Biocatalysis and Agricultural Biotechnology 21 (2019) 101301

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Study on recognition of novel RACK1 protein inhibitors for small cell lung cancer outlined by pharmacophore based virtual screening and Molecular Docking

Langeswaran K [a,*], Suganya N [b], Sangavi P [a]

[a] Cancer Genetics & Molecular Biology Laboratory, Department of Bioinformatics, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India
[b] Department of Department of Biotechnology and Bioinformatics, Bishop Heber College (Autonomous), Tiruchirappalli, Tamil Nadu, India

ARTICLE INFO

Keywords:
RACK1 inhibitor
Molecular docking
Pharmacophore based virtual screening
Lung cancer
3D models

ABSTRACT

An effort has been initiated to design pharmacophore-based 3D models pertaining to human RACK1 protein, (receptor of activated kinase-1) inhibitors were generated with accurate predictability. Such lead compounds were subsequently validated by screening measures to determine its biological activities and its adaptable stability in the systems. The benchmark for virtual screening was also treated to examine the selective RACK1 inhibitors. As a result of virtual screening experiment and in vitro evaluation of lead candidate compounds, resulted novel and appropriate RACK1 inhibitors were recognized. Therefore, the findings of the present investigation will be through a light on an innovative strategy for identifying biological adaptable RACK1 inhibitors to control or arrest the proliferation of tumor cells using in vitro studies. Molecular Docking studies were performed to understand the capability of RACK1 inhibitor activity and to explore its high interactive accord or affinity.

1. Introduction

Lung cancer is a chronic fatal disease and represents in the globe wide due to lack of effective therapeutic medication to cure or extend life expectancy. Lung cancer remains the leading cause of cancer related mortality worldwide for both men and women (Siegel RL et al., 2017). A recent study estimated that the incidence of lung cancer death was 21, 700 numbers while combined mortality and morbidity caused by colorectal, breast cancer was found to be 18,500. Small cell lung cancer (SCLC) is a type of cancer related diseases and is estimated around 13% attributed and determined by exposure of tobacco and smoking. It is interesting that 90% of the death caused by man, whereas 80% in women (World Health Organization, 2017). Receptor of activated C Kinase (RACK1), is a cytosolic protein with a subunit of G-protein (Ron et al., 1994). Shou Shi et al. (2012) have reported that RACK 1 is responsible for interacting mediate Gli1-dependent transcription in NSCLC cells. A study of the mechanism of intrinsic activation of epidermal growth factor receptor (EGFR) signalling pathways, has conferred and played a role in the occurrence of small cell lung cancer (SCLC). Several earlier studies demonstrated RACK1 has potential to induce in the proliferation of cancer cells and exposure was upregulated in various types of cancer related diseases covering lung cancer (Cao et al., 2011). Previously, RACK1 was recognized as anchoring protein and its role was believed to be involved gradual progression and expression of cancer cells (Csukai and Mochly-Rosen, 1999). RACK 1 has been identified as multiple coins and receptor and exerted various vital responses (Li et al., 2019). Currently, Small cell lung cancer cells showed a trend that RACK1 protein is a potent in accelerating or inhibiting effects on cancer cells (Yi et al., 2016). Another cancer related study has reported that RACK 1 protein was recognized and noticed over exposure in colorectal cancer cells compared to precancerous tissues and it showed a strong correlation with lymph nodes and spreading process (metastasis) and involving cancer cells differentiation.

Since the 21st century, tremendous changes have occurred in life science and a transformation of classical or traditional methods was minimized and system integration methods have evolved in favor of accuracy. Virtual screening is an advanced computer-based tool used in the drug designing process. This serves and guide to formulate or design innovative therapeutic agents with appropriate qualities. One main activity of using this tool was to analyses of interaction between drugs and

* Corresponding author. Cancer Genetics and Molecular Biology Laboratory, Department of Bioinformatics, Science Campus, Alagappa University, Karaikudi, 630 003, India.
E-mail address: langeswaran@alagappauniversity.ac.in (L. K).

https://doi.org/10.1016/j.bcab.2019.101301
Received 1 June 2019; Received in revised form 19 August 2019; Accepted 21 August 2019
Available online 27 August 2019
1878-8181/

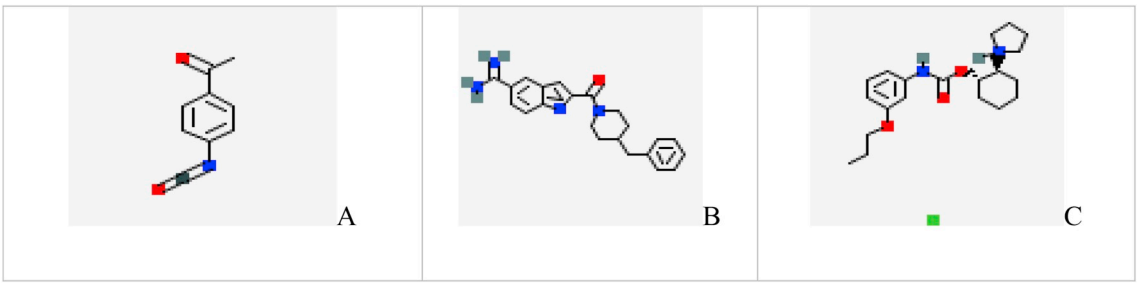


Fig. 1. The chemical structures of lead compounds.

its receptor sites. Optimum affinity with its receptors indicates its resemblance and its chemical determined of the target receptor. Pharmacophore based virtual screening has been recognized as a hotspot in drug discovery paradigm and screening inhibitors (Liew et al., 2010). Therefore, in the present study, pharmacophore guided virtual screening was employed for docking and molecular dynamics simulations were performed to recognize and understand RACK1 protein inhibitors, compounds to reduce progressive effects on proliferation of (SCLC) Cells in vitro studies.

2. Materials and methods

By extensive search of literature and text mining process, the RACK1 protein target structure was derived from (RCSB) Protein Data Bank, by thorough screening in drugability of the receptor, binding site options were performed. Selection of the most appropriate protein structure was imported from PDB into Maestro in the protein preparation wizard. Its results, hydrogen atoms display with polar option. Further, an interactive optimization of hydrogen-bonded species were selectively optimized RMSD value of the atom was minimized in Angstrom unit, subsequently optimized potentials for liquid simulations 2005 force field was determined. Ligand structure preparation was performed with high quality of 2D, 3D structure of huge numbers of drug like molecules were proceeded. The force field geometry and partial atomic changes were calculated using OPLS-2005 force field. Four grid boxes were generated using tools, with 4 taps such as Receptors, Sites, Constraints and Rotatable groups. All docking calculations were performed with maestro packages.

Virtual screening at the active sites were carried out using Glide, Schrodinger suite, virtual screening workflow docking program. The virtual screening workflow was designed for analyses large collection of compounds against the target RACK1 protein. Workflow covers ligand preparation using ligand peptide. Filtering method was used in Qikprop characterization at Glide Docking with HTVS, SP and XP accuracy level analysis. Ligands were sourced from the database, such as May bridge, Enamine and Life chemical. Virtual screening offers three options for pre-filtering ligands using the Lipinski Rule of 5, deleting ligands with reactive functional groups using a custom filter.

Binding free energy was calculated by using prime MM-GB/SA approach. It could be predicted free energy of binding of ligands to receptors, energies of complex were performed by using OPLS-AA 2005 force field and GB/SA continuum solvent following the formula $\Delta Gbind = \Delta E + \Delta Gsolv + \Delta GSA$. Simulations were performed using GB/SA continuum model (Nisha Chandna et al., 2015) in prime version 2.2 (prime, 2010). Prime use a surface GB model of Schrodinger. TIP31-2005 force field was used to analyze protein interactions and solved SPC incorporated DPPC membrane model overlapping molecules were deleted and system neutralized with Na + ions.

Subsequently, protein-ligand complex was placed on set MD simulation stability analysis. Various interaction diagrams were generated, trajectory and plots were also obtained. The trajectory was formed to serve stable and confirm appropriate docking of the ligand and protein. Such obtained complex was further subjected to the ADMET Property analysis using Qikprop module. This was essential to evaluate physiochemical significant descriptor and pharmacokinetically relevant properties. Acceptability of analogues was performed based on Lipinski's rule of 5 covering Adsorption, Distribution Metabolism and Excretion.

The materials used to perform all the techniques is Schrödinger suite, USA (see Fig. 1).

Table 1
showing Top hits compounds identified inhibitors both in ENAMINE and LIFE CHEMICAL database in Molecular Docking against RACK1 Protein.

S.No	Inhibitor coding id	Docking score	Glide energy	Glide emodel
ENAMINE database- compound ID				
1	99452	-6.906	-53.132	-74.337
2	1278	-6.693	-54.593	-70.615
3	37937	-6.668	-43.627	-54.176
LIFE CHEMICAL database- Compound ID				
4	33314	-8.722	-62.419	-96.360
5	96874	-8.211	-71.248	-97.520
6	87686	-7.607	66.789	-90.803

3. Results and discussion

3.1. Virtual screening

Virtual screening based on pharmacophore device and docking simulation was carried out in the present investigation using library comprising of huge number of small molecules and compounds. As a result, uncountable number of potential RACK1 inhibitors were obtained using the Schrodinger software within the limit of the Lipinski rule of 5 Top three compounds identified from virtual screenings on the basis of docking score and glide energy. Top three docked ligands were 99452, 1278 37937 and 33314,96874,87686 from ENAMINE and LIFE CHEMICAL database, shows a high docking score of about -6.906,-6.693,-6.668 and-8.722,-8.211,-7.607 respectively using potent approaches of the scoring method (Fig. 1 & Table 1). The chemical structures of these lead compounds are illustrated in Fig. 2. Top three compounds docked with protein are represented in Fig. 3. Two-dimensional ligand interactions with the protein of the LIFE CHEMICAL database is shown in Fig. 4. Summary of ENAMINE database compounds represented in Fig. 5.

3.2. Binding free energy

The prime MM-GBSA method was performed based on the docking complex and used to know the binding free energy (d Gbind) of the ligands. Prime MM-GBSA salvation energy (dG bind) was assessed and resulted the followings were categorized, 33314_lifechem, 96874_lifechem, 87686_lifechem ranged (Table 3). The role of water as a ligand is mandatory to adjust both increase or decline binding energy of a drug (Thilagavathi and Mancera, 2010).

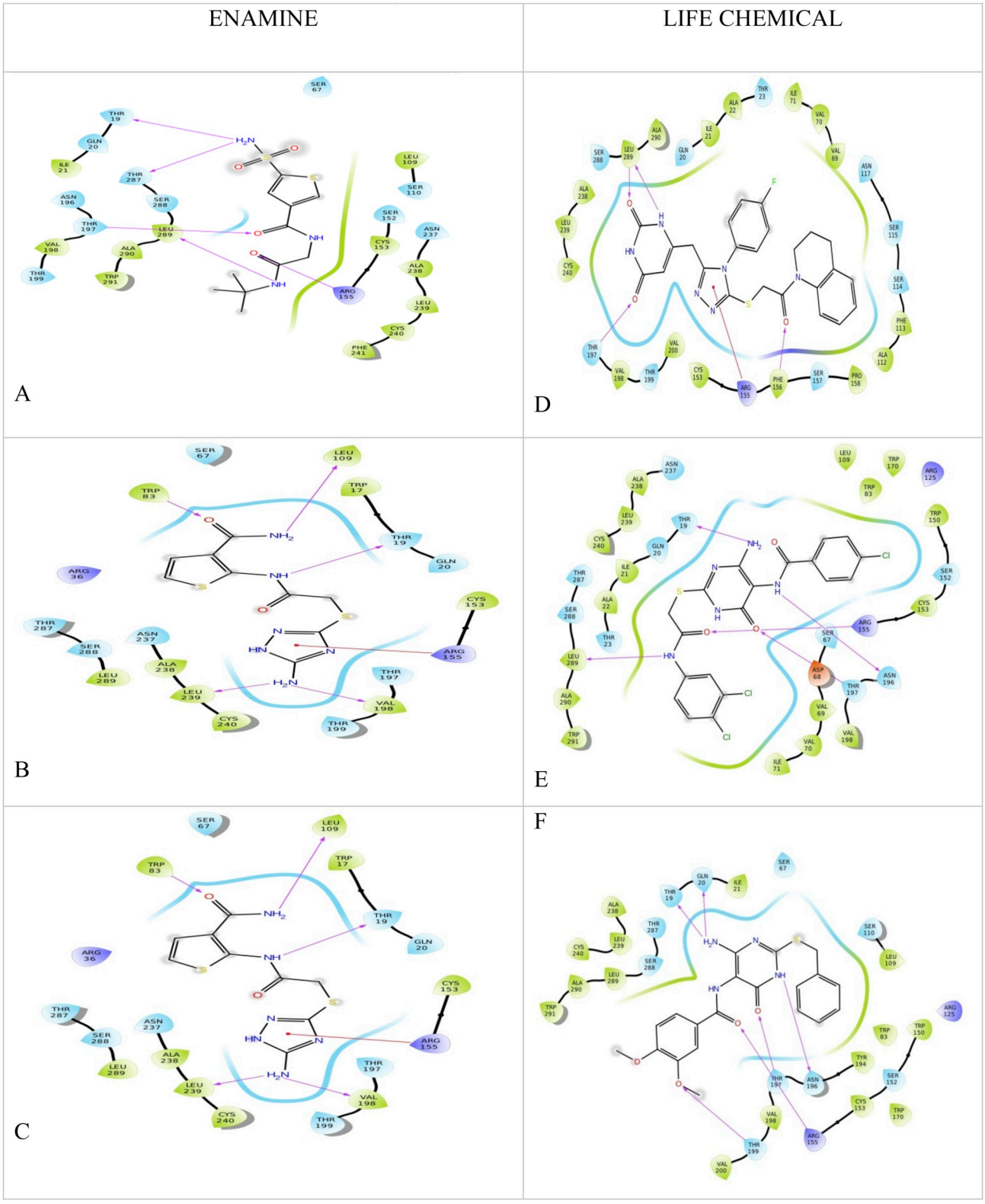


Fig. 2. Two dimensional ligand interactions with protein (ID Code)(A: 99542, B: 1278, C: 37937, D: 33314, E:96874, F:87686).

3.3. Molecular dynamics

The present study shows the top lead ligands were identified from the structure library (Yadav et al., 2014) such based on the hits molecule were technically coded as 99542 from ENAMINE database and others coded as 33314 from LIFE CHEMICALS. All two complexes were simulated for a period of 10ns. On the basis of active site observed, data sets are further bifurcated into catalytic site, peripheral and deal sites (Schrodinger, 2013). The changes in the structural features have been analyzed through the calculations of the root mean square deviations (RMSD) and the root mean square fluctuations (RMSF) over the backbone atoms.

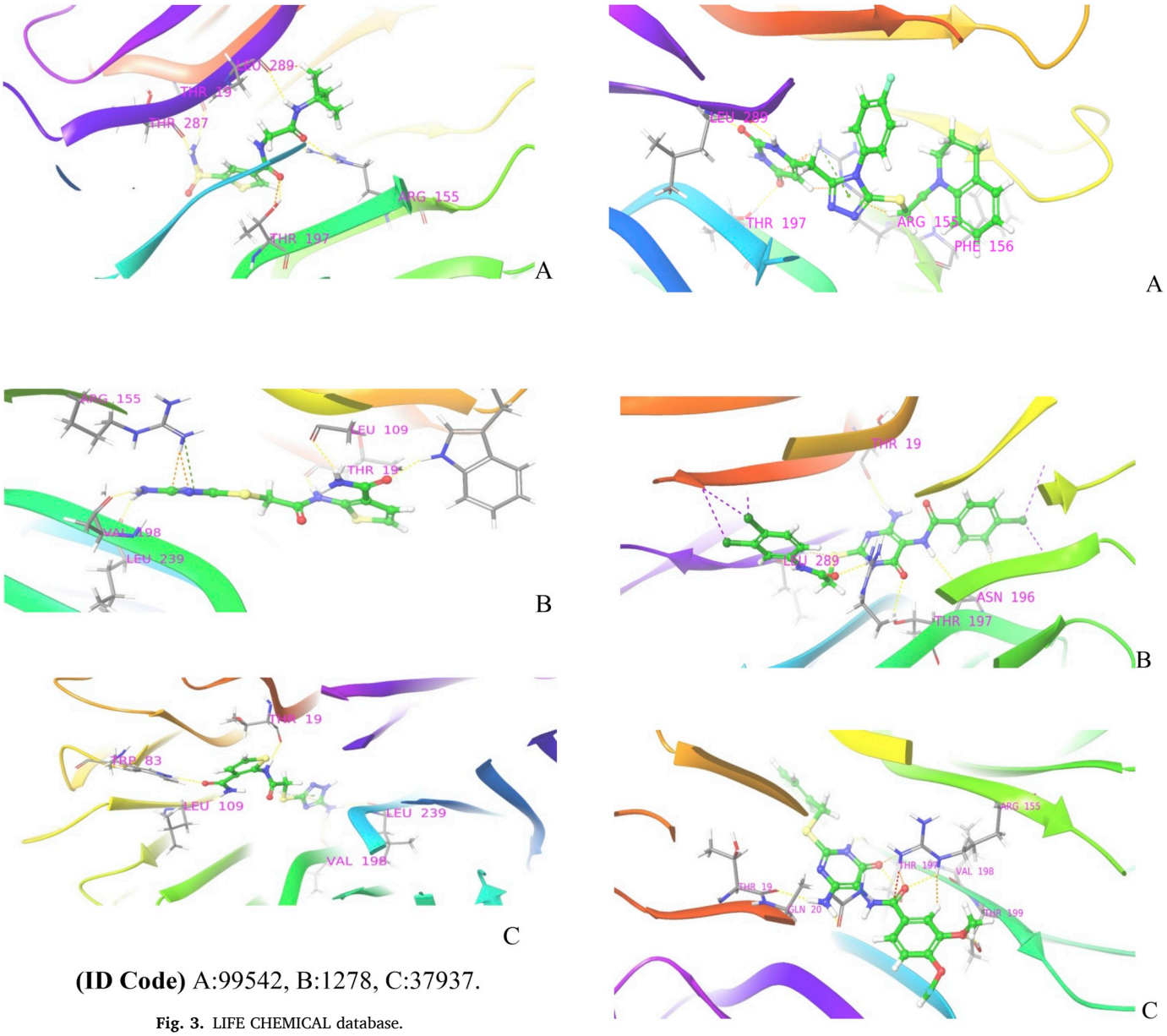


Fig. 3. LIFE CHEMICAL database.

Fig. 4. Summary of LIFE CHEMICAL database compounds.

3.4. Root mean square deviations

The stability of protein-ligand (RACK1-99542 and RACK1-33314) complexes were studied using MD simulations. Each system was subjected to positions restraint simulations for 10ns. RMSD of backbone atoms were calculated over the entire MD simulation trajectories. RMSD results were shown in Fig. 6.

3.5. Root mean square fluctuation

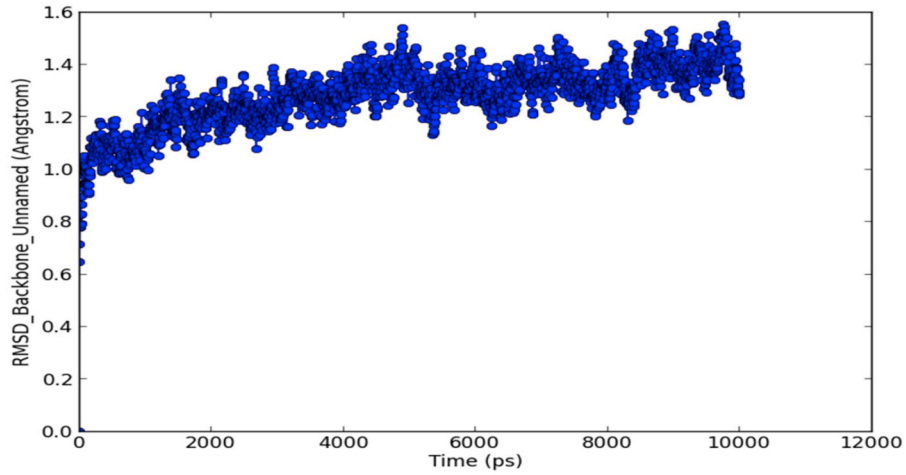
RMSF was estimated in the average fluctuations of the macromolecular target protein at the residue level during the period of stimulation. Peaks in the RMSF plot indicate residues of the protein fluctuate many times. In general, the higher fluctuations found at the ends of the plots correspond to the termini. This stabilization of residues in the active site is an essential feature for any good inhibitor. The RMSF results were shown in Fig. 6. The RMSD and RMSF of the complexes are in good agreement with one another. Overall, the current simulation studies convincingly show that the 99542 and 33314 protein complex is stable at the active site and can serve as a better inhibitor for inhibiting in Non-small cell lung cancer.

3.6. ADME prediction

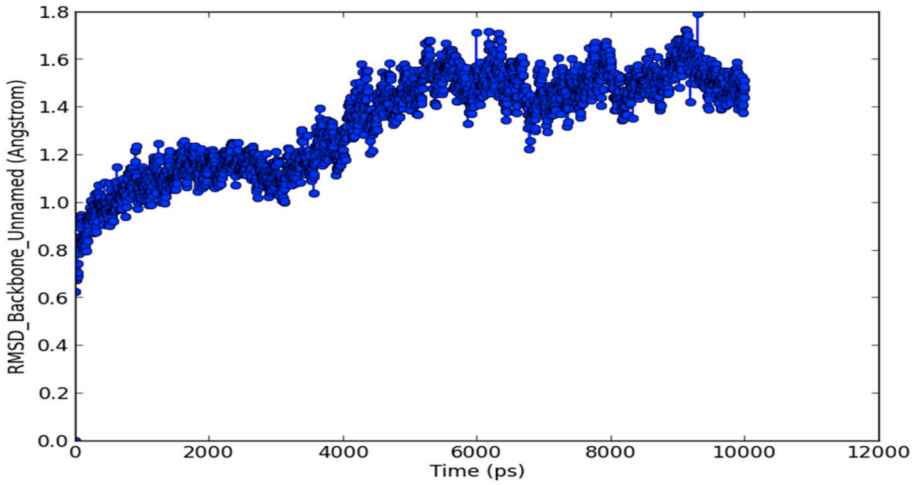
ADME prediction was performed to find the pharmaceutical properties of the lead compounds. The lead compounds have acceptable ADME properties which was tabulated in Table 2.

4. Conclusion

In the present investigation, receptor of activated kinase 1 (RACK1) was targeted using *in silico* predictive ability with a cause for preventing the proliferation and growth factors of tumor cells. Using energy minimization and molecular dynamics simulations, predicted model was found to be stable. MD simulations indicated that the theoretical prediction was more consistent and matched with the known set of



A



B

Fig. 5. The RMSD analysis of two complexes A) RACK1-99452, B) RACK1-33314.

Table 2
showing ADME properties of ENAMINE and LIFE CHEMICAL database using QikProp.

S.No	Compound Id	MW	Donor HB	AcceptHB	%HOA	QPlogPo/w	QplogHE RG	QPP Caco	QPPMD CK
				ENAMINE database					
1.	99452*	319.3	3.250	8.750	57.537	-0.208	-3.453	59.880	59.299
2.	1278	298.3	5.000	7.000	33.616	-0.387	-4.554	16.722	13.116
3.	37937	268.3	3.00	4.250	91.908	2.280	-5.186	764.780	370.226
				LIFE CHEMICAL database					
4.	33314*	492.5	2.000	8.500	81.702	3.490	-6.209	82.708	78.814
5.	96874	498.7	4.250	8.250	82.732	3.515	-6.891	92.651	772.992
6.	87686	412.4	3.250	3.250	89.510	3.224	-6.677	275.934	192.075

* Represents high potency to inhibit RACK1 protein and believed to be a promising inhibitor for therapeutic usage.

experimental results. Using structure based virtual screening and docking approach, identified six best ligand molecules showing better binding affinities to RACK1 protein and other molecular interactions within the active site of the stable receptor were also documented. The identified compounds were coded as 99542 and 33314 exerted promising and candidate molecules to inhibit activity of the Receptor of activated kinase 1 protein. Therefore, our findings confer significant novel inhibitors for RACK1 and it would serve as a new therapeutic alternative to cure small cell lung cancer, however, the result of the interaction profile required subsequent validation using in vitro studies.

Table 3
showing Binding free energy of identified compound sourced from Database.

Compound code	Docking score	MMGBSA dG Bind(NS)	MMGBSAdG Bind(NS) Coulomb	MMGBSAdGBind(NS) Hbond	MMGBSAdG Bind (NS) Lipo	MMGBSA dG Bind (NS) Solv GB	MMGBSAn dG Bind (NS) vdW
33314	-8.722	-97.432	-34.250	-2.007	-28.346	-26.182	-58.051
96874	-8.211	-100.296	-28.676	-1.201	-45.190	-36.094	-60.238
87686	-7.607	-85.037	-29.851	-1.977	-29.179	-37.845	-61.276

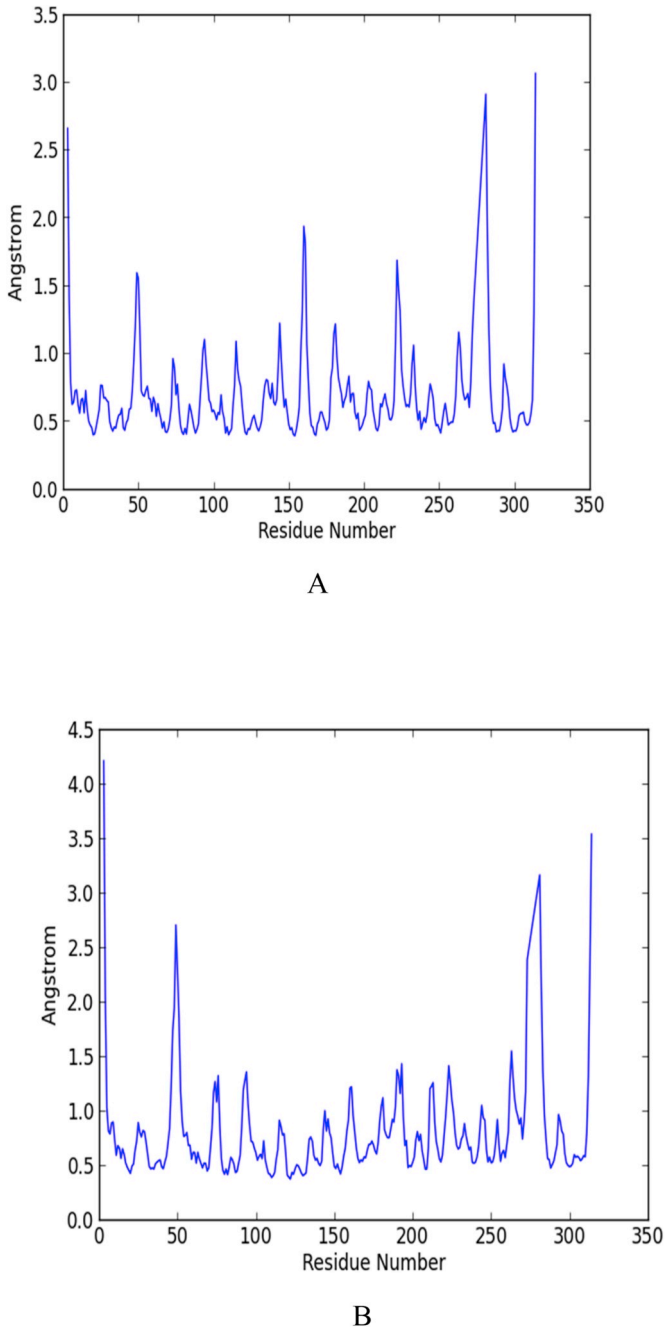


Fig. 6. The RMSF analysis of two complexes A) RACK1-99542, B). RACK1-33314.

Conflicts of interest

The authors have declared that there is no conflict of interest.

Acknowledgement and Funding

The authors would like to thank Alagappa University for financial support of RUSA – phase 2.0 grant sanctioned vide Letter No.F.24-51/2014-U policy (TNMulti – Gen), Dept of Edn. Govt of India Dt, 09.10.2018.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101301.

References

Cao, X.X., Xu, J.D., Xu, J.W., Liu, X.L., Cheng, Y.Y., Li, Q.Q., Xu, Z.D., Liu, X.P., 2011. RACK1 promotes breast carcinoma migration/metastasis via activation of the RhoA/Rho kinase pathway. Breast Canc. Res. Treat. 126 (3), 555–563.

Csukai, M., Mochly-Rosen, D., 1999. Pharmacologic modulation of protein kinase C isozymes: the role of RACKs and subcellularlocalisation. Pharmacol. Res. 39 (4), 253–259.

Li, X.Y., Hu, Y., Li, N.S., Wan, J.H., Zhu, Y., Lu, N.H., 2019. RACK1 acts as a potential tumor promoter in colorectal cancer. Gastroenterol. Res. Pract. 5625026.

Liew, F.Y., Pitman, N.I., McInnes, I.B., 2010. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat. Rev. Immunol. 10 (2), 103–110.

Nisha, Chandna, Kotni, Meena Kumari, Chetan, Sharma, Manga, Vijjulatha, Kapoor, Jitander K., Sharma, Pawan K., 2015. QM/MM docking strategy and prime/MM-GBSA calculation of celecoxib analogues as N-myristoyltransferase inhibitors. Virol. Mycol. 4 (1), 1–8.

Ron, D., Chen, C.H., Caldwell, J., Jamieson, L., Orr, E., Mochly-Rosen, D., 1994. Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. Proc. Natl. Acad. Sci. U S A. 91 (3), 839–843.

Schrodinger, L., 2013. Small-Molecule Drug Discovery Suite 2013–3: Glide, Version 6.1. Schrödinger, LLC, New York.

Shuo, Shi, Yue-Zhen, Deng, Jiang-Sha, Zhao, Xiao-Dan, Ji, Jun, Shi, Yu-Xiong, Feng, Guo, Li, Jing-Jing, Li, Di, Zhu, Koeffler, H. Phillip, Yun, Zhao, Dong, Xie, 2012. RACK1 promotes non-small-cell lung cancer tumorigenicity through activating sonic hedgehog signaling pathway. J. Biol. Chem. 287 (11), 7845–7858.

Siegel, R.L., Miller, K.D., Jemal, A., 2017. Cancer statisitcs, 2017. Ca - Cancer J. Clin. 67 (1), 7–30.

Thilagavathi, R., Mancera, R.L., 2010. Ligand-protein cross-docking with water molecules. J. Chem. Inf. Model. 50 (3), 415–421. https://doi.org/10.1021/ci900345h.

World Health Organization, 2017. Literature Review on the Health Effects of Smoke-free Policies in Light of the WHO FCTC. (Accessed 30 August 2017).

Yadav, D.K., Dhawan, S., Chauhan, A., Qidwai, T., Sharma, P., Bhakuni, R.S., Dhawan, O. P., Khan, F., 2014. QSAR and docking based semi-synthesis and in vivo evaluation of artemisinin derivatives for antimalarial activity. Curr. Drug Targets 15 (8), 753–761.

Yi, Yang, Na, Wu, Zhiyong, Wang, Fei, Zhang, Ran, Tian, Wei, Ji, Xiubao, Ren, Ruifang, Niu, 2016. Rack1 mediates the interaction of P-glycoprotein with Anxa2 and regulates migration and invasion of multidrug-resistant breast cancer cells. Int. J. Mol. Sci. 17 (10), 1718.