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DESIGN AND ANALYSIS OF NOVEL INHIBITORS AGAINST THE KRAS MUTATIONS IN LUNG CANCER THROUGH COMPUTER AIDED DRUG DESIGNING TECHNIQUES

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ABSTRACT

Background: Lung cancer is known as universal and common disease and the first leading reason for the death of males and females. Worldwide death causes due to lung cancer each year is 1,370,000. There is still deficiency to recognize the effective compounds against KRAS protein involved in Lung Cancer. Methods: In this research, lead compounds against the KRAS mutations were identified through pharmacophore modeling and virtual screening approach and then lead compounds were docked with KRAS protein for confirmation to be used as lead compounds. Results: 50 hits, compounds were obtained by Virtual Screening; Compounds that fulfill all properties of Lipinski rule were docked with protein. Two Compounds were demonstrated ideal docking result. They fit appropriately in the pocket of proteins, demonstrated the soundness, and stability of ligand compounds. Conclusion: On the basis of docking results, it is suggested that these two identified compounds may be able to be used in the treatment of KRAS mutations for Lung Cancer. Novel compounds can be designed on the basis of sharing common feature Pharmacophore model for the treatment of KRAS mutations in Lung cancer.

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Introduction

Cancer is an ailment, in which unusual cells multiply rapidly and form the tumor. These odd cells have now unequal function like ordinary cells. The most cancers, which influence the tissues of the lung, typically peculiar cell growth within the cell lining air passages is known as lung cancers [1]. Lung Cancer, starts inside the cell lining bronchi and alveoli, that absorbs oxygen from inhaled air into the blood. In a lung cancer cell develops out of control and unfold from the lungs to lymph nodes. Lung cancer also can have an effect on the liver, stomach and the brain. A cancer that can start from the lungs is called the primary lung cancer. If the most cancers start from other parts of the body and may reach into the lungs, known as secondary lung cancers [2] Lung cancer affects either the only one or both lungs. People with lung cancers won't display signs and symptoms in their early stage. Lung cancer is a prevalent malignancy worldwide. The ratio of the lung cancer is higher in Asia. According to National Cancer Institute, 221,200 new instances of lung Cancer had been identified and there were 158, 040 lung cancer-related deaths in the USA in 2013 [3]. But, in Pakistan, the prevalence rate of lung cancers is

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higher from 1990 to 2013, up from 77, 00 to 15,500. The mortality rate in women and men is higher due to the lung cancers worldwide. Deaths caused due to lung cancer each year is 1,370,000 [4].

There are two types of the lung cancers: Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). Small Cell Lung Cancer (SCLC) is the gathering of small abnormal cells that multiply unexpectedly and result in cancers. SCLC is the major competitive type of cancer than Non-small cell lung cancers. SCLC accounts 20% of the lung cancers instances. Smoking is the major causative factor of SCLC this is responsible for chest ache, coughing and shortness of breath [5]. Non-Small Cell Lung Cancer (NSCLC) is the most common kind of lung cancer. It grows and spreads slowly than SCLC. It accounts 80% of lung cancers instances [6]. It usually occurs in adults. Several mutations occur in a lung cell's gene that makes the cell unable to perform normal functions; thus, results in the development of lung cancer. These mutations are due to different reasons. Major reason is inhaling carcinogenic substances such as cigarette, asbestos, gamma and X-ray [7]. Although most lung cancers are a result of smoking, approximately 25% of lung cancer cases worldwide are not attributable to tobacco use, accounting for over 300,000 deaths each year. Striking differences in the epidemiological, clinical and molecular characteristics of lung cancers arising in never smokers versus smokers have been identified, suggesting that they are separate entities [8].

Lung cancer can be diagnosed by CT-Scan, MRI, and PT-Scan of chest. Once lung cancer diagnosed, it can be treated in different ways. The treatments of lung cancer depend on their type, stage, status, age, health and personal characteristics. It can be treated by surgery, chemotherapy, Radiations, Lobectomy and cancer pain medication. The KRAS mutations accounts 30% in Non-Small Cell Lung Cancer cases [9]. The full form of KRAS is V-Ki-RAS2 Kirsten rat sarcoma viral oncogene homolog. KRAS protein is encoded by KRAS gene: a member of RAS family [9]. KRAS protein performs essential function in a normal signaling pathway. But if mutations occur in KRAS, they will result in lung cancer development [11]. KRAS is a GTPase [12]. KRAS gene encodes KRAS protein that is involved in regulation of cell division. In a RAS signaling pathways, protein transfer signals from outside the cell to cell's nucleus. These signals allow cell to grow, divide and perform essential functions in the cells [13].

In a healthy cell, the KRAS protein is turned on, when signal reaches to protein, it sends these signals to RAF protein, which further sends them on to MEK protein. Then MEK protein passes these signals to ERK protein. ERK is the last protein in the pathway [14]. ERK turns on gene in a nucleus, which allows cells to grow normally and perform essential functions to survive. After completing their cycle, protein is turned off when the signals stop [15]. KRAS mutations are the most common in NSCLC. Changes in KRAS protein may cause KRAS Q61R mutation. 61 means mutation at position 61 of the amino acid. In KRAS, Q represents glutamine and R represents arginine [16]. Mutations in KRAS protein most commonly occurs at amino acid positions 12, 13 and 61. These three amino acid positions perform important functions in turning on gene [17].

2. Materials and Method

An ideal drug target is one that involved in the human disease. A mutant protein is the one that is encoded by a mutated gene, proteins act as a good drug target [18]. The structure of KRAS protein with 4OBE id was downloaded from protein Data Bank (PDB). The Protein Data Bank is the only worldwide repository for the structural protein data [19]

2.1 Physiochemical Analysis of Kras

To analyze the general properties of KRAS protein, an online tool of expasy ProtParam was used. ProtParam analysis also provides information about the number and percentage of amino acids in the respective protein [20].

2.2 Molecular Docking

The docking process involves the prediction of Ligand conformation and orientation within a targeted binding site. 22 ligands were selected from a standard literature source [21]. Protein-Ligand Docking was performed through PyRx. The PyRx is a tool for VS. PyRx VS tool for Computational Drug Discovery which is used to screen the drug libraries of compounds against the potent drug targets. Pyrex Wizard feature uses a chemical spread sheet and user interface that's valuable for Rotational Drug Designing [22].

Ten best compounds that scored lowest binding energy were selected and Protein-Ligand interactions were checked by the UCSF Chimera for further analysis. UCSF Chimera is an extremely extensible program for interactive visualization and analysis of molecular structures, including density maps, super molecular assemblies, sequence alignment, docking results, trajectories, and conformational ensembles. High-quality images and animation can be created by using UCSF Chimera 1.8. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics [23].

2.3 Pharmacophore Model generation

After docking, Pharmacophores of the top compounds were built through Ligand scout. Ligand Scout is a software tool that allows rapid and transparent driving of 3D Pharmacophores from structural data of macromolecule/Ligand complexes in a fully automated and convenient way [24]. A pharmacophores describes the structural arrangement of the essential molecular features of interactions between the ligand and receptor. A Pharmacophore is a set of common properties. The Pharmacophore is basically a bridge of future analysis for the virtual screening.

2.4 Virtual Screening against shared feature Pharmacophore to Obtain Hit Compounds

Virtual Screening was performed to find the inhibitor that might be able to stabilize the expression of KRAS. The LigandScout was used to prepare Pharmacophore. The built Pharmacophore was used for Virtual Screening (VS). Virtual Screening was performed by ZINCPharmer. ZINCPharmer is free Pharmacophore search software for screening the purchasable subset of the ZINC database. ZINC Pharmer can import MOE and Ligandscout Pharmacophore definition, as well as identify Pharmacophore features directly from the structure. ZINC is a comprehensive collection of commercially available biologically relevant compounds suitable for screening. The ZINCPharmer library is synchronized with the ZINC library on a monthly basis [25]. In the result of Virtual Screening, 50 hit compounds were produced as an output for further docking analysis. These 50 hit compounds were selected on the basis of Lipinski rule.

2.5 Docking of Hit compounds with KRAS protein

The main purpose of docking was to find the number of interactions between the protein and ligand molecules. Docking was performed on KRAS focused library to locate the appropriate binding orientations and conformations of various inhibitors in the KRAS binding pocket using PyRx. The selected compounds were docked with KRAS and best compounds were selected on the basis of lowest binding energy.

2.6 Protein-Ligand Interaction

The characterization of interactions in protein–ligand complexes is essential for research in structural bioinformatics, drug discovery and biology. Protein-Ligand interactions were predicted using UCSF chimera.

2.7 Toxicity prediction

After the detailed analysis of docking and protein-ligand interactions, compounds were further screened on the basis of drug-likeliness, drug score and toxicity characteristics through AdmetSAR. In AdmetSAR, over 210 000 ADMET annotated data points for more than 96 000 unique compounds with 45 kinds of ADMET-associated properties, proteins, species, or organisms have been carefully curated from a large number of diverse literatures. The database provides a user-friendly interface to query a specific chemical profile, using CAS registry number, common name, or structure similarity. In addition, the database includes 22 qualitative classifications and 5 quantitative regression models with high predictive accuracy, allowing estimating ecological/mammalian ADMET properties of novel chemicals [26].

2.8 Lead Compound Identification

After the detailed analysis of docked score, ligand-receptor interactions and the toxicity studies, the most active inhibitor was identified. The compound that demonstrated the best interactions among all has been identified as the lead.

4. Results

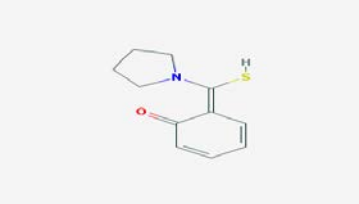
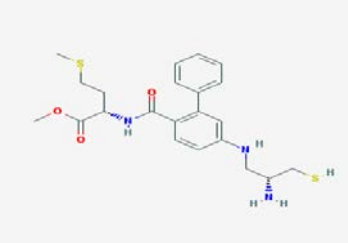
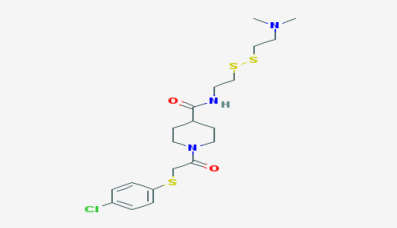
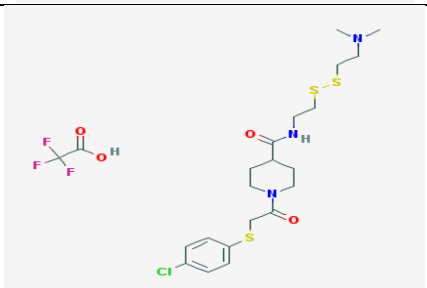
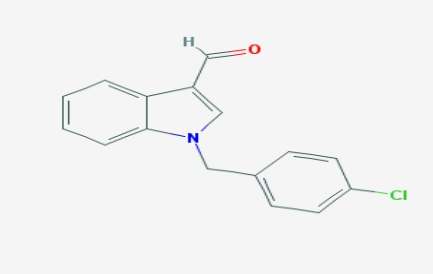
The aim of this study was to identify the lead compound that acts as inhibitor using ligand based Pharmacophore modeling approach with virtual screening and molecular docking strategies. ProtParam analysis was done to analyze general properties of KRAS Protein including number, percentage and hydrophobicity or hydrophilicity of protein. The amino acid names, numbers and their percentages are shown in table 1.

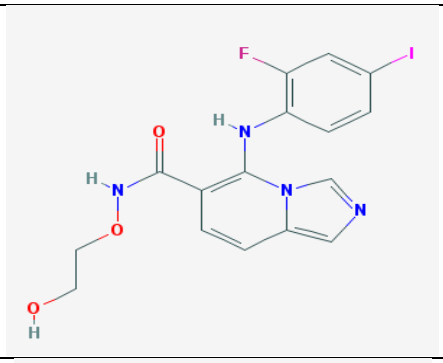
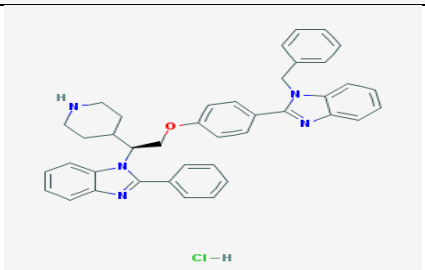
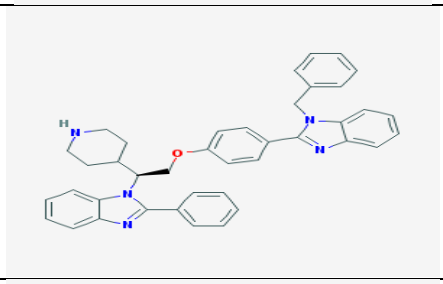
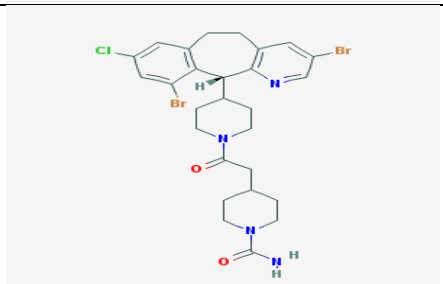
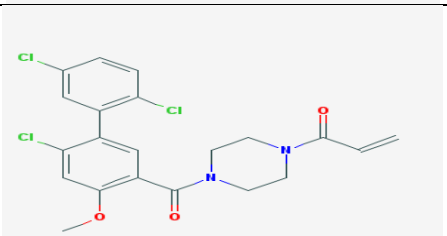
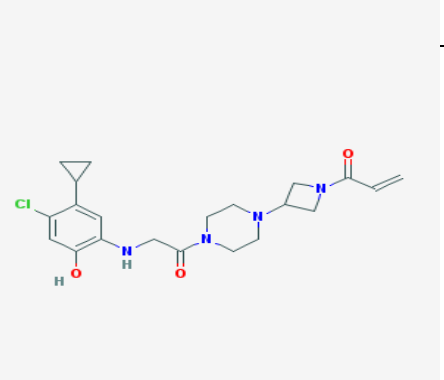
Table 1: The ProtParam analysis of KRAS protein, including Amino acids, their numbers and percentages

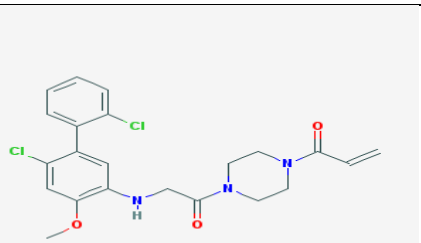
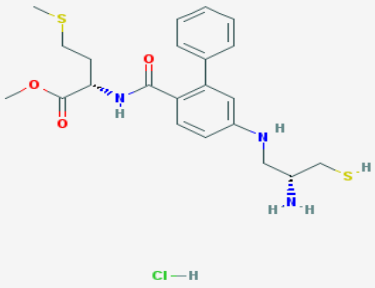
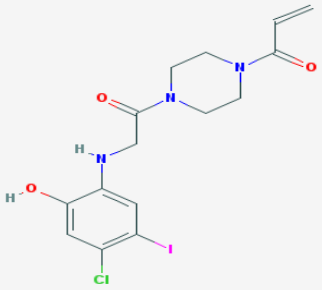
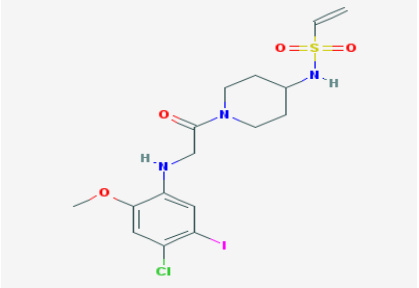
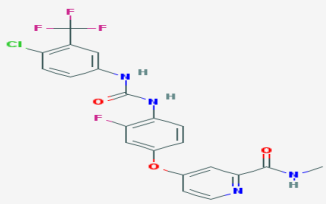
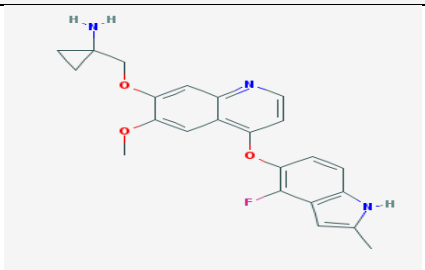
Amino Acid	No.	Percentage
Ala	9	5.3%
Arg	10	5.9%
Asn	4	2.4%
Asp	15	8.8%
Cys	3	1.8%
Gln	9	5.3%
Glu	13	7.6%
Gly	12	7.1%
His	4	2.4%
Ile	11	6.5%
Leu	11	6.5%
Lys	12	7.1%
Met	4	2.4%
Phe	6	3.5%
Pro	4	2.4%
Ser	8	4.7%
Thr	12	7.1%
Tyr	8	4.7%
Val	15	8.8%

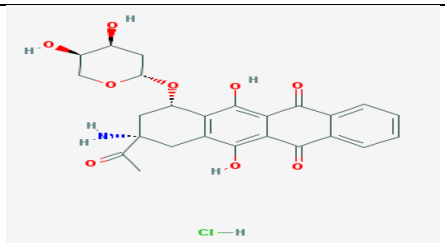
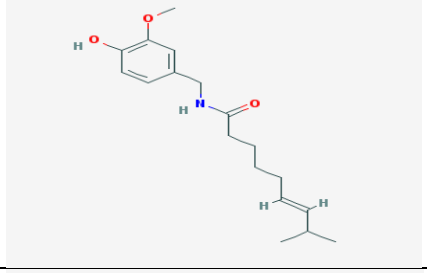
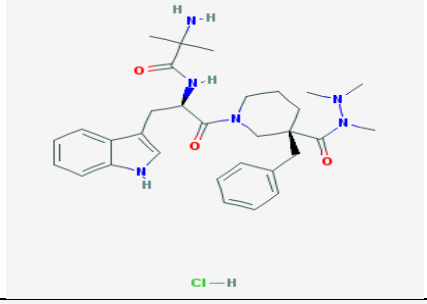
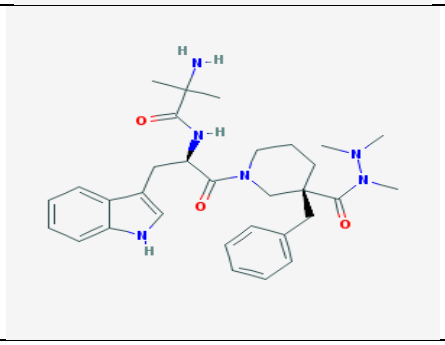
It was observed that the total number of Amino Acids was 170 in the protein. Total percentages of amino acid residues involved in KRAS protein showed hydrophobic residues and hydrophilic residues. The higher percentages represent hydrophobic amino acids while lower represent hydrophilic amino acid residues. Atomic compositions of atoms were different. Molecular weight of protein was found to be 19302.80 Da. The total atomic composition of the protein obtained through ProtParam tool demonstrated that the total number of atoms involved in protein is 298. The number of Carbon atoms was 847, the Hydrogen 1343, Oxygen 268, Nitrogen 233 and 7 atoms of sulfur. Lipinski's rule of five also referred to as the Pfizer's rule of five or the Rule of 5 is a rule of thumb to assess drug-likeness or decide if a chemical compound with a sure pharmacological or organic activity has characteristics that might make it a probable oral energetic drug in people. The rule was formulated with the aid of Christopher A. Lipinski in 1997, based totally on the observation that most orally administered drugs are relatively small and fairly lipophilic molecules ([27,28]. Ligand molecules were selected on the basis of Lipinski Rule of five for the analysis. The selected Inhibitors are shown in Table II.

Table II: 2D structures of ligands which were selected on the base of Lipinski Rule.

Inhibitors Name	Molecular Weight	Structure
K-Ras-IN-1	207.291 g/mol	
FTI-277	447.612 g/mol	
6H05	476.109 g/mol	
6H05 (Trifluoroacetate)	450.132 g/mol	
Oncrasin-1	269.728 g/mol	

GDC-0623	456.216 g/mol	
Deltarasin (Hydrochloride)	440.228 g/mol	
Deltarasin	403.77 g/mol	
Lonafarnib	438.829 g/mol	
K-Ras G12C-IN-3	453.744 g/mol	
K-Ras G12C-IN-2	418.922 g/mol	

K-Ras G12C-IN-1	448.344 g/mol	
FTI-277 hydrochloride	484.07 g/mol	
K-Ras(G12C) inhibitor 12	449.673 g/mol	
K-Ras(G12C) inhibitor 9	413.775 g/mol	
Regorafenib	482.82 g/mol	
Anlotinib	407.445 g/mol	

Amrubicin Hydrochloride	459.931 g/mol	
Capsaicin	305.418 g/mol	
Anamorelin Hydrochloride	483.174 g/mol	
Anamorelin	450.716 g/mol	

The docking process involves the prediction of ligand conformation and orientation within targeted binding sites. The main purpose of docking is to find interaction between ligand and protein [29]. The docking results of the selected inhibitors are shown in fig 1.

From the figure 1, it was observed that Lonafarnib, Amrubicin, Amrubicin_Hydrochloride, Deltarasin, Regorafenib, Deltarasin (Hydrochloride), Anamorelin, KRAS_G12C_IN_1, KRAS_G12C_IN_3, KRAS_G12C_IN_1 represented them as the best compounds, selected on the basis of lowest binding energy by docking and their aligned Pharmacophore was designed through ligand scout 3.12. Pharmacophore is a set of common properties. Pharmacophore model was designed to merge the properties of inhibitors in order to get the novel compound specific for target protein that have less toxic effect and high efficiency as compared to previously designed compound. The merged pharmacophore is shown in fig 2.

The pharmacophore of Lonafarnib, Amrubicin, Amrubicin_Hydrochloride, Deltarasin, Regorafenib, Deltarasin (Hydrochloride), Anamorelin, KRAS_G12C_IN_1, KRAS_G12C_IN_3, KRAS_G12C_IN_1 were generated on the basis of fit scores shown in table 3. and the pharmacophoric features of these compounds are shown in table IV.

Table III: Pharmacophore fit score of Ligands

Ligand Name	Pharmacophore fitness Score
Deltarasin	45.8200
Anamorelin	45.6700
Regorafenib	58.2100
Amrubicin	46.1400
KRAS_G12C_IN_1	57.0300
KRAS_G12C_IN_2	57.8300
KRAS_G12C_IN_3	58.3500
Lonafarnib	52.2700

A pharmacophore is an abstract description of molecular features that are necessary for molecular recognition of a ligand by a biological macromolecule [30]. Usually the pharmacophore features include hydrophobic, aromatic rings, hydrogen bond acceptors or donors, cations, and anions.

Table IV: Pharmacophoric Features of best Inhibitors

Compound Name	Hydrophobic	HBA	Aromatic
Lonafarnib	Three	Two	None
Regorafenib	Three	Two	One
Anlamorelin	Two	Two	None
Ambrubicin	Two	Two	None
KRAS G12C IN 1	Three	Two	One
KRAS G12C IN 3	Three	Two	One
KRAS G12C IN 2	Three	Two	One
Deltarasin	Two	Two	None

From the table 4, it was observed that none of the compounds have any hydrogen bond donor. the shared feature of the pharmacophore generated by aligning the pharmacophores of best compounds consists of one aromatic ring, two hydrogen bond acceptors and three hydrophobic regions.

Virtual screening is an important part of drug discovery. The main objective of virtual screening was to identify bioactive compounds through computational means, by employing knowledge about the protein target and known bio active ligands. Pharmacophore model was built through ligand scout. The Pharmacophore was used for virtual screening. Virtual Screening of Pharmacophore model was performed to identify the chemical compounds which have similar features to those of Pharmacophore model. According to Pharmacophore features, Virtual Screening was done through Zinc Pharmer. After Virtual screening, top 50 outputs were selected for further docking analysis. These 50 outputs were selected on the basis of Lipinski rule. The main purpose of docking was to find the best interactions between ligands and KRAS protein. The compounds retrieved from zinc Pharmer were docked with KRAS protein and result showed the residue that interacts with ligand molecules. The compounds which were selected after a virtual screening are shown in fig 3.

Figure 3: compounds were selected after virtual screening and their docking results

Top ten compounds were selected from the 50 hit compounds, on the basis of lowest binding affinity and their interactions with protein were checked by UCSF Chimera 1.8. Among these 10 selected best compounds only two compounds demonstrated better interactions with the protein. These two compounds were identified as a lead compound. The best interactions are shown in figure 4.

Figure 4: the interactions formed by the best hit compounds with KRAS Protein

Toxicity is the diploma to which a substance can damage an organism. There are usually four kinds of toxic entities; chemical, organic, bodily and radiation. The toxicity analysis of these to best hit compounds is shown in table V.

Table V: Toxicity risk of top two compounds

Compound Name: ZINC19714527	
Blood Brain Barrier	0.9717
Human Intestinal Absorption	0.9776
Caco-2 Permeability	0.6362
Carcinogens	0.6233
Acute Oral Toxicity	0.5802
H-Bond Donors	1
H-Bond Acceptors	5
X log P	2.43
Molecular weight	374.437
Compound Name: ZINC76573745	
Blood Brain Barrier	0.9636
Human Intestinal Absorption	0.9970
Caco-2 Permeability	0.6136
Carcinogens	0.6118
Acute Oral Toxicity	0.5597
H-Bond Donors	1
H-Bond Acceptors	5
X log P	2.45
Molecular weight	342.436

These two compounds ZINC76573745 and ZINC19714527 have been identified as the most active from all molecules after toxicity analysis. These ligands have shown strong hydrogen and hydrophobic interactions with the target receptor. These two compounds have been identified as efficient compounds.

Discussion

KRAS is a member of RAS family. RAS family included HRAS, NRAS and KRAS. KRAS protein is encoded by KRAS gene. KRAS protein performs essential function in normal signaling pathway. But if mutation occurs in KRAS, it causes lung cancer. KRAS mutation in lung cancer usually arises at codon 12, 13 and 61. KRAS is a GTPase (it converts GTP into GDP). KRAS mutation is most common in non small cell Lung cancer (NSCLC) [11]. KRAS have 177 amino acid lengths. Change in KRAS protein caused KRAS Q61R mutation. 61 means mutation at 61 amino acid position. If KRAS have glutamine at 61 amino acid position, it will perform normal function. It is denoted by Q. But if KRAS have arginine at 61 amino acid position, it will cause lung cancer. It is denoted by R. That's why it is called KRAS Q61R mutation [14]. The structure is already predicted and was retrieved from RCSB PDB. 4OBE is PDB code for KRAS. The structure of original protein was purified by removing unique ligands, HOH and adding hydrogen molecules. Ligands for KRAS were searched out by standard literature survey. These ligands were docked with KRAS protein and selected top compounds having lowest binding energy. These top compounds were utilized for further analysis.

Kandakatla and Ramakrishnan [31] have used performed ligand based pharmacophore modeling and virtual screening techniques to find inhibitors against the histone deacetylase enzyme. Therefore, the mixtures of pharmacophore modeling, virtual screening, and molecular docking fruitfully give more likely inhibitors that may have abundant impact of the upcoming experimental studies in diseases associated with KRAS inhibition. All the studies regarding Ligand based pharmacophore modeling and virtual screening proved that pharmacophore modeling is a influential apparatus in calculating activities and setting significances for virtual screening.

Conclusion

This study aimed at finding an efficient compound that acts as inhibitors against KRAS through Pharmacophore modeling with virtual screening. The result concluded that the selected inhibitor stabilizes KRAS. It could serve as a lead compound for further study on RAS family. This study of lead compound for KRAS protein can be used for further drug designing and experimentation. This study might provide a basis for combating irregularities and cancer caused by KRAS. These findings will be beneficial for the scientific community and can aid in the design of new compounds against Lung Cancer.

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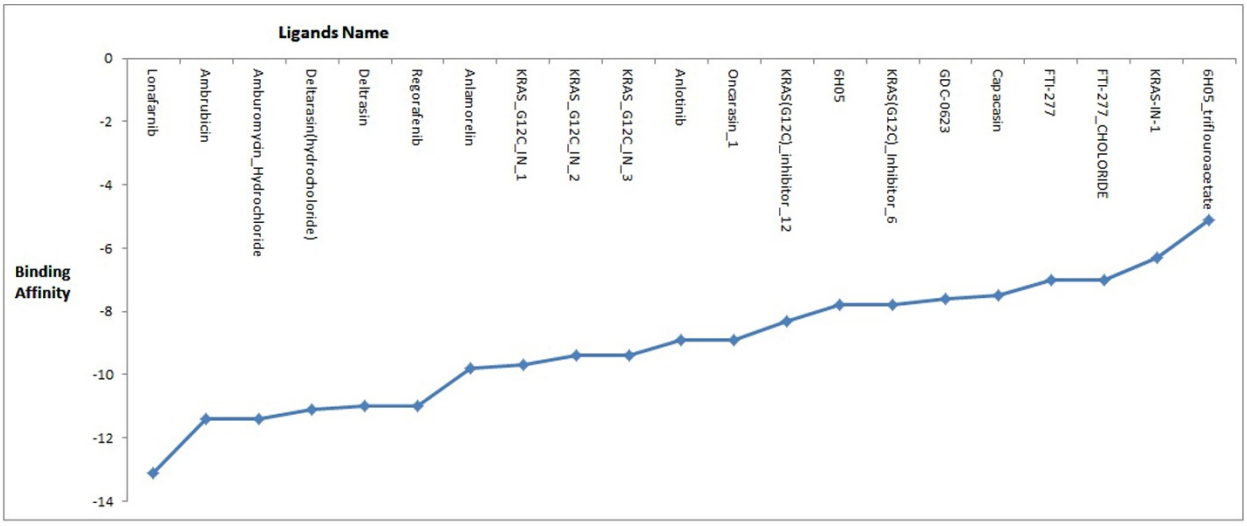

Figure 1: The graph of the Docking results of Ligands, selected on the basis of Lipinski Rule of Five

Figure.2|: Ligand based merged Pharmacophore, designed by the selected inhibitors

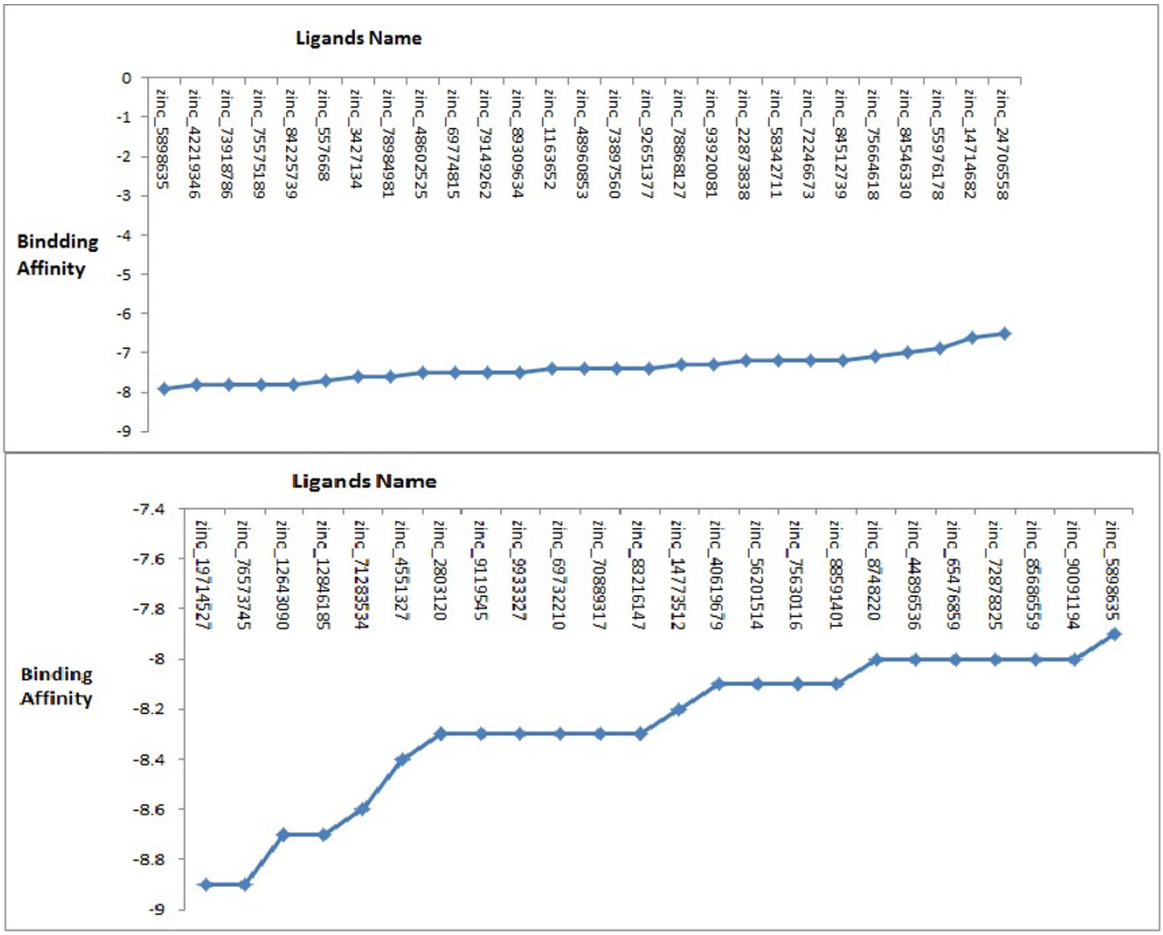

Figure 3: compounds were selected after virtual screening and their docking results

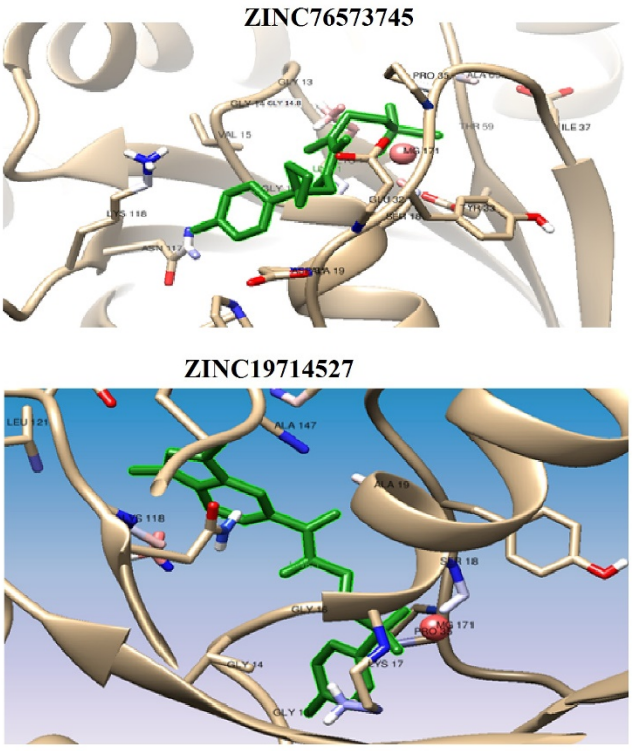

Figure 4: the interactions formed by the best hit compounds with KRAS Protein