International Journal on Recent Trends in Life Science and Mathematics (IJLSM)
Volume: 2 Issue: 2

ISSN: 2349-7955
011 – 016

Structural Analysis of Tyrosine SpecificProtein Kinase (TPK) and Interaction Studies with Inhibitors for Lung Cancer.

Aishwarya Hipparagi [1], Shambhu M G [1], Kusum Paul [1]
[1]Department of Biotechnology, The Oxford College of Engineering, Bangalore 560068, India
Correspondence should be addressed to Aishwarya Hipparagi:*aishwarya.malde@gmail.com*

Abstract:-The developments of drugs that inhibit target tyrosine specific protein kinase receptor (TPK) provide important insight for treatment of non-small cell lung cancer (NSCLC), as well as other cancer types. The TPK is involved in different signalling pathways within the cell function to regulate cellular proliferation from external and internal stimuli. Based on pathophysiology, the mutation of tyrosine kinase receptors may over express in cellular growth and cell proliferation that tightly trigger cellular process which mediates in developing malignancy in lungs and other internal and external organ. Our research is focusing on ROS1, ErbB2 and PTK2B of protein-protein interaction (PPI) and to regulate normal cell in to cancer and determine how these TPK pathways may lead to better therapeutic targets to developing small molecular inhibitors to control tumor growth and disease progression. We used different protein-protein interaction and signaling pathway databases to understand TRK interactions with different proteins and also integrate in structural studies of receptor tyrosine kinase might therefore provide new therapies for diseases.

Keywords*: tyrosine kinase, lung cancer, NSCLC, protein-protein interaction, ROS1, ErbB2, PTK2B*

*****

I. INTRODUCTION

Non-small cell lung cancer (NSCLC) is a malignant lung tumor characterized by uncontrolled growth of cells in lung tissues [1]. Based on molecular biology NSCLC is caused by activation of oncogenes that may cause genetic mutations in several membrane associated signaling proteins that regulate cell growth, cell proliferation, apoptosis and tumor invasion [2].Themutations in ROS1 tyrosine kinase receptor rearrangement is mostly observed in NSCLC of light/never smokers and young patients [3, 4]. The ROS1 receptor tyrosine kinase plays an important role in cell differentiation and proliferation that mediates PI3K signaling pathways [5]. The ROS1 is highly expressed in tumor cells of PTPN6 networks to regulate dephosphorylation of tyrosine proteins in hematopoietic cell signaling pathways and is potential targets against ROS1 inhibitors development [6,7].

Another gene such as ErbB2 is a member of human growth factor receptor tyrosine kinase neuro/glioblastoma cell lines (HER2/neu) is over expressed in oncogenes [8, 9]. The ErbB2protein has no ligand binding domain of EGF receptor enhancing kinase mediated downstream signaling cascades regulated outgrowth and stabilizing peripheral microtubules [9]. Upon ErbB2 of allelic variation of amino acids Ile654 and Ile655 phosphorylate tyrosine kinase receptor with EGF receptors that block GSK-3β and APC of signaling cascades overexpress in oncogenesis, this is the potential targeted drugs for mutations [10, 11, 12]. The PTK2B encodes cytoplasmic protein kinase which regulates calcium ion channels to regulate NMDA receptor and map-kinase receptor of different signaling pathways [13, 14, 15, 16]. In this current study, the allelic variation of proto-oncogene ROS1, ErbB2 of transmembrane receptor and PTK2B receptors is mainly involved in different signaling cascades in NSCLC and is used as a potential drug targets.

II. MATERIALS AND METHODS

2.1 Preparation of dataset. We have obtained 54 genes in the human non-small cell lung cancer (NSCLC) [17] and we have screened these 54 genes using advanced search database Uniprot with following condition: Protein name: *Tyrosine kinase*AND Pathology and Biotechnology: *lung cancer*AND Reviewed: *Yes*. Based on these criteria,three tyrosine kinase proteins obtained,namely ROS1, ErbB2 and PTK2B,these proteins are found to be highly involved in lung cancer.

2.2Sequence retrieval. The protein sequences of target tyrosine kinase protein receptors such as PTK2B, ROS1 and ErbB2 sequences were retrieved from Uniprot Protein sequence database. The protein ROS1 sequence (Uniprot id: P08922) has 2347 amino acids contains 1945-2222 region has tyrosine kinase domain of mutagenesis of loss of phosphorylation at tyr2274phe amino acids and shows loss of interaction with PTPN11. The PTK2B protein sequence (Uniprot id: Q14289) has 1009 amino acid of domain has 801-1009 amino acids of TGFB1I1 of phosphorylation observed in 819, 834, 842, 849 and 881 amino acids. The pro859ala has loss of interaction with NPHP1 and tyr881phe shows loss of phosphorylation site is strongly interacts with GRB2. The ErbB2 protein (Uniprot Id:P04626) of 1255 amino acids of which 753 position has ATP binding siteand 845 amino acid position has active site of proton acceptor. The protein has 720 987 amino acids of protein kinase receptor.

2.3Template selection and model building. The target protein sequences such as PTk2B, ErbB2 and ROS1 proteins were used to predict templates using BLASTp and Swiss Model server. The Swiss model template library was search with evolutionary related structures matching the target sequences. For each identified templates quality has been predicted from features of the target-template alignment. The template with the highest quality has then been selected

for model building. The global model of protein quality has been assisted using the QMEAN scoring function. For improved performance, weights of the individual QMEAN terms have been trained specifically for SWISS-MODEL. The coordinates of both target and templates of both side chain and backbone chains are the rebuilt based on geometry of the resulting model is regularized by using a force build, the overall models is designed using Swiss PDB viewer.

*2.4Quality analysis of protein structure and active site prediction.*Using SAVES served (Structural analysis and verification server) to identify the stereochemical quality of amino acids based on residue by residue geometry is analysed based on PROCHECK. The estimated quality of protein structures of psi and phi angles of all peptide structures is complexly arranges with 3D structure along with quality factor were observed. The complex model structure shows >80% is good structure and is further used for molecular docking. The sites of enzyme or ligand activity are lying in cavities or packets. The size and shape is all protein pockets predict the 3D confirmations of ligand binding. The binding of a substrate is typically serves as chemical modifications or conformational changes in protein structure. The CASTp calculation of concave surface regions on 3D with surface area and surface volume of selected protein pockets is frequently associated with strong ligand binding events. It is also calculates mouth opening of individual pockets for better accessibility of protein and ligand.

*2.5Ligand preparation.*The NSCLC of tyrosine kinase inhibitors of lead compounds is selected based on target protein. The anticancer drugs such as Crizotinib reference drug is used for ROS1 inhibitor, Erlotinib and gefitinib is used for ErbB2 inhibitors and leflunomide drug is used for PTK2B inhibitors, we searched these reference compounds with their similar compounds using Pubchem compound database. The Crizotinib has 811 similar compounds of which only 507 compounds alone is accepted for drug likeliness properties. Based on pharmacological action of Crizotinib has only 19 compounds with ALK tyrosine kinase receptor binding properties, out of 19 compounds we selected only 5 compounds for molecular docking.
The other reference compounds such as Erlotinib has only 23 similar compounds of which only 10 compounds is accepted for drug likeliness properties. Using pharmacological properties of these compounds shows only 10 compounds is accepted for ErbB2 binding and these compounds is potentially used for NSCLC. The gefitinib has 10 similar compounds of which only 6 compounds is accepted for drug likeliness properties. The pharmacological property of ErbB2 inhibitors of bioassay properties shows only 5 compounds is used for small molecule inhibitors on NSCLC. The Leflunomide compounds have 13 similar compounds of which only 12 compounds is accepted for drug likeliness properties. Using pharmacological properties of PTK2B inhibitor of only 8 compounds is accepted for best small molecular inhibitor against NSCLC.

2.6Molecular docking. The target protein structures such as ErbB2, ROS1 and PTK2B is docked with selected lead molecules using Auto Dock 4.2. There are various parameters were adjusted to macromolecule such as addition of Gasteiger charges, addition of hydrogen bonds with polar groups, atomic salvation parameters and fragmental volumes were assigned to the protein. To run AutoDock to search Auto grid by creating Grid map of different grid points and keeping ligands to cover binding pockets based on active site amino acids within the selected protein. A set of Lamarckian genetic algorithm was substantially used for molecular docking simulations. The simulation is carried about 10 times which ultimately yielded 10 docked conformations. From this, the lowest energy conformations were regarded as the best binding conformations. In the end, the reverse validation processes ensured the identified hits that fitted with generated Pharmacophore models and active sites of both targets. Since all the parameters were required for molecular docking and Pharmacophore mapping were consequently fixed and used in regular process.

III. RESULTS AND DISCUSSION

The NSCLC target tyrosine kinase receptor proteins such as ROS1, ErbB2 and PTK2B proteins is mainly involved in different signaling pathways such as MAP kinase, EGFR, GSK-3β of Wnt and other transmembrane signaling pathways. The ROS1 has 100% similar to 3zbf_A template with the sequence coverage of ROS1 protein kinase domain 1934-2232 amino acids. The ROS1 kinase domain has mutation acquired on gly2032arg substitution may cause NSCLC and is potentially used for molecular docking. The ErbB2 protein has 100% similar to 3PP0.2.A template of sequence coverage from720-987 amino acids. The PTK2B protein of tyrosine kinase domain is present in 425-683 amino acids of which 3fzt template shows 100% similarity. The overall sequence similarity of protein structures is used for molecular modelling and Docking.

3.1 Homology modelling. The three dimensional structures of protein sequences were predicted using SWISS MODEL and the resultant structures were used for homology modeling. The target and the template sequences were aligned using a comparative protein modeling program Swiss Model.The theoreticalmodels were subjected to model using Swiss-PDB-viewer. The protein structures were superimposed in order to deduce structural alignment. The 3D modelled structures of PTK2B, ERbb2 and ROS1 were expressed 100% similarity to their selected templates of 3FZT, 3PP0 and 3ZBF structures. Thus, the geometric accuracy of the theoretical 3D modelled proteins was rectified to analyse the quality of proteins. The reliability of protein structures were validated chiefly by using SAVES. The structural statistical measures of modelled proteins were derived from X-ray crystallographic data.

3.2Protein Quality Analysis.

*Active site and ligand binding sites identification.*The reliability of protein structures can be validates using protein structural analysis and verification server (SAVES). The geometry of both target and template models with Q mean score predicts -2.89 of 3zbf.A template model with target ROS1 protein and the overall quality shows 93.333 and verify 3D of 97.15%, this results shows ROS1 protein is good model with template structure and potentially used for molecular docking. The another protein Erbb2 has -0.60 of Q mean values with 100% similar to 3PP0 template, the model is verify the quality of structure shows 98.364 and verify3D of 85.47%. The PTK2B is model with 3fztA template with Q mean of -0.77 shows 99.160 quality factor and 98.46% of verify 3D. The overall results shows all the model structure is more complex with residue-by residue geometry and is potentially used for molecular docking.

The complex structure of both ROS1, PTK2B and ErbB2 protein have more number of active sites and ligand binding sites. The ROS1 has 39 pockets with surface area of 599.3Å^2 and surface volumes of 1066.8 Å^3 have 31 active site and ligand binding site amino acids. The PTK2B protein has 29 pockets of which the surface area of 1066.8Å^2 and surface volume 1572.6Å^3 shows 46 active site and ligand binding amino acids. The ErbB2 has 92 pockets of which 48 active sites and ligand binding site amino acids occupied in surface area of 1161.6Å^2 and surface volume of 1468.6Å^3.

Table 1 Summary of active site residues

Protein	Surface area (Å^2)	Surface Volume (Å^3)	Active site amino acids
ROS1	599.3	1066.8	Leu2086, Glu2027, Arg2083, Asp2033, Leu1951, Gly2032, Leu2028, Met2029, Ala1978, Leu2026
ErbB2	1161.6	1468.6	Asp863, Met774, Phe864, Thr798, Ser783, Leu852, Gln799, Met801, Ala751, Leu796, Val734, Thr862, Arg849, Leu800, Gly804, Lys753, Asn850, Gly729, Leu726
PTK2B	1066.8	1572.6	Ile477, Arg572, Asp576, Ser473, Leu481, Met478, Ile486, Val487, Leu565, Gly566, Asp567, Leu556, Glu503, Leu504, Tyr505, Phe568, Met502, Lys457, Glu474,

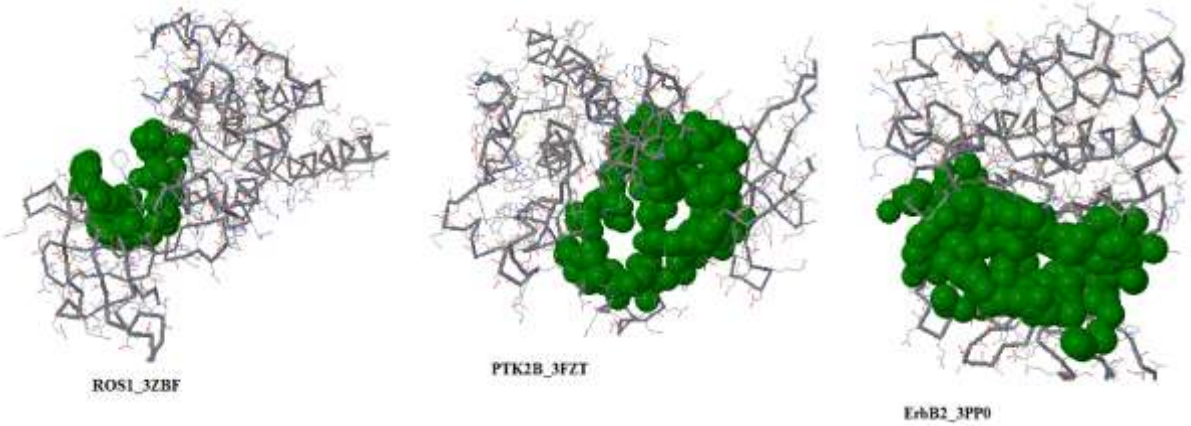

Figure 1 Active sites prediction of target proteins using CASTp

*3.3 Ligand preparation and Pharmacophore properties.*The NSCLC of tyrosine kinase inhibitors such asCrizotinib, Erlotinib, Gefitinib and leflunomide compounds along with their selected lead compounds is used for Pharmacophore analysis (Table: 2). The Compound CID254182616 and CID16038120 have highest molecular weight and hydrogen bond acceptors is not accepted for drug likeliness properties. The compound CID11511120 has miLogp of 5.019 also have highest soluble in aqueous media and this compound also not accepted for drug likeliness properties. Using molecular docking techniques to screen the compounds which show no interaction with target protein also with less energy binding compounds.

Table 2 Molecular properties of ligands obtained from Molinspiration

Compound Name	miLogP	TPSA	natoms	Mol Wt	nON	nOHNH	nviolations	nrotb
Crizotinib	4.006	78.002	30.0	450.345	6	3	0	5
CID72710568	2.069	132.215	33.0	470.53	9	4	0	7
CID25182616	3.648	127.508	39.0	532.58	11	4	2	8
CID16038120	5.662	102.928	42.0	614.216	10	2	2	9

ISSN: 2349-7955
011 – 016

Gefitinib	4.192	68.751	31.0	446.91	7	1	0	8
CID176870	2.795	74.747	29.0	393.443	7	1	0	10
CID76317764	4.095	70.592	34.0	453.546	7	1	0	5
Erlotinib	2.795	74.747	29.0	393.443	7	1	0	10
CID11511120	5.019	79.381	33.0	469.948	7	2	1	7
CID10184653	4.215	88.615	34.0	485.947	8	2	0	8
Leflunomide	2.358	55.13	19.0	270.21	4	1	0	3
CID1234281	4.471	64.699	25.0	330.366	4	2	0	2
CID6375556	1.336	72.444	20.0	309.368	5	1	0	4
CID1331726	4.129	98.233	32.0	423.428	7	1	0	5
CID5329102	1.954	80.989	29.0	398.482	6	3	0	7

*3.4 Molecular docking.*The target proteins such as ROS1, ErbB2 and PTK2B proteins with selected reference lead molecules along with their pharmacological similar compounds is used for molecular docking. Using Lamarckian genetic algorithm to run both target protein along with lead compounds with 10 simulation runs to interpret the results. The docking of Crizotinib is strongly interacts with target protein ROS1 by forming 2 hydrogen bonds with energy of -18.662 kcal/mol within active site amino acids Glu46 and Asp135. The pharmacological property of lead compounds such as CID72710568 has 4 hydrogen bonds with energy of -19.0629 kcal/mol, the CID25182616 has 2 hydrogen bonds of energy -23.3976 kcal/mol and CID11626560 has 1 hydrogen bonds of -12.4396 kcal/mol of energy. The overall results shows all the compounds Crizotinib compound along with their pharmacological similar compound CID72710568 and CID25182616 is strongly interacts with target protein ROS1 and these compounds is potentially used for further clinical investigations.

Table 3 Ligand of ROS1 protein was retrieved from the PubChem Ligand database

Ligands	No. H bonds	Bond Energy	Amino Acid
CID72710568	4	-19.0629	Glu46, Glu49,Asp135, Lys173
CID11626560	1	-12.4396	Glu46
CID25182616	2	-23.3976	Arg163, Leu178
Crizotinib	2	-18.662	Glu46 Asp135

Figure 2 Interaction between ROS1 and ligand (Compounds CID72710568 and CID25182616 is strongly interacts with target Protein ROS1)

The Gefitinib and Erlotinib of compound is strongly interacts with ErbB2 by forming 2 hydrogen bonds of -13.5944 and -8.35318 kcal/mol within active site amino acids Ile886, Lys887, these compounds is already used for treatment of NSCLC of ErbB2 protein. The Pharmacological property of CID123631 and CID10184653 kcal/mol of 4 hydrogen bonds within active site amino acids of Gln902, Cys965, Pro967, Arg968 and Glu971. The overall results shows CID123631 and CID10184653 is also used for potential treatment for ErbB2 protein structure.

Table 4 Ligand of ErbB2 protein was retrieved from the PubChem Ligand database

Ligands	No. H bonds	Bond Energy	Amino Acid
CID 176870	1	-18.1365	Glu971
CID 123631	4	-8.20112	Arg968, Glu971, Cys965,
Gefitinib	2	-13.5944	Ile886, Lys887,
Erlotinib	2	-8.35318	Ile886
CID 11511120	1	-6.95753	Ala710
CID 10184653	4	-6.72492	Gln902, Glu971, Pro967, Cys965

ISSN: 2349-7955
011 – 016

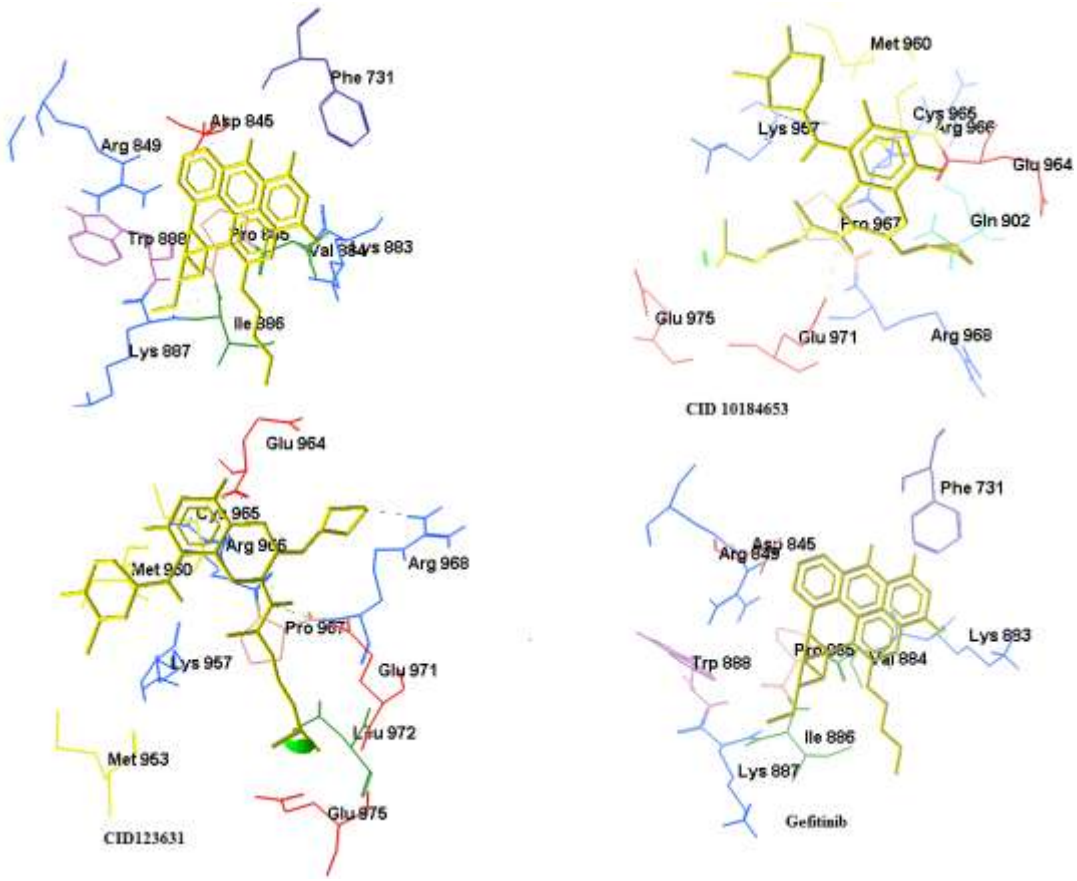

Figure 3 Interaction between ErbB2 and ligand (Compounds CID10184653 and CID123631 is strongly interacts with target Protein ErbB2 with 4 hydrogen bonds each)

The Leflunomide compound is strongly interacts within active site amino acid of PTK2B protein by forming 2 hydrogen bonds of energy -10.2984 kcal/mol. The pharmacological property of CID6375556 compound has 4 hydrogen bonds of energy of -18.6752 kcal/mol within active site amino acids Arg129, Glu193, Lys199 and Gln200. Other compounds such as CID 1234281, CID 1331726, CID 72710568 and CID 5329102 have 2 hydrogen bonds within active site amino acids. The overall results show all these compounds is potential drug targets on PTK2B protein.

Table 5 Ligand of PTK2B protein was retrieved from the PubChem Ligand database

Ligands	No. H bonds	Bond Energy	Amino Acid
Leflunomide	2	-10.2984	Tyr115
CID 1234281	2	-26.4043	Ser196, Lys199
CID 6375556	4	-18.6752	Arg129, Glu193,Lys199, Gln200
CID 1331726	2	-28.6526	Gly198, Lys199
CID 72710568	2	-26.3117	Asn43, Glu151
CID 5329102	2	-37.7501	Tyr149, Tyr155

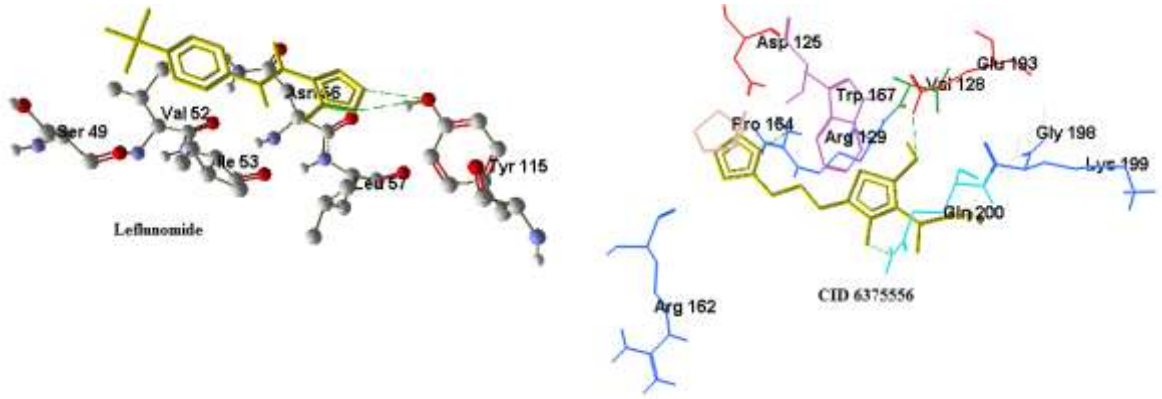

Figure 4 Interaction between PTK2B and ligand (Compounds CID6375556 strongly interacts with PTK2B with 4 hydrogen bonds and energy -18.6752)

IV. CONCLUSION

The tyrosine kinase proteins ROS1 ErbB2 and PTK2B were highly involved in lung cancer and the three dimensional structures of protein sequences were predicted using SWISS MODEL based on features of template-target alignment and QMEANS scoring function.The reliability of protein structures were validated using protein structural analysis and verification serverSAVES then active sites were predicted by surface area and surface volume. Among identified 19 compounds of which Crizotinib, Gefitinib, Erlotinib, and Leflunomide already used for treatment of NSCLC of target proteins ROS1, ErbB2 and PTK2B. We used pharmacological and bioassay properties of these reference compounds is selected from Pubchem compound database and used for molecular docking. The Overall docking results shows CID72710568, CID25182616, CID 123631, CID 10184653, CID 1234281, CID 6375556, CID 1331726 and CID 5329102 compounds are potential on NSCLC of target protein structures are potentially used for further clinical practice.

V. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

VI. ACKNOWLEDGMENT

The authors thank TOCE, Bangalore for the supportand infrastructure provided and for their undiminished encouragement and valuable inputs in presenting the work

REFERENCES

[1] Sgambato A1, Casaluce F,Maione P, Rossi A, Rossi E, Napolitano A, Palazzolo G, Bareschino MA, Schettino C, Sacco PC, Ciadiello F, Gridelli C. (2012). The role of EGFR tyrosine kinase inhibitors in the first-line treatment of advanced non small cell lung cancer patients harboring EGFR mutation. Curr Med Chem. 2012;19(20):3337-52.
[2] Davies, K. D. et al., Identifying and Targeting ROS1 Gene Fusions in Non-Small Cell Lung Cancer. Clin Cancer Res 2012; 18:4570-9.
[3] Hunter T. Signaling-2000 and Beyond. Cell. 2000; 100:113–127.
[4] Schlessinger J. Cell Signaling by receptor tyrosine kinase. Cell. 2000; 103:211–225.
[5] Bergethon, K. et al., ROS1 Rearrangements Define a Unique Molecular Class of Lung Cancers. J Clin Oncol 2012; 30:863-70.
[6] Shaw, A. et al., Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangements. J Clin Oncol 30, 2012 (suppl; abstr 7508).
[7] Rong-jun He, Zhi-hong Yu, Ruo-yu Zhang and Zhong-yin Zhang, (2014). Protein tyrosine phosphatases as potential therapeutic targets. Acta Pharmacologica Sinica 35, 1227-1246.
[8] Hegedus, L. Orfi, A. Seprodi, A. Varadi, B. Sarkadi, G. Keri, (2002). Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1Biochim Biophys Acta, 1587, pp. 318–325
[9] Coussens L., Yang-Feng T.L., Liao Y.C., Chen E., Gray A., McGrath J., Seeburg P.H., Libermann T.A., Schlessinger J., Francke U., Levinson A., Ullrich A.(1985). "Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene."Science 230:1132-1139(1985).
[10] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. LancetOncol 2011; 12:175–80.
[11] Buchdunger E, et al. “BCR-ABL inhibition as a modality of CMLtherapeutics”. Biochim Biophys Acta. 2001 1551

[12] Kim D, Ahn M, Yang P, et al. Results of a Global Phase II Study with Crizotinib in Advanced ALK-positive Non-small Cell Lung Cancer (NSCLC). ASCO Annual Meeting 2012; 2012:ix400-46.
[13] Wong DW, Leung EL, Wong SK, et al. A novel KIF5B-ALK variant in nonsmall cell lung cancer. Cancer 2011; 117:2709-18.
[14] Verbeek HH, Alves MM, de Groot JW, et al. The effects of four different tyrosine kinase inhibitors on medullary and papillary thyroid cancer cells. J Clin Endocrinol Metab 2011; 96:E991-5.
[15] Lee JS, Hirsh V, Park K, et al. Vandetanib Versus placebo in patients with advanced non-small-cell lung cancer after prior therapy with an epidermal growth factor receptor yrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). J Clin Oncol 2012; 30:1114-21.
[16] Dawson SJ, Conus NM, Toner GC, et al. Sustained clinical responses to tyrosine kinase inhibitor sunitinib in thyroid carcinoma. Anticancer Drugs 2008; 19:547-52.
[17] Bi-Qing Li, et al."Identification of lung-cancer-related genes with the shortest path approach in a protein-protein interaction network. BioMed Research International 2013;267375