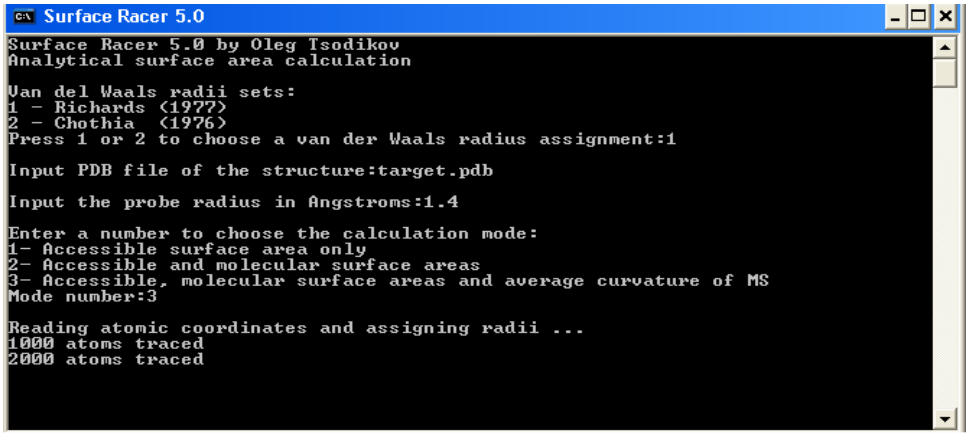
FEATURES	SWISS MODEL	MODELLLER
Number of residues in favoured region (~98.0% expected)	270 **(88.2%)**	283 **(89.9%)**
Number of residues in allowed region (~2.0% expected)	24 (7.8%)	1 7 (5.4%)
Number of residues in outlier region	12 (3.9%)	15 (4.8%)

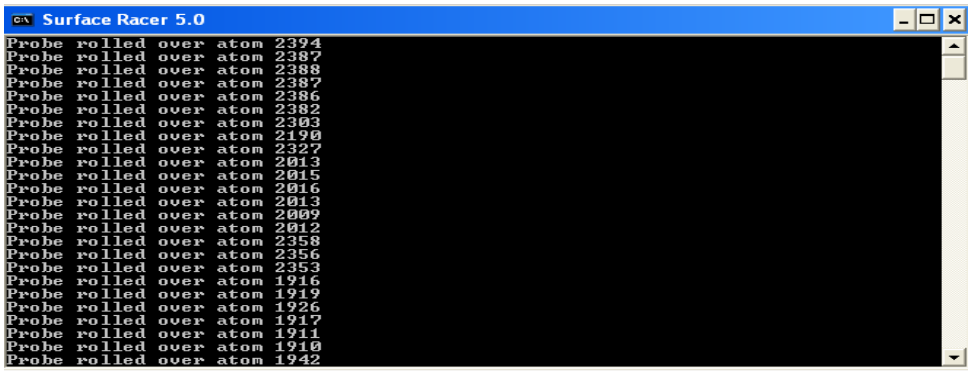
The 3D Structure was validated and evaluated by Ramachandran Plot. The modeled 3D Structure of Swiss Model and Modeller were considered to be Good Model (88.2 & 89.9%).

PROTEIN STRUCTURE ANALYSIS

ACTIVE SITE PREDICTION SURFACE RACER

Figure 3: Surface Analysis of SETX – Surface Racer

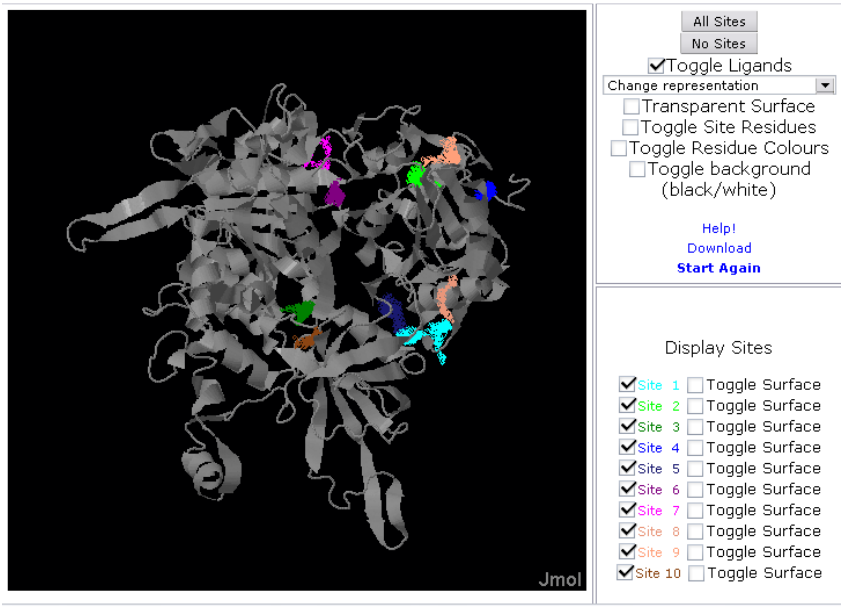




The surface area of target
Number of non-HOH, non-H atoms=2467
Time used=18.000000 sec
Probe radius=1.00
TOTAL ASA=27300.10 TOTAL MSA=0.00
Polar ASA=11064.95 Non-polar ASA=16235.16
Polar MSA=0.00 Non-polar MSA=0.00
Total backbone ASA=6614.31 Total backbone MSA=0.00
Polar backbone ASA=4454.61 Non-polar backbone ASA=2159.69
Polar backbone MSA=0.00 Non-polar backbone MSA=0.00
Polar side chain ASA=6610.34 Non-polar side chain ASA=14075.46
Polar side chain MSA=0.00 Non-polar side chain MSA=0.00
+charge ASA=1905.00 -charge ASA=1128.17
+charge MSA=0.00 -charge MSA=0.00
Structure contains 19 cavities

The result of surface racer displayed the Cavities, Accessible surface area(ASA), Molecular surface area(MSA) and charges of protein.

Figure: 4 Q Site Finder



The Q site finder was used to identify the active sites of target. The 10 sites were colored in different colours.

DRUG SCREENING: LIGAND SELECTION – PUBCHEM:

Table 2: List of existing drugs with its smiles and binding energy.

Existing drugs	Binding energies	Smiles
Melatonin	-233	CC(=O)NCCC1=CNC2=C1C=C(C=C2)OCCN1C=NC2=C1C(=O)N(C(=O)N2C)C
Benzamide	-233	C1=CC(=CC(=C1)C2C(C(C(O2)CO)O)O)C(=O)NCC(=O)OC1=CC=CC=C1C(=O)O
Camptothecin	-355	CCC1(C2=C(COC1=O)C(=O)N3CC4=CC5=CC=CC=C5N=C4C3=C2).[Na+]
Cardamonin	-249	COC1=CC(=CC(=C1C(=O)C=CC2=CC=CC=C2)O)OCC(=O)OC1=CC=CC=C1C(=O)O

The above drugs based on molecular weight were taken from pubchem compound for denovo design. The table shows the binding energy and Canonical smiles of existing drugs.

XEMISTRY WEB SKETCHER DEMONSTRATION – LIGAND DESIGNING AND DEVELOPMENT

Table 3: List of existing drugs with agents (to add) and its action.

DRUGS	AGENTS	ACTION
Melatonin	caffeine	Central Nervous System Stimulant.
Benzamide	aspirin	prototypical analgesic
Camptothecin	Sodium	Pain Killer
Cardamonin	aspirin	prototypical analgesic

The table shows the agent that is added to the respective drug for the development of new ligands.

TOXMATCH

Table 4: Pharmacophore features of all denevo ligands – A Comparative study.

DESCRIPTORS	Drug 1	Drug 2	Drug 3	Drug 4
Aromatic atom counts	9	6	10	12
Aromatic bond counts	10	6	11	12
LogP	1.601	-0.6480	1.119	3.38
Molecular Surface area	54.120	113.01	59.50	66.78
Molecular Weight	232.1211	253.0950	357.1211	270.08920
Number of H atom acceptors	3	6	5	1
The number failures of the Lipinski's Rule Of 5	0	0	0	0
The number of atoms in the largest chain	14	18	26	6
The number of atoms in the largest pi system.	10	9	17	19
The number of rotatable bonds	7	7	2	7
Topological polar surface area	54.120	113.01	59.50	66.76

Almost all the drugs qualified the criterions of Lipinski's rule of five like the number of hydrogen donors and the number of hydrogen acceptors, molecular weight, logP values. The logP values of drug1 complex (1.601), drug 2 complex(-0.6480), drug 3 complex (1.119), drug 4 complex(

3.38) were found, almost all drugs were in acceptable range of logP value and all of the drugs had molecular weight below 500. Thus all the drugs showed satisfactory results on all the parameters.

TOXTREE:

Table 5: Toxicity of denevo ligands.

DESCRIPTORS	Drug 1	Drug 2	Drug 3	Drug 4
Normal constituent of the body	No	No	No	No
Contains functional groups associated with enhanced toxicity	No	No	No	No
Contains elements other than C,H,O,N,divalent S	No	Yes	No	Yes
CLASS	LOW(Class I)	High (Class III)	High (Class III)	High (Class III)

The main kind of biological activity is a substance's toxicity. The designed ligand is applied to Toxtree server in order to predict its toxicity. The toxicity classification result is shown in graphical form (green highlight for class I, yellow highlight for class II and red highlight for class III), as well as in text form.

LIGAND DOCKING STUDIES – PATCHDOCK:

Table 6: Binding energies of denevo ligands

DOCKING – DRUG INTERACTION STUDIES			
Existing drugs	**Binding affinities**	**De novo drugs**	**Binding affinities**
Melatonin	**-175.01**	**Drug 1**	**-385.02**
Benzamide	**-183.61**	**Drug 2**	**-233.97**
Camptothecin	**-140.21**	**Drug 3**	**-233.49**
Cardamonin	**-169.77**	**Drug 4**	**-249.43**

MOLEGRO - DRUG INTERACTION STUDIES:

Figure: 5 Docking View of GNB2L1----- Drug Complex

DE NOVO drug 1 – TARGET COMPLEX DE NOVO drug2 – TARGET COMPLEX

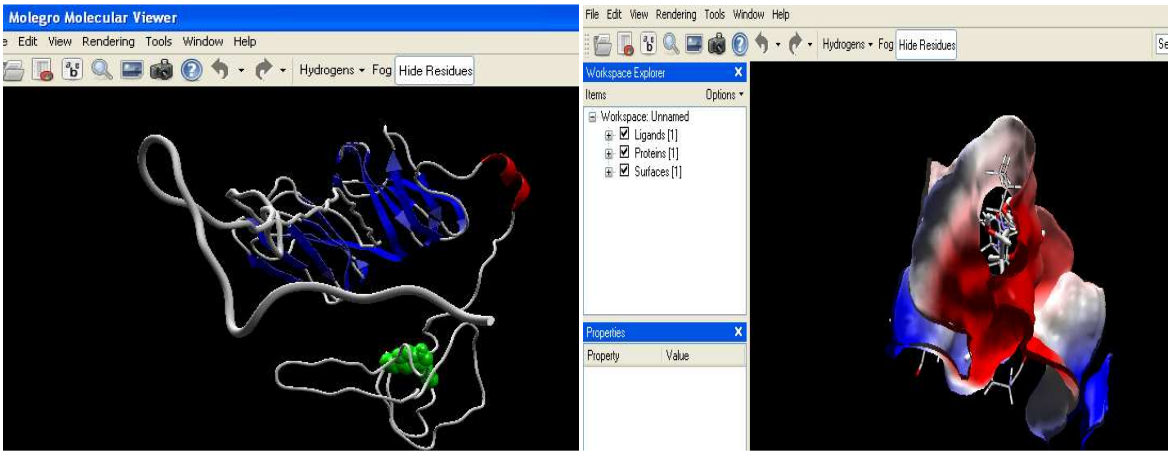
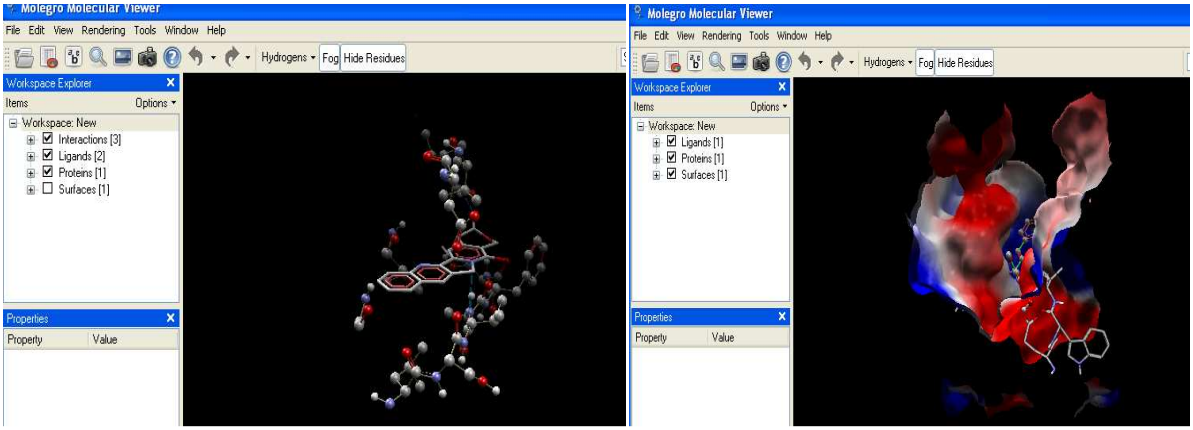


Figure 6: Docking View of GNB2L1----- Drug Complex

DE NOVO drug3 – TARGET COMPLEX DE NOVO drug4 – TARGET COMPLEX



The Docking Results of all the target- denevo ligands were viewed in Molegro Viewer. Here the Proteins were viewed in Stick Model and colored by Amino Acid Type with Labels, Ligands in Ball and Stick with Electrostatic surface around ligand.

CONCLUSION

Molecular Modelling and Chemoinformatics are becoming essential component in drug discovery. Determining the structure and function of a novel protein is a cornerstone of many aspects of modern biology. The three dimensional structure of the target were modelled in MODELER and SWISS MODEL..The following denevo ligands drug 1, drug 2, drug 3, drug 4 were designed and docked with target (GNB2L1) in Patchdock server.

Toxicity prediction is the basis of Drug validation. The efficiency is a drug is determined by drug validation. Our study shows that the designed ligands are the best candidate for curing the disease. Thus the novel drugs designed which would bind with the mutated gene of the coded protein and thus inhibit the expression of the gene

The molecular docking studies were performed to predict whether a given molecule will bind to a target and if so how strongly. It is clear that all the denevo ligands satisfied almost all properties like drug likeness value, drug score, lower logP values, and Lipinski's rule of five and has better binding affinity than the existing drugs.

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