J. Chosun Natural Sci.
Vol.15, No.4 (2022) pp.171-177
https://doi.org/10.13160/ricns.2022.15.4.171

Identification of inhibitors against ROS1 targeting NSCLC by In- Silico approach

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Abstract

ROS1 (c-ros oncogene) is one of the gene with mutation in NSCLC (non-small cell lung cancer). The increased expression of ROS1 is leading to the increase proliferation of cell, cell migration and survival. Crizotinib and Entrectinib are the drugs that have been approved by FDA against ROS1 protein, but recently patients started to develop resistance against Crizotinib and there is a need of new drug that could act as an effective drug against ROS1 for NSCLC. In this study, we have performed virtual screening, where compounds are taken from Zinc 15 dataset and molecular docking was performed. The top compounds were taken based upon their binding affinity and their interactions with the residues. The compounds stability and chemical reactivity was also studied through Density Functional theory and their properties. Further study of these compounds could reveal the required information of ROS1-inhibitor complex and in the discovery of potent inhibitors.

Keywords: ZINC 15, Molecular docking, Density Functional theory, Drug likeliness

(**Recceived** November 23, 2022; **Revised** December 23, 2022; **Accepted** December 23, 2022)

1. Introduction

The ROS1 rearrangement is a kind of chromosome abnormality that may additionally have an effect on cells of Non-small cell lung cancer (NSCLC). Chromosomal translation of the gene encoding ROS1 proto-oncogene receptor tyrosine kinase (ROS1) outline a good molecular subtype of non-small cell lung cancers that can also be inclined for treating ROS1 kinase inhibition. [1] Chromosomal disruption containing the ROS1 gene had been initially determined in NSCLC in 2007. ROS1 transcriptions recognized in 1 to 2%of sufferers with non-small cell lung cancer [2] Lung cancer that is ROS1 positive is a type of aggressive malignancy that spreads swiftly. New, targeted treatments can delay the progression of this form of lung cancer for some duration that giving patients a better prognosis than past origination. [3] Patients with ROS1 transcription share many information's in frequent with ALK-positive patients. Few more, ROS1-positive patients in this collection had adenocarcinoma background.

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ROS proto-oncogene 1 belongs to the subfamily of tyrosine kinase insulin receptor. The tyrosine kinase insulin receptor plays an important role in the embryonic development and show homology similarity with ALK receptor [4]. The genomic alteration of ROS1 is well known and they lead to the gene fusion with several other fusion partners that are oncogenic drivers [5-8]. As a result, ROS1 will be activated continuously leading to the increased cell proliferation, migration and survival due to the upregulation of AKT/PI3K pathway and MAPK-ERK signaling pathway [4].

Crizotinib is a known FDA drug that works against several target proteins which includes ROS1, ALK and MET [9]. The main function of the Crizotinib is that it binds to the ATP-dependent site of the respective protein kinase domain, leading to the suppression of these protein expression [10]. Crizotinib was the first line of treatment for NSCLC targeting ROS1, which showed a disease progression and genomic alteration, later there was an evident shrinkage of the malignant lesions [11]. But the fact is that, patients started to develop resistance over Crizotinib and there was no effective progression. A new generation of drugs that are capable of ROS1 inhibition is required for the therapy. Therefore, in our research we have virtually screened drugs against ROS1 protein and have found a potent inhibitor based upon their binding affinity and the interaction with the protein. These drugs where then analyzed by Density Functional Theory to know their chemical reactivity and stability.

2. Materials and Method

2.1 Preparation of protein and ligand for Molecular docking

Ligands were downloaded from Zinc 15 database in sdf format. The first step is to energy minimize the compounds, it was performed using PyRx software and they were rewritten in pdbqt format [12]. The targeted protein ROS1 was downloaded in RCSB (Structural Bioinformatics in Protein Data Bank) in PDB format. The protein was optimized by removing the water molecule and the co-crystal ligand. To the protein molecule the charges were added, Kollman and Compute Gasteiger charges, polar hydrogen bonds and atoms were added and saved in pdbqt format.

2.2 Molecular Docking

In In-Silico approach docking is one of the most important methodology used in finding the behavior of the active site of the targeted protein and the affinity of the ligands that binds to the targeted site [13-14]. The grid box was generated at the active site of the protein, with three dimensions x, y and z which is the parameter of the grid box. The dimension of the protein was at x= 35.83, y=11.58 and z = 1.73 and the size of the box was x=65, y=65 and z=65, these parameters were saved.

2.3 Density Functional Theory

DFT is one of the widely used technique to study the nature of the compounds. Gaussian was used to optimize the compounds. It is based on Hohenberg and Kohn theorem [15].

The calculations were carried out using B3LYP (Becke 3-Lee-Yang-paar) method, with 6-31G (d, p) basis set. Frontier molecular orbital [16] analysis is carried out, where the HOMO and LUMO values are calculated. The energy gap between the HOMO and LUMO, softness, absolute hardness, electronegativity, electrophilicity index [17-19] were the other descriptors that were calculated.

2.4 Drug likeliness Analysis

The drug likeliness property of the ligands was checked in Drug Likeliness Tool (DruLiTo; http://www.niper.gov.in/pi_dev_tools /DruLiToWeb/DruLiTo_index.html), developed by the Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India n open-source drug-likeness software. The pharmacokinetics properties like absorption, distribution, metabolism, excretion and toxicity were analysed [20]. The compounds were also analysed to checked whether these compounds violate the Lipinski rule of five and pass Ghose and Veber filter.

3.Results

3.1 Molecular docking

Molecular docking results showed a binding affinity of -10.3 kcal/mol for the compound 1 with the optimized structure of the protein. The top 10 compounds were taken. The binding of those compounds are ranging from -9.9 to -8 kcal/mol. Along with the binding affinity the interactions with the protein was also analysed. The interactions revealed that compounds are having the interactions with residues of the active sites. The hydrogen bonds were formed between Leu-1951, Lys 2040, Asp-2033, Met-2029, Leu-2026, and Glu-2027 which are the active site residues of ROS1 protein. Out of these compounds the top 10 were considered for C-DFT study and drug likeliness analysis using DrugLiTo and ADMET properties. The conformations were visualized using the Pymol software.

3.2 Conceptual DFT

The top ten compounds were taken for DFT analysis. The HOMO (Higher molecular orbital) values denotes the electron donating capacity, higher the HOMO value (small negative value) more will be the capacity to donate the electron. In case of LUMO (Lowest molecular orbital), it denotes the electron acceptance. The energy gap indicates the strength and the reactivity of the molecule, the lowest energy gap is found in between compound 4 and 5. The energy of the small band gap denotes the high nature stability and reactivity. In addition, the other descriptors like hardness, global softness, electronegativity, chemical potential, electrophilicity index were calculated using equations and the results are tabulated.

3.3 Drug likeliness prediction

In this study, the physiochemical profile and ADME properties of the compounds were studied. The molecular weight (MB) $\leq$ 500, the value of logP compounds lie in the range between 0 to 9 , number of Hydrogen bond donors $\leq$ 5 and the number of hydrogen bond acceptor $\leq$10.6 , and rotatable bonds less than 10 which showed that these can be a drug candidate. A polar surface area not greater than 10 Å is predicted to have good

oral availability. Accordingly, if any of the drugs violates 2 or more rules, these compounds could not be administered orally. The values imply the tedious of the Lipinski rule of five. All the values are tabulated.

4. Discussion

Molecular docking, Density Functional Theory, and ADMET study were performed for the compounds. The analysis of these compounds revealed a good docking score and reidentified the residues forming H-Bond with the protein interactions reactivity and the stability of the compounds was analyzed through DFT. The properties of these compounds included molecular weight where it is below 500 and the log P , HBD , HBA and RB are lying in the correct range and following the Lipsinki rule , where these hits can be a potent candidate. The most important violation of the Lipinski rule is checked and tabulated because these are novel compounds, as any violation of the Lipinski rule the drugs cannot be tested further as it will be ruled out. Further research could give us an idea of how these compounds would be an effective inhibitor of ROS1 protein.

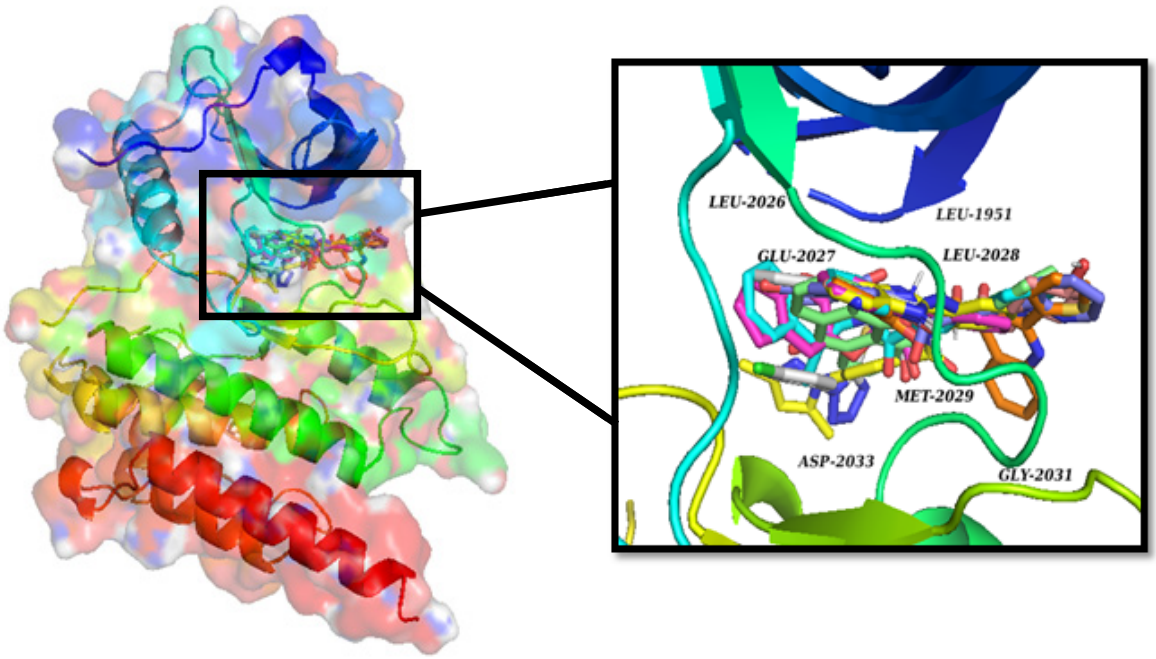

Image 1. a) Docked pose of ROS1 protein with the top compounds
b) The top compounds around the active site residue

Table1. Binding affinity of the top compounds and their H-bond interactions

Compounds	Binding Affinity kcal/mol	H-bond interactions
1	-10.3	Glu 2027,Met 2029
2	-9.9	Glu2030
3	-9.5	Asp2033
4	-9.4	Leu1951
5	-9.2	Met2029
6	-9	Met2029
7	-9	Asp2033,Met2029
8	-8.9	Leu1951
9	-8.5	Met2029,Glu2027,Lys2040
10	-8.1	Leu2028,Leu1951

Table 2. Statiscal analysis of HOMO and LUMO and other descriptors of top compounds.

Compounds	Total Energy (E y) (in eV)	Molecular dipole moment (Debye)	EHOMO	ELUMO	HOMO /LUMO Gap (AE)	Absolute Hardness (n)	Global Softness (P)	Electrone gativity (x)	Chemical potential	Electroph ilicity index
1	-52473.34	7	-5.43	-1.0	4.43	2.2	0.22	-3.22	3.22	-3.56
2	-156498.3	12.92	-0.14	-0.71	0.56	0.28	1.75	-0.430	0.43	0.326
3	-52507.08	6.43	-5.55	-4.94	0.61	0.30	1.63	-5.24	5.24	-0.79
4	-1880.3	3.86	-0.2	-0.04	0.16	0.08	6.25	-0.12	0.12	-4.52
5	-4.3823	4.01	-0.13	-0.09	0.04	0.02	25	-0.11	0.11	0.30
6	-50000.1	7.76	-0.22	-0.05	0.17	0.08	5.88	-0.135	0.165	0.10
7	-51163.66	5.77	-5.97	-1.25	4.71	2.35	0.21	-3.61	3.61	-4.6
8	-77754.3	13.56	-6.05	-2.45	3.60	1.80	0.27	-4.25	4.25	-3.83
9	-53083.1	8.59	-6.01	-2.25	3.79	1.89	0.26	-4.14	4.14	1.09
10	-49645.	4.14	-5.63	-1.91	3.72	1.86	0.268	-3.77	3.77	3.82

Table 3. Molecular properties of top hits.

Compounds	Molecular weight	LogP	HBD	HBA	TPSA A2	Rotatable bonds
1	364.29	0	2	3	50.36	6
2	388.32	0	2	3	75.6	5
3	347.26	0	3	4	95.5	4
4	462.8	0	1	6	132.14	6
5	461.37	0	2	7	139.5	6
6	439.34	3.38	1	5	89.02	4
7	445.38	3.34	1	4	104.37	4
8	383.27	2.93	1	5	93.45	3
9	136.23	2.70	0	0	0.00	1
10	156.27	2.61	1	1	20.23	4

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