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DOCKING STUDIES OF NOVEL 1, 2, 4- TRIAZOLE CONTAINING 1*H*-INDOLE-2, 3-DIONE ANALOGUES AS ANTICANCER PROPERTIES

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ABSTRACT

Cancer is a disease characterized by uncontrollable, irreversible, independent, autonomous, uncoordinated and relatively unlimited and abnormal over growth of tissues. 1*H*-Indole-2, 3-Dioneis an important class of heterocyclic compound which possess interesting biological activities like anti-cancer, anti-microbial, anti-fungal, anti-tubercular, anti-malarial, anti-convulsant, anthelmintic, anti-viral, analgesic and anti-inflammatory activity. The drugs containing triazole groups were the first effective chemotherapeutic agents which were systematically proved for the prevention and cure of bacterial infection in human beings. For the treatment of cancer, Indoleamine 2, 3-dioxygenase (IDO) is emerging as an important new therapeutic drug target characterized by pathological immune suppression. Recent understanding of the molecular pathophysiology of cancer have highlighted that many tyrosine kinases are found upstream or downstream of epidemiologically relevant oncogenes or tumor suppressor, in particular the receptor tyrosine kinases. The present research study is focused on the design of 1*H*-Indole-2, 3-Dione analogues containing triazole, docking against the known anti-cancer targets like indoleamine 2, 3-dioxygenase (IDO) and EGFR tyrosine kinase. Twenty Five compounds of 1*H*-Indole-2, 3-Dione analogues were designed and docked against indoleamine 2, 3-dioxygenase (IDO) and EGFR tyrosine kinase using Auto dock (version 4.2). Among the docked compounds five compounds (Tri1, Tri6, Tri12, Tri16, and Tri20) were showed a significant docking score against target enzyme compared to the standard. The present study concluded that 1*H*-Indole-2, 3-Dione analogues will be a significant lead for further investigation of anti-cancer properties.

KEYWORDS: 1*H*-Indole-2, 3-Dione, 1, 2, 4-Triazole, indoleamine 2, 3-dioxygenase, EGFR tyrosine kinase, Anti-cancer properties.

INTRODUCTION

The global burden of cancer continues to increase largely because of aging and growth of the world population. Based on the GLOBOCAN estimates, about 12.7 million cancer cases and 7.6 million cancer deaths have occurred in 2008.[1] The development of new anticancer agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to develop more potent molecules with higher specificity and reduced toxicity.

Isatin's are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry.

Isatin (1H-indole-2,3-dione) is an endogenous compound, identified in humans and possesses a wide range of biological activities such as anxiogenic, sedative, anticonvulsant activities and acts as a potent antagonist on atrial natriuretic peptide receptors *in vitro*. Derivatives of isatin have been reported to have cytotoxicity against human carcinoma cell lines derived from breast, prostrate, human acute lymphoblastic leukemia (MOLT-4), colon and lung.[2]

IDO is an intracellular enzyme that catalyzes tryptophan at the initial and rate-limiting step. Evidence for an immunosuppressive function of IDO was first documented in the mouse placenta, where IDO prevents rejection of the allogeneic fetus during pregnancy. Subsequent studies have clarified the mechanisms of IDO immunosuppression in tumors. First, IDO expressed by tumor cells depletes tryptophan locally and produces a toxic tryptophan catabolite kynurenine, which causes growth arrest and the apoptosis of effector T-cells or natural killer (NK) cells that are extremely sensitive to tryptophan shortage, and also suppresses their killer functions. Secondly, IDO expressed by antigen-presenting Dendritic Cells (DCs) within tumor-draining lymph nodes induces tolerance to tumor-derived antigens. Lastly, IDO expressed by plasmacytoid DCs plays a critical role in conversion of CD4+CD25- T cells intoCD4+CD25+ Foxp3+ regulatory T (Treg) cells, directly activating mature Tregs. These findings suggest that IDO, in cooperation with Treg (and possibly MDSC and immunosuppressive cytokines), induces the immune tolerogenic microenvironment, which leads to tumor progression.[3]

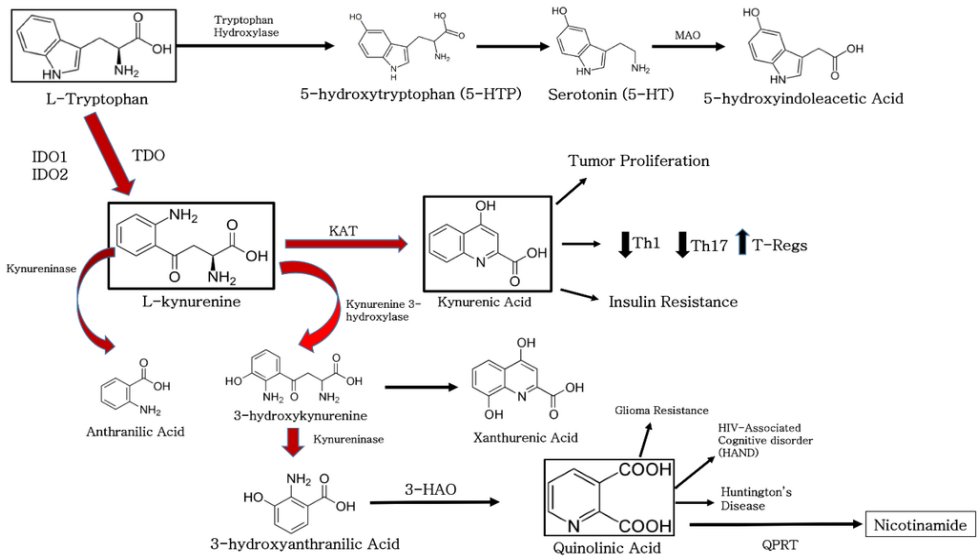

Figure No.1: Kynurenin pathway of tryptophan catabolism in mammalian cells.

Protein kinases were proved to be a viable target for anticancer drug development Kinases are involved in many pathophysiological problems especially cancers where their overexpression can lead to different types of malignancies. In addition, EGFR-TK is one of the most important kinases that plays a fundamental role in signal transduction pathways. EGFR and its ligands, epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) have been implicated in numerous tumors of epithelial origin Therefore, the design of inhibitors that target EGFR-TK is an attractive approach for the development of new therapeutic agents. Gefitinib and erlotinib were approved as EGFR-TK inhibitors for the treatment of non-small cell lung cancer.[4]

The 1,2,4-triazole(4H) derivatives, which are used in this study, play an important role in medicinal chemistry. Although they have been known from long ago to be biologically active, their varied biological features are still of great scientific interest. Their derivatives have a variety of biological properties, such as anti-microbial and anti-inflammatory, antidepressant, antifungal, anti- convulsant, and antitumor propertie. Due to their antitumor effect, 1,2,4-triazole(4H) derivatives are attracting more interest. Until now, there have been only a few computational studies on 1,2,4-triazole(4H) derivatives; also, the protein targets of 1,2,4-triazole(4H) derivatives have not yet received a great deal of attention.[5]

The Objective of the study is too carried out the docking studies of 1*H*-Indole-2, 3-Dione analogues containing 1,2,4-triazole groups with known anti-cancer targets like indoleamine 2, 3-dioxygenase (IDO), EGFR tyrosine kinase by using Auto dock programmes.

MATERIALS AND METHODS

Preparation of protein structure

The crystal structure of the indoleamine 2, 3-dioxygenagse 1 (PDB ID: 5ETW) in complexed with NLG919 analogue and the crystal structure of EGFR tyrosine kinase (PDB ID: 5HIC) domain mutant "TMLR" with a imidazo pyridinyl-amino pyrimidine inhibitor analogue (Fig. 9) solved by X-ray crystallography at 2.30Å was retrieved from the Protein Data Bank (http://www.pdb.org/pdb/home/home.do). Energy minimization of all 3D structure of proteins by Chimera 1.6.1.

Preparation of ligand structures

Marvinsketch is a tool for drawing chemical structures, adding or deleting functional group or atoms, queries and reactions.

Molecular Docking

The twenty five ligands were drawn in Marvinsketch assigned with proper 3D orientation and the structure of each compound was analyzed for connection error in bond order.

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The docking was done by using Autodock software. Then the pre-screened ligands were validated using Autodock version 4.2 which is more efficient. In Autodock, the proteins were refined by removing water molecules and polar hydrogen's and kollmann charges were added. Grid box for docking simulations were constructed with 50 points in x, y, and z direction to be centered in the active site using Autogrid utility of the Autodock programme. The target ligand complex was subjected to 2.5 million evaluations. The binding energies are compared with the docking score of the standard ligands, Rosmarinic acid and Gefitinib.

RESULT AND DISCUSSION

Docking Analysis

The docking scores were obtained from the analogues against indoleamine 2, 3-dioxygenagse 1 and EGFR tyrosine kinase receptors. The output of all ligands was given by energy values in kcal/mol as shown in Table 1. All the compounds show good docking scores when compared to standard drugs. Docking score of the compounds targeted indoleamine 2, 3-dioxygenagse 1receptor was compared with the score of the drug **Rosmarinic** which is used as a potent drug for the tumoral immune tolerance and docking score of the compounds

targeted EGFR tyrosine kinase was compared with the score of the drug **Gefitinib** which is used as drugs to treatment of cancer. In Auto dock, Tri 6 shows the highest docking score than the standard drugs against the receptor IDO 1 and EGFR tyrosine kinase than the standard drugs, **Rosmarinic** and **Gefitinib.** Next comes, all the 24 analogues with high docking score against the receptor IDO 1 Triazole derivatives were docked with the crystallographic structures of the targets by Autodock version 4.2 screening programme as shown in Table 1. The analogues were examined for their binding energies and hydrogen bonding. The conformations with highest binding energies and greater number of hydrogen bonds of all the ligands were taken in consideration for ranking the analogues. All the analogues show higher docking scores when compared to standard drugs. Tri1, Tri6, Tri12, Tri16 and tri20 shows higher docking scores with both IDO and EGFR tyrosine kinase receptors. Studies have proved that compounds showing good tumoral immune tolerance can also be considered as good agents for anti cancer therapy. The interactions were stronger (energetically lesser) for all the ligands which are used for docking simulation.

TABLE No. 1: Binding Energy and Inhibition Constant of the compounds and the standard drug.

S.No	Compound code	5ETW		5HIC	
		Binding Energy (kJ mol^{-1})	Inhibition Constant (µM)	Binding Energy (kJ mol^{-1})	Inhibition Constant (µM)
1.	**Tri 1**	-11.21	6.03	-5.81	55.43
2.	**Tri 2**	-6.37	21.03	-5.71	65.77
3.	**Tri 3**	-7.04	6.9	-6.08	35.13
4.	**Tri 4**	-7.42	3.66	-5.96	42.82
5.	**Tri 5**	-6.43	19.34	-5.93	45.35
6.	**Tri 6**	-11.68	2.73	-7.59	2.72
7.	**Tri 7**	-7.14	5.84	-5.49	94.25
8.	**Tri 8**	-6.95	8.09	-5.03	204.97
9.	**Tri 9**	-7.14	5.84	-6.36	21.81
10.	**Tri 10**	-6.67	12.88	-5.62	76.41
11.	**Tri 11**	-5.71	65.48	-5.99	40.62
12.	**Tri 12**	-10.43	22.69	-7.48	3.3
13.	**Tri 13**	-6.55	15.84	-5.52	90.58
14.	**Tri 14**	-6.93	8.32	-4.98	222.02
15.	**Tri 15**	-10.64	15.8	-8.56	532.15
16.	**Tri 16**	-10.97	9.17	-7.43	3.59
17.	**Tri 17**	-5.06	196.31	-7.34	4.17
18.	**Tri 18**	-3.72	1.88	-5.63	74.27
19.	**Tri 19**	-4.36	642.07	-6.33	22.92
20.	**Tri 20**	-10.6	17.1	-6.32	23.37
21.	**Tri 21**	-6.94	8.14	-6.35	22.15
22.	**Tri 22**	-5.99	40.54	-3.81	4.64
23.	**Tri 23**	-3.93	1.32	-7.29	4.52
24.	**Tri 24**	-6.86	9.33	-6.75	11.29
25.	**Tri 25**	-6.94	8.12	-5.53	88.11
26.	**Rosmarinic acid**	-5.14	169.43	----------	----------
27.	**Gefitinib**	----------	----------	-5.08	188.13

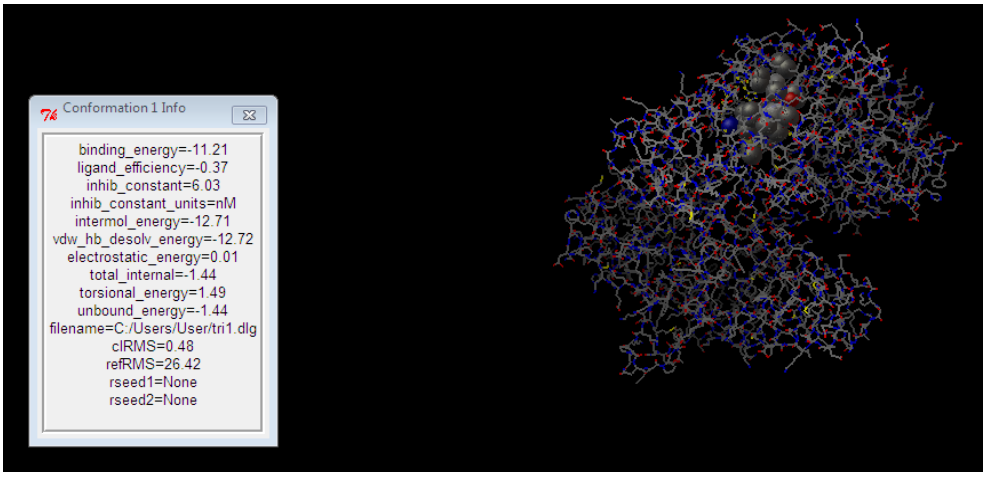

Figure No. 2a: Compound Tri 1 docked at the receptor of indoleamine 2, 3-dioxygenagse 1.

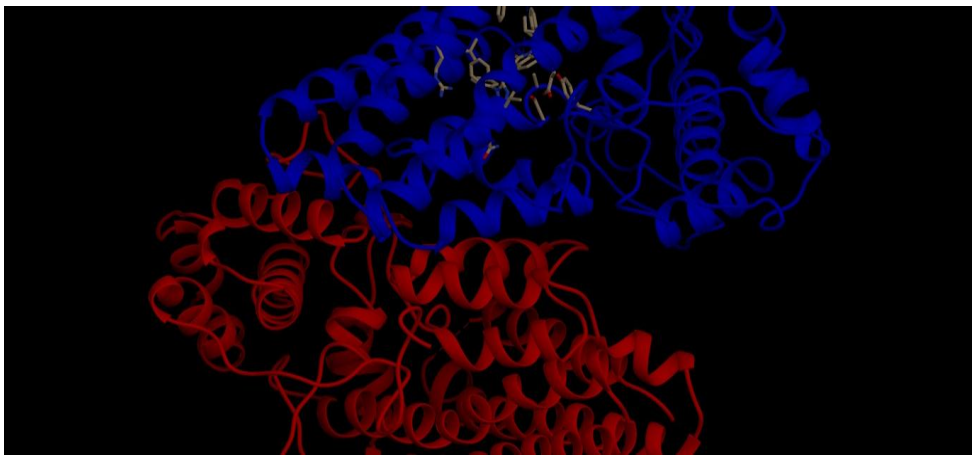

Figure No. 2b: Compound Tri 1 docked at the receptor of indoleamine 2, 3-dioxygenagse 1 in chimera view.

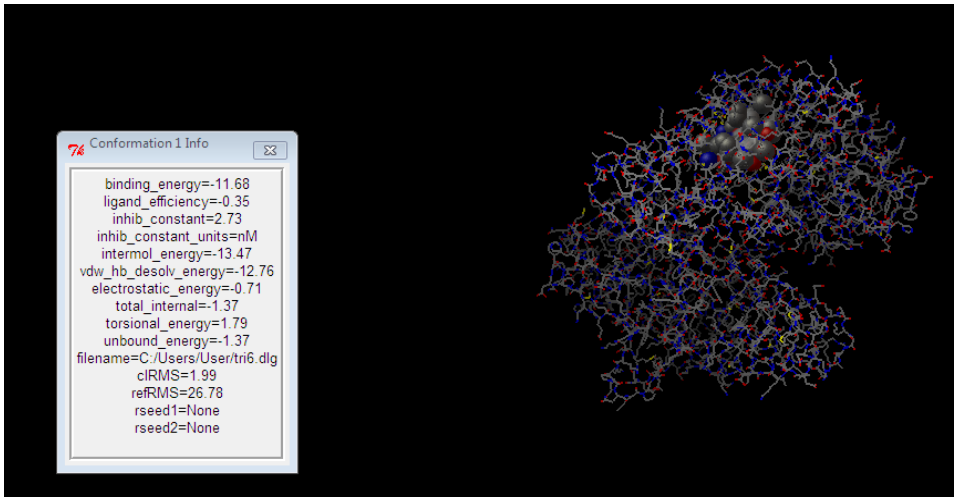

Figure No. 3a: Compound Tri 6 docked at the receptor of indoleamine 2, 3-dioxygenagse 1.

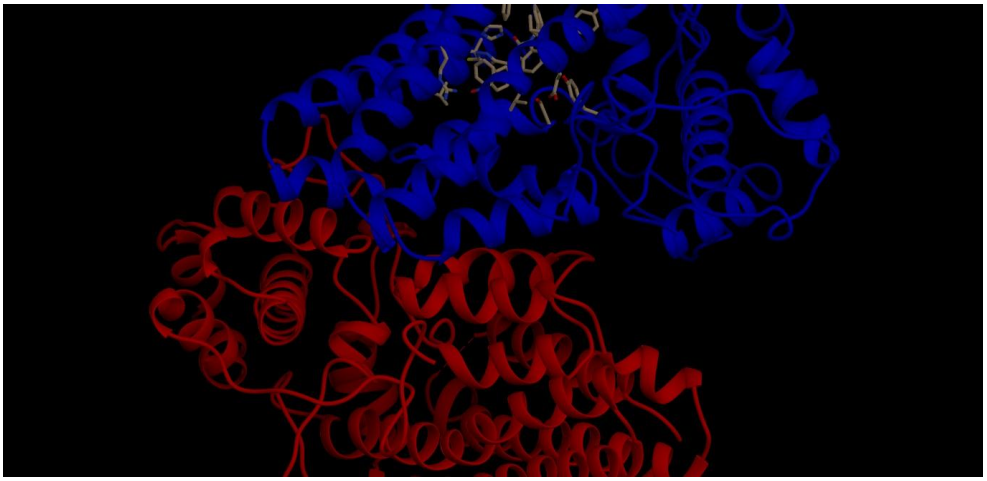

Figure No.3b: Compound Tri 6 docked at the receptor of indoleamine 2, 3-dioxygenagse1 in chimera view.

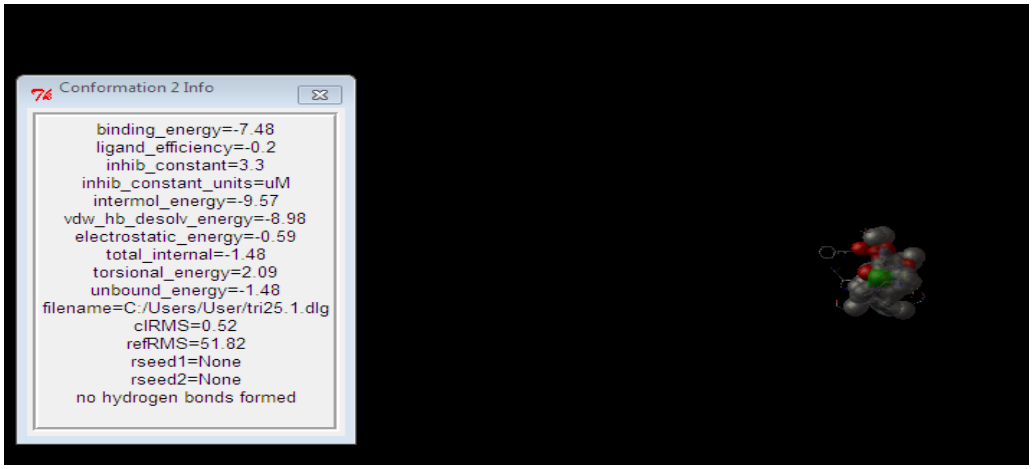

Figure No.4a: Compound Tri 12 docked at the receptor of EGFR–TK.

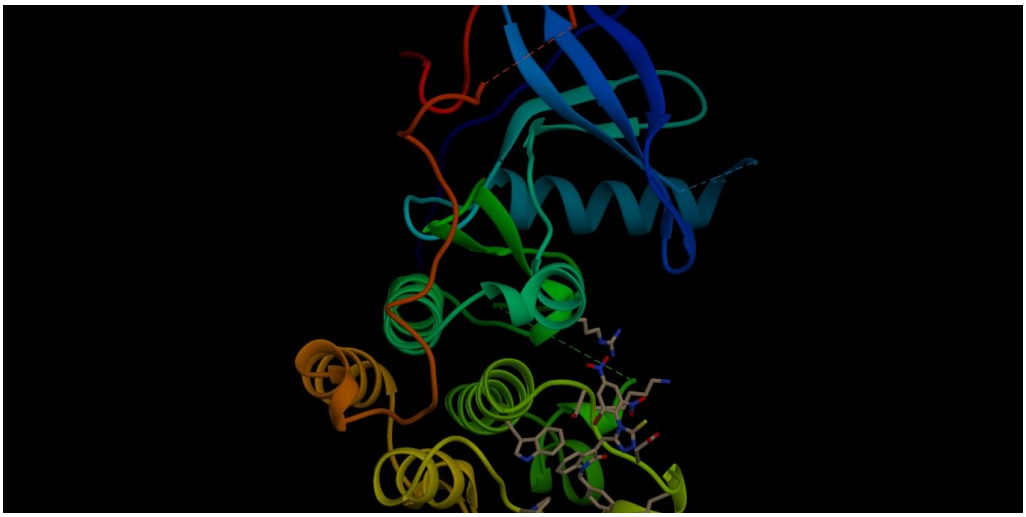

Figure No.4b: Compound Tri 12 docked at the receptor of EGFR–TK in chimera view.

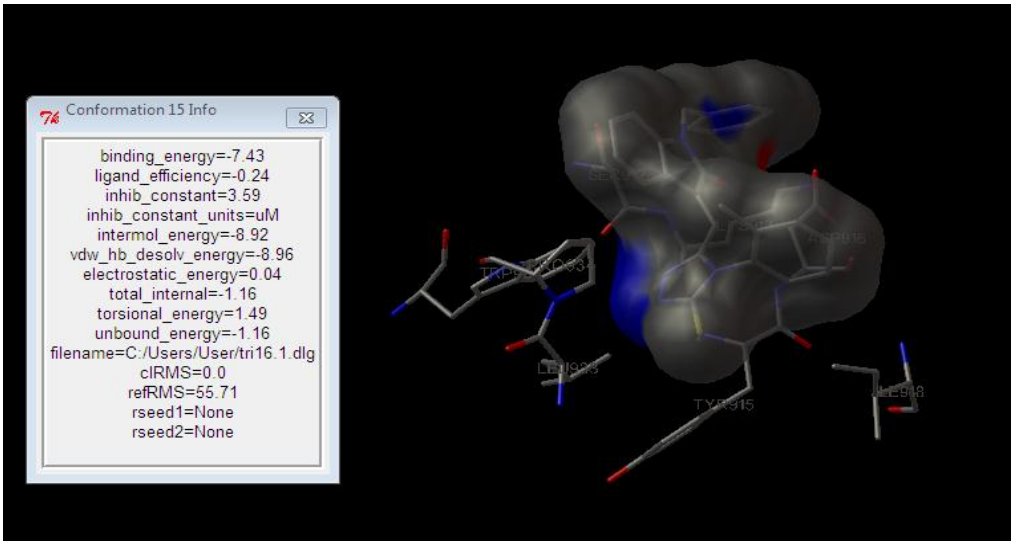

Figure No.5a: Compound Tri 16 docked at the receptor of EGFR–TK.

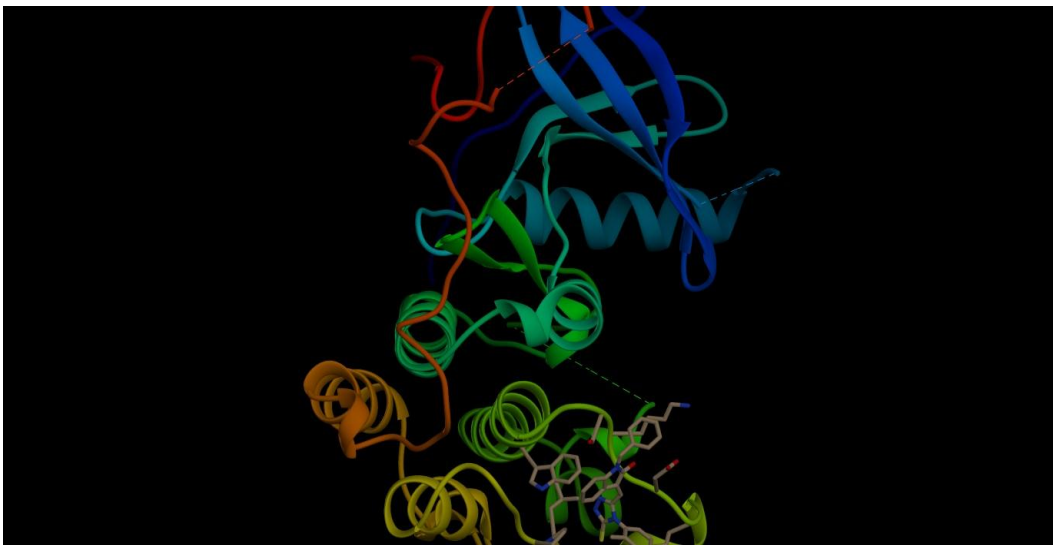

Figure No.5b: Compound Tri 16 docked at the receptor of EGFR–TK in chimera view.

Validation of Ligands

QSAR and toxicity studies was performed to obtain the molecular properties of all ligands as shown in Table 2. QSAR studies reveals that all ligands was passed and acted as a drug molecule by their adherence to the properties such as Absorption, Distribution, Metabolism and Excretion (ADME) as per the Lipinski Rule Of 5. The results shows that all the values of analogues were relays within the optimal range. Also the compounds have molecular weight less than 500 Daltons and number of hydrogen bond donors and hydrogen bond acceptors of all the analogues is below 5 and 10 respectively.

All the values of partition coefficient and number of rotatable bonds were coming under the limit of 5. All these data indicates that the analogues shows no more violations likely to be an orally active drug.

TABLE No. 2: Analysis of Lipinski rule of 5 for the novel proposed analogues.

S.No	Compound code	Molecular weight	No. Of Hba	No. Of Hbd	ClogP	No. Of Rot.b	n violation
1.	**Tri 1**	410.49	5	0	4.41	4	0
2.	**Tri 2**	444.94	5	0	5.28	4	1
3.	**Tri 3**	444.94	5	0	5.28	4	1
4.	**Tri 4**	444.94	5	0	5.09	4	1
5.	**Tri 5**	489.39	5	0	5.22	4	1
6.	**Tri 6**	455.49	8	0	4.37	5	0
7.	**Tri 7**	455.49	8	0	4.37	5	0
8.	**Tri 8**	455.49	8	0	4.37	5	0
9.	**Tri 9**	424.52	5	0	4.86	4	0
10.	**Tri 10**	440.52	6	0	4.47	5	0
11.	**Tri 11**	479.38	5	0	5.91	4	1
12.	**Tri 12**	489.93	8	0	5.08	5	0
13.	**Tri 13**	489.93	8	0	5.18	5	1
14.	**Tri 14**	500.49	8	0	4.47	6	2
15.	**Tri 15**	581.39	8	0	5.59	5	2
16.	**Tri 16**	424.52	5	0	5.02	4	0
17.	**Tri 17**	453.56	6	0	4.51	5	0
18.	**Tri 18**	467.59	6	0	5.10	5	1
19.	**Tri 19**	438.54	5	0	5.63	4	1
20.	**Tri 20**	513.83	5	0	4.72	4	0
21.	**Tri 21**	647.18	5	0	7.12	4	2
22.	**Tri 22**	524.38	8	0	6.00	5	2
23.	**Tri 23**	534.93	8	0	5.28	6	3
24.	**Tri 24**	613.28	8	0	6.26	5	2
25.	**Tri 25**	579.38	8	0	5.42	6	3

CONCLUSION

Rigid docking of ligand to receptor molecules is an emerging approach and is extensively used to reduce cost and time in drug discovery. In this study the approach utilized is successful in finding potent inhibitors against indoleamine 2, 3-dioxygenagse 1 and EGFR tyrosine kinase receptors. All the compounds show lowest docked energy and hydrogen bonding stabilizes the interactions. The analogues of 1, 2, 4 - Triazole showed good receptor binding with the selected targets. Heterocyclic triazole ring is having tumoral immune tolerance and anti-cancer activity.

The final assessment of drug-likeness and its related parameters helps to confirm the oral activity of compounds. The study concluded that all 1, 2, 4-triazole derivatives will be significant lead for further investigation of anti-cancer and immunomodulater activity.

Future studies are needed to elucidate the structures of synthesized compounds and finally screen for their in-vitro anti-cancer effect. This study could be utilized for the designing of effective drug for the treatment of cancer.

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