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In-Silico Docking studies of thymoquinone as potential anti-cancer drug target on Lung Cancer Cells

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Abstract: Understanding the inhibitory mechanism of thymoquinone targeting proteins involved in lung cancer. Thymoquinone were reported as possible anti-cancer drug targeting the gene containing protein like GTPase KRas in the cancer inducing pathways and we have used molecular docking in order to understand the underlying mechanism. The target proteins were preferred from various studies in cancer inducing pathways were docked by thymoquinone and also with its analogue poloxime. Protein-ligand complexes were selected based on the binding energy ranked from lowest to highest according to thymoquinone. Our results identified that thymoquinone inhibiting GTPase KRas, Sir-2, ALK5 and β-Catenin,. The docking study also establishes the multifaceted role of thymoquinone as a chemo preventive anti-cancer agent against lung cancer. These in-silico study report would be the substantial platform for the drugs to be focused in future for in-vitro and in-vivo studies for exploring its multitude function for providing a functional strategy for using as a therapeutic agent against cancer.

Keywords: Thymoquinone, Molecular docking, GTPase KRas, Poloxime

1. INTRODUCTION

Cancer is a disease involving abnormal cell growth would be invaded to other parts of the body. There are more than 100 different types of cancers are known to affect the human. Among that, the Lung cancer has been with the highest mortality rate in the world. The lung cancer is classifying into non-small lung carcinoma (NSCLC) 85% of the people are get affected (**1**). Whereas 35% of the people are affected by small-cell lung carcinomas (**2**).The progression of

disease is due to changes in signaling pathways such as extracellular signal regulated kinase (ERK)-MAPK cascade. In many cascade the mutated growth factor activates the oncogenic pathway and also a gene encodes a protein called B-Raf which alters the gene expression for lung malignant transformation (**3**).The abnormal activation of these signaling cascades leads to uncontrolled proliferation of cells, inhibition of apoptosis induces the oncogene pathway for increasing the severity of disease condition (**4**).As many therapeutic agents are available in treatment of cancers are high priced moreover also has toxicity effect over the noncancerous tissues. To overwhelm, the new therapeutic agents with cost effective & bioavailability nature of drug candidate are focused in therapeutic approaches.

Plants are the precious source with many phytochemical components its potential helpsto cure various diseases. *Nigella sativa* (Black cumin seeds) belongs to Ranunculaceae family which is an annual remarkable herbal plant contains more valuable benefits as pharmacological activity. Among the constituents of Black cumin seeds, thymoquinone plays a numerous pharmacological activities. In view of that, to explore the potential of this plant in chemo preventive treatment, the black cumin seed containing bioactive compound of thymoquinone are preferred.

Thymoquinone (IUPAC name:2-methyl-5-isopropyl-1, 4-benzoquinone) is the major active component in *N.sativa* exhibits anticancer activity was determined in various studies.The anticancer activity of thymoquinone reducing the progression of lung cancer mechanism is not clear, hence we focused on various target proteins in signal cascade pathway in lung by molecular docking studies for identifying its multitude function by assessing its binding energy and affinity which further helps to explore the mechanism of thymoquinone (**5**).

Based on the literature, thymoquinone shows a responsive medication for the treatment of lung cancer (**6 & 7**). Our proposed study identifies some fifteen proteins in the regulatory mechanism of tumor causative for lung cancers were examined with thymoquinone and its analogue poloxime through in-silico approach. Using the computational process of searching the ligand with its ability to fit both geometrically and energetically to the binding site of a protein using the molecular docking tools. The molecular docking results in finding the best orientation of the ligand interaction to the target protein of interest.

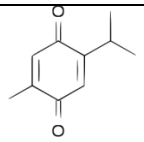
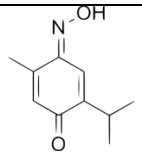
2. MATERIALS AND METHODS

All molecular modeling calculations were performed using AutoDock 4.2 (**8)** installed on a Linux environment.

Preparations of ligands for docking

Structures of ligands viz., thymoquinone and its analogue poloxime were obtained from PubChem ID (NCBI Pubchem) 10281 and 75393, respectively and converted into SDF files using ‘Online SMILES convertor and Structure file generator (**9**) as shown in **Table 1** Then, the energy minimization was carried out using Avogadro software with MMF force field. The minimization was accomplished using steepest descent algorithm with parameter of 200 steps at RMS gradient 0.1. Finally, minimized ligands were subjected to docking studies.

Table 1 Ligands used for Docking against the proteins in the pathway of lung cancer

S.NO.	LIGAND	MOLECULAR FORMULA	MOLECULAR WEIGHT (amu)	2-D STRUCTURE
1.	**Thymoquinone** (2-Isopropyl-5-methylbenzo-1,4-quinone)	$C_{10}H_{12}O_2$	164.204	
2.	**Poloxime** (2-Isopropyl-5-methyl-4-nitrosophenol)	$C_{10}H_{13}NO_2$	179.22	

Structure Preparation

The three-dimensional structure of fifteen various target receptor of lung cancer has been retrieved from the protein data bank and prepared for further study. Thymoquinone and its analogue (Poloxime) were targeted to the following protein based on the prediction with SWISS Target Prediction Tool. The following fifteen proteins as listed **Table 2** were carried for the structure preparation for molecular docking. These structures were further utilized to remove the co-crystallized ligand molecule and the leaving some residues the others were built manually. All water molecules were removed and hydrogen atoms were added to the molecule using Pymol (**10**). The structure needs to be energy minimized in order to remove bad contacts. Localized strain can be present due to small errors in the original structure such as bad Van der Waals contacts. The structures were refined by performing energy minimization in a vacuum assumption to relax from the strain. Steepest descent algorithm and Gromos96 force field of GROAMACS simulation package (**11**) was used for this refinement procedure and energy minimization was performed for 1000 steps. The optimized peptide molecules with partial atomic charges were used in further docking study to predict the preferred orientation of the molecules inside the receptor active site and also to identify the key residues involved in contact with the ligand molecules.

Table 2. List of proteins in the pathway of lung cancer with their PDB-ID.

S.No	PROTEIN	PDB ID
1	G12C Oncogenic Mutant of Human GTPase KRas	4LDJ
2	Human ALK2 kinase domain	6ACR
3	EGFR 696-1022 T790M/V948R	5X2F
4	BRAF Kinase Domain	5ITA
5	Sir2-p53	1YC5
6	Core/latch dimer of Bax in complex with BimBH3	4ZIE
7	p53 core domain	3ZME
8	RGS-HOMOLOGOUS DOMAIN OF AXIN	1DK8
9	β-Catenin	3OUX
10	Caspase-3	2XYG
11	ALK5	5FRI
12	PTEN Tumor Suppressor	1D5R
13	P53 cancer mutant Y220C	5AOI
14	MDM2/P53 Peptide	4HFZ
15	Human ROS1 Kinase Domain	4UXL

Molecular Docking Protocol

Binding mode and selectivity of target proteins with thymoquinone and its analogues (poloxime) were studied by Auto dock 4.2 (**12**) .AUTODOCK is a source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. Autodock can be regarded because of its utility for docking purpose by docking programs (**13**). Hydrogen atoms and the active torsions of peptide were assigned using AUTODOCK 4.2 tools and were docked into the binding site. The binding pockets for the docking studies were obtained from the literature studies (**14 & 15**). The binding site region was created within a range of distance 5 Å. Grid map were generated utilizing the autogrid program. The grid was focused in the centered of active site region, thus map was formed around the active site using 50 x 50 x 50 points and a grid spacing of 0.375 Å. In the current investigation, the docking parameters modified by creating a number of individuals in the Population (set at 150), maximum of 2500000 energy evaluations, maximum number of generations (set at 27,000), and number of GA runs (set at 100). The final structures were clustered and ranked according to the Auto dock scoring function. The docking results were compared with excellent conformational pose and high docking score were selected for analysis within the top clusters. The interactions and the binding affinities of the ligand targeting the lung cancer site for reducing protein-protein interaction were analyzed by the estimation of ligand binding energy, contact analysis, and clustering of docked poses **(16).**

3. RESULTS AND DISCUSSION

In order to reduce the progression of cancer, Thymoquinone was docked with various targets of lung cancer as enlisted in **Table 2**. The binding site of protein were identified and docked with thymoquinone with the binding energy (kJ/mol) of acceptable range between them. The same

docking protocol was carried out to identify the binding affinity of poloxime at the binding site of the targets. We have primarily focused on docking studies of thymoquinone along with these proteins to enumerate about binding capacity targeting the binding site over poloxime. Since, poloxime is an analogue of thymoquinone and many studies have not carried out with this synthetic analogue. The results were examined based on binding energy and H-bond interaction with the binding site residues. The binding energy for the docked proteins and the ligands shows less than -10 kJ/mol value which indicates that the scores is feasible and worth for further investigation. According to the binding energy, top four protein viz., G12C Oncogenic Mutant of Human GTPase KRas, Sir2-p53, ALK5 and B-catenin were taken to examine the result. From the result, thymoquinone recorded lowest value of binding energy with Human GTPase and poloxime with β-Catenin. The docking interaction of thymoquinone exhibited high binding affinity in target site of lung cancer protein with low dock score (-8.0 kJ/mol) compared to other proteins were shown in the **Table 3.** Based on the binding energy ranking form lowest to highest, the top four proteins with lowest binding energy were chosen for further investigation of the binding site residual interaction. The H-bond interactions measuring the distance between its donors and acceptors for various targets were shown in **Table 4.** The residual interaction of the selected four protein were discussed below and thus confirming that the thymoquinone plays a significantly role in decreasing the progression of lung cancer condition.

Table 3. The interaction of protein-ligand is ranked according to the binding energy of Thymoquinone.

S. No.	PROTEIN	BINDING ENERGY (kJ/mol)	
		THYMOQUINONE	POLOXIME
1	**G12C Oncogenic Mutant of Human GTPase KRas**	**-8.00**	**-7.55**
2	**Sir2-p53**	**-7.45**	**-6.56**
3	**ALK5**	**-7.38**	**-7.38**
4	**B-catenin**	**-6.9**	**-8.47**
5	EGFR 696-1022 T790M/V948R	-6.46	-6.97
6	BRAF Kinase Domain	-6.25	-7.45
7	Core/latch dimer of Bax in complex with BimBH3	-5.94	-7.12
8	p53 core domain	-5.72	-6.63
9	RGS-HOMOLOGOUS DOMAIN OF AXIN	-5.55	-6.69
10	Caspase-3	-5.12	-6.39
11	Human ALK2 kinase domain	-5.03	-6.53
12	PTEN Tumor Suppressor	-4.99	-6.36
13	P53 cancer mutant Y220C	-4.86	-4.70
14	MDM2/P53 Peptide	-4.72	-5.62
15	Human ROS1 Kinase Domain	-4.42	-5.64

G12C oncogenic mutant of Human GTPase KRas

The docking interaction of TQ exhibited highest binding affinity among the fifteen proteins at binding site of the human GTPase Kras causing lung cancer with -8.0 KJ/mol as shown in Table 3. The docking interaction of thymoquinone binding with G12C oncogenic mutant of Human GTPase KRas is shown in Figure 1. The key residues of G12C oncogenic mutant KRas protein

involves in high affinity binding with the oxygen of 4-benzoquinone of TQ by establishing hydrogen bond with Val14, Gly15 and Lys16 residues at a distance of 2.9 Å, 3.0 Å and 3.2 Å, respectively. Similarly, the oxygen of 4-benzoquinone of thymoquinone with Lys16 forms a conditional hydrogen bond at a distance of 2.8 Åto increase the stability of ligand-protein interaction.

Table 4. The hydrogen bond interaction with distance of TQ against the top four proteins docked.

S.NO.	PROTEIN	BINDING ENERGY (kJ/mol)	HYDROGEN BOND INTERACTIONS D-H…A	DISTANCE(Å)
1	GTPase KRas	-8.0	(Val -14) N-H…O (Gly -15) N-H…O (Lys -16) N-H…O (Lys -16) N-H…O	2.9 3.0 3.2 2.8
2	Sir^2 Protein	-7.45	(Ile-100) N-H…O (Phe-33) N-H…O	2.1 1.8
3	ALK 5	-7.38	-	-
4	Beta catenin	-6.9	-	-

. Additionally, the 2-Isopropyl of TQ stabilizes by hydrophobic interaction with Ala11, Pro34, and Cys12 residues which contributes for binding affinity and electrostatic interaction with Gly13 (at a distance 3.5 Å) and the oxygen of 1-benzoquinone of thymoquinone Tyr32 which inhibits the functions of mutant G12C oncogenic protein. The mutant KRas interacting with the other ligand at the catalytic site bearing Mg^{2+} ion is thus adhered due to the occupancy of TQ at the binding site. This creates strong evidence that the ligand significantly blocks the mutant activity of target protein & suggesting possible inhibition leading to suppression of progression of cancer.

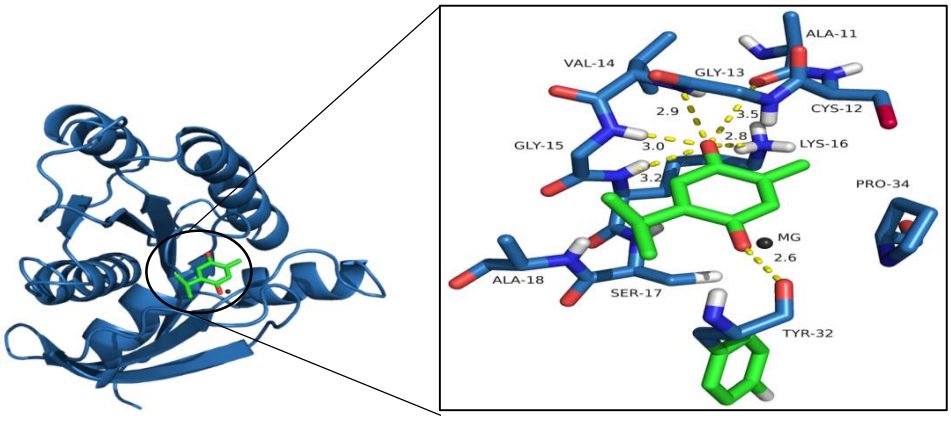


Figure 1. Docked Complex of thymoquinone (green) against G12C Oncogenic Mutant of

Human GTPase KRas (blue) occupying the binding pocket lined by bulky hydrophobic residues of the receptor.

Among the protein binding affinity with TQ synthetic analogue Poloxime, Human GTPase KRas shows binding energy of -7.55 kJ/mol and its interaction with binding residues is shown in **Figure 2**. The oxygen of 4-nitrosophenol of poloxime establishes hydrogen bond with the residues Gly10 and Val9 at a distance of 2.5Å and 2.3Å, respectively. Like TQ, the synthetic analogue poloxime also binds onto the same site of the target protein & exhibits the electrostatic interaction with the residue Gly 60 Arg68, Tyr71, His 95 and Tyr96. This provides information that the poloxime also has same role in the inhibition of Human GTPase KRas.

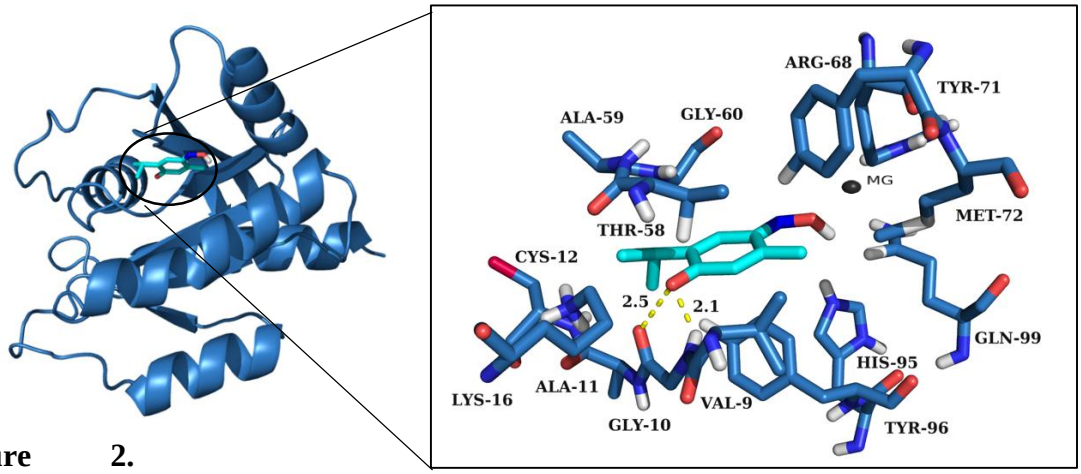


Figure 2. Docked Complex of Poloxime (cyan) against G12C Oncogenic Mutant of Human GTPase KRas (blue) occupying the binding pocket lined by bulky hydrophobic residues of the receptor similar to thymoquinone.

Sir2 Protein

The Sir2 protein docked with TQ shows binding energy of -7.45 kJ/mol and the interaction are shown in **Figure 3**. The oxygen of 1, 4-quinone of TQ binds onto the binding site residues Ile100 and Phe33 establishing hydrogen bond interaction with the distance of 2.1 Å and 1.8 Å, respectively. This interaction facilitates the drug-like activity to stimulate p53 action by locking the Sir2 protein (**17**). Further, the down regulating Sir2 Protein for reducing the progression of cancer in target site. Also generating other hydrophobic and electrostatic interaction of 2-Isopropyl of TQ with the residues Ala22, Ser25, Thr26, Ile30, Pro31, Asp32, and Asn99 which are found occupying at the binding catalytic site of receptor. These interactions significantly enhance the binding affinity of ligand towards the Sir2-protein. Hence this study, creates great evident that the TQ drug can be targeted on its site for reducing protein–protein interactions & exhibits its function very well by declining the progression of lung cancer.

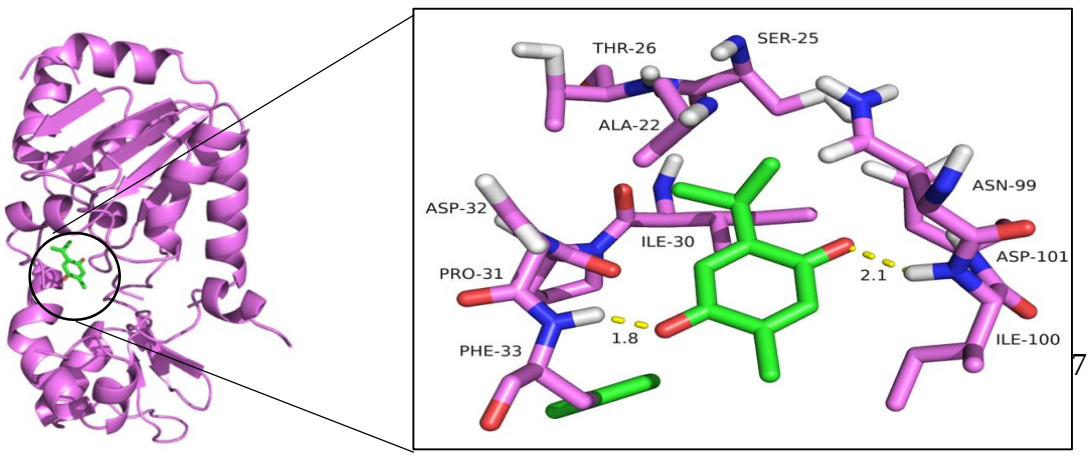


Figure 3. Docked complex of thymoquinone (green) interacting with the binding site-residues of Sir2 (pink).

ALK-5

Focusing, on the ALK-5 activity expression by binding of TQ with the binding energy of -7.38 kJ/mol as shown in Figure 4. The interaction pattern results that unlike other protein, ALK -5 does not establish any hydrogen bond interaction, even though it produced negative score binding energy. Interestingly, 2-isopropyl of TQ exhibits hydrophobic or electrostatic interaction with binding site residues between Gly214 and Asn338.

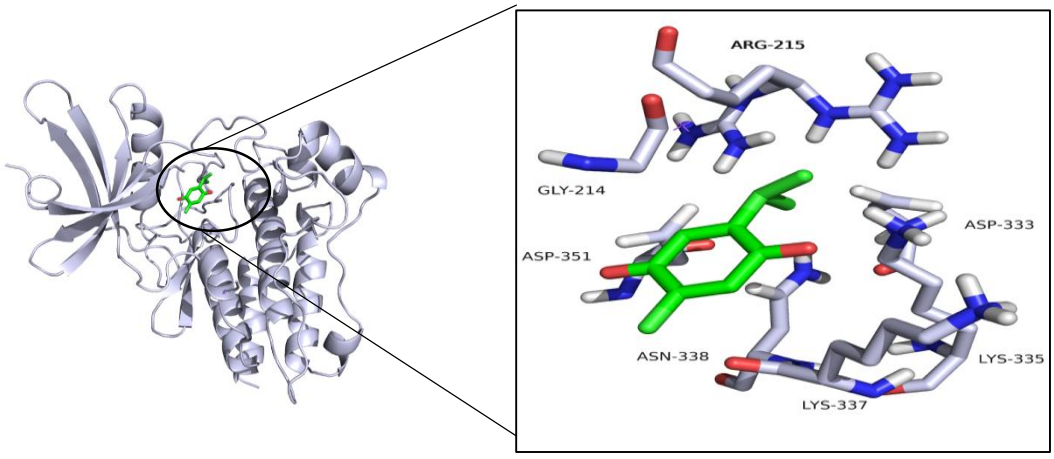
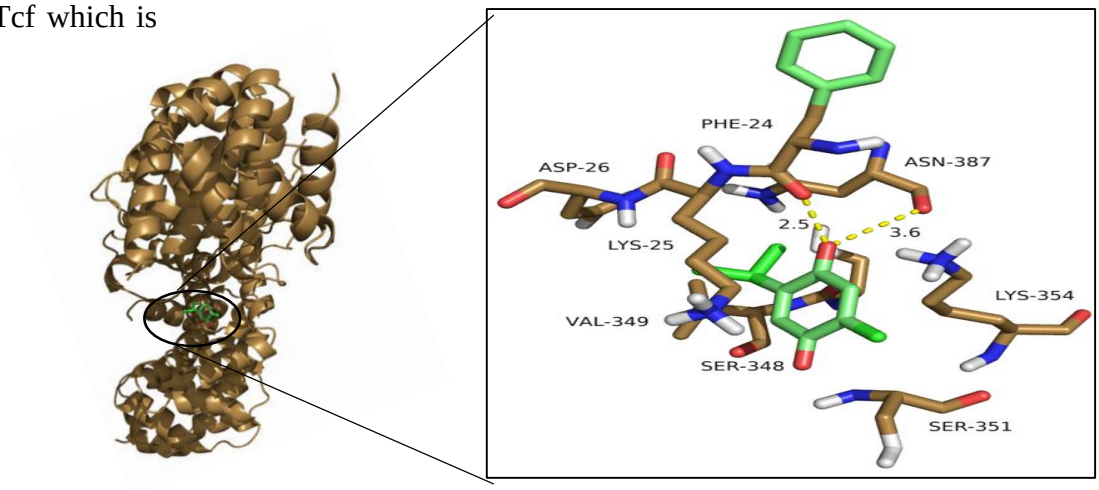


Figure 4. Docked Complex of ALK-5 (grey) with thymoquinone (green) which lacks to establish hydrogen bonding but gets fit into the binding cavities through the electrostatic interactions at the binding site of protein.

On the other, a group of residues Arg215, Asp351, Asp333, Lys337 & Lys335 stabilizes 5-methyl-1,4-benzoquinone of TQ between each other and involved in generating for some tight binding interaction with the drug-likeliness. These results would suggest that the ligand binds moderately to the transcription factor and potentially downplay the gene activity to block the progression of disease by occupying the catalytic site of the protein

Beta-Catenin

Finally, the docking study of TQ with β-Catenin shows a binding energy of -6.9kJ/mol. The binding site residues of beta catenin involved in interacting with TQ causing transcription cofactors is shown in Figure **5**. The ligand binds to the protein & generates electrostatic interaction among the binding site residues Phe24 and Asn387 with the oxygen of 1-benzoquinone of thymoquinone. Interaction of **β**-Catenin with strong binding affinity between Lef/Tcf which is the



transcriptional cofactor of target site makes the **β**-Catenin to be act as proto-oncogene (**18 & 19**). This strongly suggests that the drug-likeliness of TQ resolves the protein-protein interaction & inhibits the oncogene effect of **β**-Catenin & rejuvenate the action of **β**-Catenin as proto-oncogene.

Figure 5. Docked complex of thymoquinone (green) against β-Catenin (brown) occupying the binding pocket lined by bulky hydrophobic residues of the receptor.

Whereas, the docking interaction of Poloxime exhibited highest binding affinity among the fifteen protein at binding site of **β**-Catenin with -8.47 kJ/mol. The docking interaction of poloxime binding with **β**-Catenin is shown in **Figure 6**. Like TQ, the synthetic analogue also binds onto the same site of the target protein & exhibits the electrostatic interaction with Phe24 and Asn387 residues. This provides information that the poloxime also has same role in exhibiting help such as regulating the cell cycle & controls the rapid cell division in any manner by promoting the action of tumor suppressor gene, activating apoptosis process **(20).** The synthetic analogue poloxime interacts with the residues of β-Catenin in similar manner to that of TQ**.**

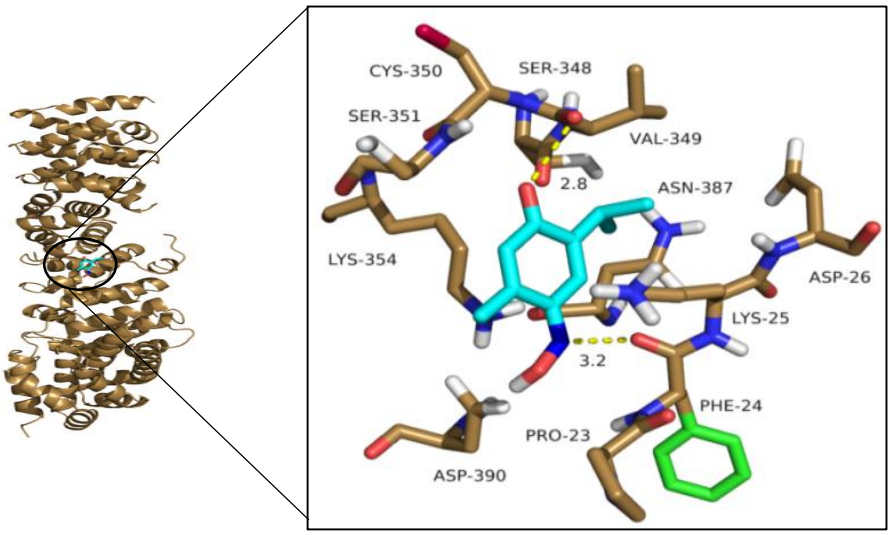


Figure 6. Docked complex of poloxime (cyan) against β-Catenin (brown) occupying the binding pocket lined by bulky hydrophobic residues of the receptor.

Only the highest and lowest the binding energy among the selected four protein-poloxime complex was studied for the residual interaction. Since the poloxime is the analogue, it's only

supportive information for the interaction pattern of TQ to the binding site of the target proteins. The docking study of Thymoquinone reveals the binding affinity of ligand interacting with protein, results in indicating that high negative scores reveals high possibility of inhibition of target protein. Thus, docking study, results suggests that the Thymoquinone & poloxime are found to be increase in binding affinity and efficiency thus can be used in therapeutic agents.

4. CONCLUSION

Based on the strength of interaction and binding energy from our docking study it has been clearly established that the anti-proliferative activity of Thymoquinone leads to the following

a) Inhibition of GTPase Kras leading to arrest activating in MAPK pathway.
b) Inhibition of Sir2 protein activates the P53 & brings the action of tumour suppressor gene leading to suppression of progression of cancer.
c) Inhibition of ALK5 & Beta catenin are also down regulates its activity by dissociating the protein –protein interaction.
d) The docking results clearly implicate the role of Thymoquinone as a p53 activator.

Further, the docking studies also establish the multifaceted role of Thymoquinone as a chemo preventive anti-cancer agent against lung cancer. These in-silico study report would be the substantial platform for the drugs to be focused in future for in-vitro and in-vivo studies for exploring its multitude function for providing a prudent strategy for using as a therapeutic agent against cancer disease.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

6. REFERENCES

[1] Gridelli C, Rossi A, Carbone DP, et al. Non small-cell lung cancer.Nat Rev Dis Primers.2015; 1:15009.
[2] Abacioglu, UfukYumuk, PerranCaglar, Hale Sengoz ,MericTurhal, Nazim Concurrent chemoradiotherapy low dose weekly gemcitabine in stage III non-small lung cancer .BMC cancer vol 5(1):71.2005
[3] Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer.Oncogene.2007;26 (22):3291-310.
[4] Connie Yip, Charlotte Dinkel, Abhishek Mahajan, MusibSiddique,GaryJ.R.Cook, & Vicky Goh, Imaging body composition in cancer patients:visceral obesity, sarcopenia and sarcopenic obesity may impact on clinical outcome. Insights Imaging (2015) 6(4):489-497
[5] .H.U.Gali-Muhtasib, W.G.AbouKheir, L.A.Kheir, N.Darwiche, and P.A.Crooks, "Molecular pathway for thymoquinone –induced cell cycle arrest and apoptosis in neoplastic Keratinocytes," Anti-cancer Drugs, vol.15, no.4, pp.389-399, 2004
[6] Deng CX SIRT 1, is it a tumor promoter or tumor suppressor? Int J Bio Sci. 2009; 5(2):147-152.
[7] Gao Y, Duran S, Lydon JP, DeMayo FJ, Burghardt Rc, Bayless KJ, Bartholin L, Li Q(February 2015)."Constitutive activation of transforming growth factor Beta receptor 1in the mouse uterus imparts uterine morphology and function "Biology of Reproduction.92 (2).34.
[8] D. Seeliger and B. L. de Groot, "Ligand docking and binding site analysis with PyMOL and Autodock/Vina," J. Comput. Aided. Mol. Des., vol. 24, no. 5, pp. 417–422, 2010

[9] Fabliha Ahmed Chowdhury Md Kamal Hossain, A.G.M.Mostofa, Maruf Mohammad Akbor and Muhammad Shahdaat Bin Sayeed (2018). Therapeutic Potential of Thymoquinone in GiloblastomaTreatment:Targeting Major Gliomagenesis Signaling Pathways. Biomed research International.

[10] Norgan, A. P., Coffman, P. K., Kocher, J. P. A., Katzmann, D. J., & Sosa, C. P. Multilevel parallelization of AutoDock 4.2. *Journal of cheminformatics*, 2011, *3*(1), 12.

[11] Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of cheminformatics*, 2012, *4*(1), 17.

[12] Tao J, Calviski DF, Ranganathan S, Cigliano A, Zhou L, Singh S, Jiang L, Fan B, Terracciano L, armeanu-Ebinger S. Ribback S, Dombrowski F, Evert M, et al .Activation of Beta catenin and Yap 1 in human hepatoblastoma and induction of hepatocarcinogenesis in mice. Gastroenterology, 2014; 147:690-701

[13] Lemkul, J. From Proteins to Perturbed Hamiltonians: A Suite of Tutorials for the GROMACS-2018 Molecular Simulation Package [Article v1. 0]. *Living Journal of Computational Molecular Science*, 2019, *1*(1), 5068 .

[14] BenardinaNdreshkjana, AysunCapci, Volker Klein, PithiChanvorachote, Julienne K.Muenzer, KerstinHuebner,SaraSteinmann,KatharinaErlenbach- Wuensch,CarolI.Geppert, Abbas Agaimy, Farah Balluot, Chirine El-Baba, HalaGali-Muhtasib, Adriana Vial Roehe, Arndt Hartmann, Svetlana B.Tsogeva, and Regine Schneider – stock. Combination of 5-Fluorouracil and thymoquinone targets stem cell gene signature in colonrectal cancer cells. Cell Death & Disease (2019)

[15] Samantha Messina , Small GTPase RAS in multiple sclerosis - exploring the role of RAS GTPase in the etiology of multiple sclerosis, Small GTPases, 2020, 11:5, 312-319,

[16] Goudie DR, D'Alessandro M, Merriman B, et. al. Multiple self-healing squamous epithelioma is caused by a disease- spectrum of mutations in TGFBR1. Nat Genet.2011; 43(4):365-369.

[17] Vogelstein B, Lane D, Levine AL. Surfing the P53 network. Nature. 2000;408(6810):307-310

[18] Khramtsov AI, Khramtsov GF, Tretiakova M, HuoD.OlopadeOI,Gross KH. Wnt/beta-catenin pathway activation is enriched in basal like breast cancers and predicts poor outcome. Am J Pathol 2010:176; 2911-2920.

[19] DamskyWE, Curley DP, Santhanakrishnan M, Rosenbanum LE, Platt JT, Gould Rothberg BE, Taketo MM. DankortD.Rimm DL. McMahon M, Bosenberg M, Beta catenin signaling controls metasis in Braf-activated pten-deficient melanomas.cancer Cell.2011; 20:741-754.

[20] Tao J, Calviski DF, Ranganathan S, Cigliano A, Zhou L, Singh S, Jiang L, Fan B, Terracciano L, armeanu-Ebinger S. Ribback S, Dombrowski F, Evert M, et al .Activation of Beta catenin and Yap 1 in human hepatoblastoma and induction of hepatocarcinogenesis in mice. Gastroenterology, 2014; 147:690-701.