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Identification of Novel Phenylquinazoline dervatives as EGFR inhibitors by using Insilico tools and Techniques

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Abstract

Epidermal Growth Factor Receptor (EGFR) is a member of the EGFR/HER family of receptor tyrosine kinases (RTKs) plays an important role in normal organogenesis and in neoplastic processes of cell proliferation, inhibition of apoptosis, angiogenesis, and metastatic spread. EGFR expression is frequent in Non-Small Cell Lung Cancers (NSCLC) and over expression is observed in 32-79% of NSCLC patients. In the present study, attempts are made to identify ligands with Phenylquinazoline moiety having better inhibition of EGFR using computational methods. A set of 27 molecules are designed and docked with the EGFR protein. ADME and Toxicity studies are performed by using Discovery Studio 2.5. 11 ligands like 1, 2, 3, 6, 7, 9, 13, 17, 19, 24 and 25 have shown better Dock score when compared to gefitinib, a marketed potent EGFR inhibitor, in which Ligand-1, N-(4-bromo-2-fluorophenyl)-6-methoxy-7-((1-methyl-1,2-dihydropyridin-4-yl)methoxy)quinazolin-4-amine is having highest Dock score of 62.131. Ligands like 1, 2 and 24 is having better docking scores and the results of Toxicity studies also supported this ligands having better drug-likeness properties, modifications to these ligands may result in better ligands than gefitinib.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide. The two major types of lung cancers are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Among these, NSCLC

accounts for more than 85% cases. Epidermal growth factor receptor (EGFR) belonging to receptor tyrosine kinases is often over expressed in NSCLC, ranging from 43% to 89%. Moreover, EGFR increased expression strong correlates with disease progression and poor prognosis. EGFR [1,2] is a transmembrane glycoprotein with an extracellular ligand-binding domain and an intracellular domain having tyrosine kinase (TK) activity. Ligand binding [3] leads to receptor dimerization resulting in activation of TK domain by autophosphorylation, which further phosphorylates intracellular substrates for downstream signaling. Currently approved EGFR inhibitors for the treatment of cancer include: cetuximab, a monoclonal antibody which blocks the extracellular ligand binding domain and gefitinib and erlotinib, both of which are competitive inhibitors of adenosine triphosphate (ATP) of the receptor's tyrosine kinase. However, a point mutation in the EGFR gene confers resistance to gefitinib. In this study a set of 27 molecules were designed and docked with the EGFR protein to evaluate the best fit molecule. ADME and Toxicity studies were also performed and compared with the currently available inhibitor Gefitinib. Our results revealed that Ligand 1 (N-(4-bromo-2-fluorophenyl)-6- methoxy-7- ((1-methyl-1, 2-dihydropyridin -4-yl) methoxy) quinazolin-4-amine) is having highest docking score of all.

METHODOLOGY

Selection of target protein

There are several PDB structures available for the EGFR protein; The PDB structure selected for our study has a better resolution of 2.50. For the prediction of the binding mechanism, PDB structure (PDB ID: 1MOX) of Human Epidermal Growth Factor Receptor was chosen [4].

Design of Ligands

27 ligands were designed keeping Phenylquinazoline as common moiety and modifications are done at R^1, R^2, and R^3 positions. All the ligands were designed by using Accelrys Symyx Draw 4.0. These ligands were designed according to the SAR properties of EGFR inhibitors. Major considerations during designing of ligands were given to increase the hydrophobic character of the molecule which was shown in Fig: 1. Modifications were given in the Table: 1

Protein Preparation

The ligands, ions and the crystallographic water molecules were removed from the protein and missing Hydrogens were added. Crystallographic disorders and unfilled valance atoms were corrected using alternate conformations and valance monitor options. Then the protein structure was subjected to energy minimization (Moleclucar Mechanics) using the CHARMm 27 Force field and RMS Gradient of 0.001[5].

Ligand Preparation

27 Ligands having common Phenylquinazoline moiety were designed by using Accelrys Symyx Draw 4.0 and subjected to geometrical optimization where the 2D moleclues convert to their least possible energy state 3D conformer structure. This energy minimization process is done by using CHARMm 27 force field. Smart Minimizer, a specialized minimization algorithm designed for Discovery studio is used to perform Geometrical Optimization of ligands [6].

ADME and Toxicity prediction

ADME properties of ligands were predicted by using "ADME Descriptors" protocol of Discovery Studio 2.5 where it predicts Intestinal absorption[7], Aqueous solubility[8] – drug likeness, Blood-Brain Barrier (BBB) penetration[9], Plasma protein binding (PPB)[10], Hepatotoxicity[11] (Dose- dependent).

Toxicity profile of each ligand was identified by using "TOXICITY PREDITION – EXTENSIBLE" protocol of Discovery Studio. Probability for Aerobic Biodegradability, Developmental Toxicity potential, Mutagenicity, carcinogenicity and ocular & skin irritancy were studied.

Molecular Properties

Molecular properties like surface area, volume, hydration energy, log p, mass, refractivity, polarizability, was studied by using Hyperchem 8.0[12, 13], for all the newly designed 27 ligands and listed in Table: 2.

Protein-ligand Interactions

All 27 ligands taken for the study were subjected to dock within the active site of EGFR using Ligand Fit docking program available with Discovery Studio 2.5. The method employs a cavity detection algorithm for detecting invaginations in the protein candidate active site regions. For docking Number of Monte Carlo steps was set to"2 500 120,4 1200 300,6 1500 350,10 2000 500,253000 750" with maximum 10 number of poses. The determination of the ligand binding affinity was calculated using Ligscore and PLP1, JAIN and Dock score were used to estimate the ligand-binding energy.

RESULTS & DISCUSSIONS

Toxicity profile

Toxicity profiles of ligands are studied by using "TOXICITY PREDITION – EXTENSIBLE" protocol of Discovery Studio. Probability for Aerobic Biodegradability, Developmental Toxicity potential, Mutagenicity, carcinogenicity and ocular & skin irritancy were studied [14]. Listed in the Table: 3

All the 27 ligands are not showing any mutagenicity and skin irritancy. All the 27 ligands are showing ocular irritancy. Ligands like 3, 6, 8, 10, 13 and 15 are showing aerobic biodegradability. 4, 17 and 25 ligands are showing developmental toxicity potential. Nearly 9 ligands are skin sensitizers. Ligands 3, 4, 5, 6, 7, 8, 9, 10, 11 and 20 are showing carcinogenicity. Ligands like 1, 2, 14, 16, 24, 26 and 27 are showing only ocular irritancy and they are not exhibiting any other toxicity.

Docking studies

All 27 ligands taken for the study were subjected to dock within the active site of EGFR using Ligand Fit docking program available with Discovery Studio 2.5. The method employs a cavity detection algorithm for detecting invaginations in the protein candidate active site regions. The determination of the ligand binding affinity was calculated using Ligscore and PLP1, JAIN and Dock score were used to estimate the ligand-binding energy.

Docking results are tabulated in the Table: 4. The analogue which is having the highest docking score is having the highest binding affinity.

From the table, ligand 1 is having the best docking score of 62.131. Ligands like 1, 2, 3, 6, 7, 9, 13, 17, 19, 24 and 25 are having better docking score compared with gefitinib the score was 41.3481, a standard marketed drug. Binding modes of gefitinib, ligands 1, 2 and 3 are visualized in Fig. no: 2, 3, 4 & 5. Ligands 1, 2 and 24 are having better dock score compared to gefitinib and they are showing only ocular irritancy modifications to these ligands may result in better ligands than gefitinib. Ligands like 16, 10, 22, 11, 27, 20, 21 and 15 are having the least Dock scores.

CONCLUSION

Present study was conducted to design and identify the potent epidermal growth factor receptor (EGFR) inhibitors for the treatment of lung cancer using InSilico tools and techniques. The interactions between EGFR and the ligands were studied by using Ligand Fit docking program available with Discovery Studio 2.5. Based on dockscores docking results were analyzed. The results were compared to gefitinib to find out the best ligand which can inhibit EGFR. The overall review of results concludes that ligands 1, 2, and 24 have shown better properties when compared to all other ligands with no toxic profile. These ligands have also shown the highest dock-scores when compared to gefitinib and other analogues. Ligand 1 is having the highest docking score compared to all other ligands. Further development and synthesis of these ligands may lead to be as better drugs for blocking EGFR in treatment of lung cancer.

Table 1: newly designed phenylquinazoline derivatives

Ligand	R^1	R^2	R^3
1	4-(methoxymethyl)-1-methylpiperidine	dimethyl ether	2- F, 4-Br
2	4-(methoxymethyl)-1-methylpiperidine	Hydroxyl group	2- F, 4-Br
3	4-(methoxymethyl)-1-methylpiperidine	dimethyl ether	2- F, 4-Cl
4	4-(methoxymethyl)-1-methylpiperidine	dimethyl ether	2- F, 4-Cl
5	4-(methoxymethyl)-1-methylpiperidine	dimethyl ether	2- F, 4-F
6	4-(methoxymethyl)-*N*-methylcyclohexanamine	dimethyl ether	2- F, 4-Cl
7	4-(methoxymethyl)-*N*-methylcyclohexanamine	dimethyl ether	2- F, 4-F
8	4-(methoxymethyl)-1-methylpiperidine	dimethyl ether	2-bromo-6-fluoropyridine
9	4-(methoxymethyl)-1-methylpiperidine	4-(methoxymethyl)-1-methylpiperidine	2- F, 4-Cl
10	4-(methoxymethyl)-1-methylpiperidine	Hydroxyl group	2-Cl, 3-F
11	4-(methoxymethyl)-1-methylpiperidine	*N*-(furan-2-ylmethyl)-2-(methylsulfonyl)ethanamine	3-prop-1-yne
12	4-(methoxymethyl)-1-methylpiperidine	4-(3-methoxypropyl)morpholine	2-F, 4-Cl
13	-H	4-(methoxymethyl)-1-methylpiperidine	3-F, 4-Cl
14	4-(methoxymethyl)-*N* methylcyclohexanamine	1,3-dimethoxypropane	4-prop-1-yne
15	1-ethoxy-2-methoxyethane	1-ethoxy-2-methoxyethane	2-Br, 4-Cl
16	1-ethoxy-2-methoxyethane	1,3-dimethoxypropane	2-F, 4-Cl
17	4-(methoxymethyl)pyridine	-H	3-F,4-3-fluorobenzyl methyl ether
18	4-(methoxymethyl)pyridine	4-(methoxymethyl)-1-methylpiperidine	2-Br, 4-Cl
19	4-(methoxymethyl)pyridine	dimethyl ether	3-prop-1-yne
20	4-(methoxymethyl)pyridine	1-ethoxy-2-methoxyethane	3-Cl, 4-Br
21	1,3-dimethoxypropane	-H	2-Br, 4-Cl
22	4-(3-methoxypropyl)morpholine	ethyl methyl ether	3-Cl,4-3-chlorobenzyl methyl ether
23	1,3-dimethoxypropane	ethyl methyl ether	4-2-F, 4-C
24	4-(3-methoxypropyl)morpholine	dimethyl ether	2-F, 3-Br
25	*N*-(furan-2-ylmethyl)-2-(methylsulfonyl)ethanamine	dimethyl ether	3, 5-Cl
26	*N*-(furan-2-ylmethyl)-2-(methylsulfonyl)ethanamine	1,3-dimethoxypropane	3,5-dichlorobenzyl methyl ether
27	4-(methoxymethyl)-*N*methylcyclohexanamine	1,3-dimethoxypropane	2-Cl, 4- prop-1-yne

Table: 2: Molecular properties of 27 ligands using Hyperchem 8.0

Ligand	M.Wt	M. Mass	M. Solubility	pKa	H. Acceptors	H. Donors	M.Volume	M. Surface Area	Energy
1	457.296	456.06	-5.669	6.55667	6	2	270.96	387.31	162.13
2	426.871	426.126	-6.031	8.595	6	1	277.82	396.2	155.86
3	430.903	430.157	-6.039	9.31	6	1	284.68	403.59	135.42
4	414.448	414.187	-5.477	7.295	6	1	269.94	388.96	136.61
5	444.93	444.173	-6.434	9.7	6	1	297.03	418.89	137.53
6	428.475	428.202	-5.867	7.685	6	1	288.8	404.26	138.74
7	476.342	475.102	-5.905	5.83667	7	1	292.92	414.83	131.12
8	462.315	461.086	-5.183	6.005	7	2	276.11	395.59	127.78
9	460.343	459.107	-6.411	4.75	6	1	282.97	401.7	125.26
10	462.315	461.086	-5.144	5.19	7	2	271.65	395.59	134.55
11	528.061	527.246	-6.911	9.12333	7	1	365.63	500.79	136.61
12	400.877	400.147	-6.096	5.155	5	1	261.7	371.04	107.7
13	559.679	559.225	-7.134	5.2525	8	3	365.98	533.79	207.74
14	581.077	580.225	-8.456	7.295	8	1	388.27	547.74	160.51
15	528.061	527.246	-6.838	5.66333	7	1	359.12	499.43	143.91
16	400.877	400.147	-6.021	5.01	5	1	260.33	371.04	123.98
17	579.503	578.169	-9.797	7.295	6	1	375.24	515.64	165.19
18	458.592	458.257	-7.045	5.8	5	1	327.56	455.36	167.54
19	536.886	535.112	-9.564	2.07	5	1	349.85	484.02	182.11
20	524.835	523.087	-7.978	11.28	7	1	331.68	471.65	141.6
21	602.538	601.206	-7.223	7.69	8	1	388.27	538.74	147.82
22	470.47	470.155	-8.184	2.89	6	1	295.32	425.3	184.02
23	568.893	567.104	-8.704	6.27	7	1	359.12	496.33	183.1
24	382.415	382.143	-5.77	5.03	6	1	247.3	366.98	158.3
25	529.813	528.056	-8.428	5.735	7	1	323.44	461.64	151.48
26	602.538	601.206	-7.223	7.69	8	1	388.27	538.74	147.82
27	471.322	470.075	-6.371	6.58	6	1	286.74	406.55	155.89

Table 3: Toxicity profile of 27 ligands using "TOXICITY PREDITION – EXTENSIBLE" protocol of Discovery Studio

Ligand	Aerobic Bio-Degradailability	AMES Mutagenicity	Developmental Toxicity Potential	Ocular Irritancy	Skin Irritancy	Skin Sensitizer	Carcinogenicity				
							Rodent	Female Mouse	Male Mouse	Female Rat	Male Rat
1	No	No	No	Yes	No	No	No	No	No	No	No
2	No	No	No	Yes	No	No	No	No	No	No	No
3	Yes	No	No	Yes	No	Yes	yes	No	No	No	No
4	No	No	Yes	Yes	No	No	No	yes	No	No	No
5	No	No	No	Yes	No	No	No	yes	No	No	No
6	Yes	No	No	Yes	No	No	No	yes	No	No	No
7	No	No	No	Yes	No	No	No	yes	No	No	No
8	Yes	No	No	Yes	No	No	No	yes	No	No	No
9	No	No	No	Yes	No	No	No	yes	No	No	No
10	Yes	No	No	Yes	No	No	No	yes	No	No	No
11	No	No	No	Yes	No	Yes	No	yes	No	No	No
12	No	No	No	Yes	No	Yes	No	No	No	No	No
13	Yes	No	No	Yes	No	No	No	No	No	No	No
14	No	No	No	Yes	No	No	No	No	No	No	No
15	Yes	No	No	Yes	No	No	No	No	No	No	No
16	No	No	No	Yes	No	No	No	No	No	No	No
17	No	No	Yes	Yes	No	No	No	No	No	No	No
18	No	No	No	Yes	No	Yes	No	No	No	No	No
19	No	No	No	Yes	No	Yes	No	No	No	No	No
20	No	No	No	Yes	No	Yes	yes	yes	yes	yes	yes
21	No	No	No	Yes	No	Yes	No	No	No	No	No
22	No	No	No	Yes	No	Yes	No	No	No	No	No
23	No	No	No	Yes	No	Yes	No	No	No	No	No
24	No	No	No	Yes	No	No	No	No	No	No	No
25	No	No	Yes	Yes	No	No	No	No	No	No	No
26	No	No	No	Yes	No	No	No	No	No	No	No
27	No	No	No	Yes	No	No	No	No	No	No	No

Table 4: Docking results of 27 ligands using ligand Fit docking program available with Discovery Studio 2.5.

S.No	Ligands	Dockscore (-)	Rotl bonds	Internal –energy
1	1	62.131	6	-3.044
2	19	61.832	7	-6.346
3	17	56.149	8	-5.532
4	24	53.208	8	-3.477
5	13	53.051	5	-4.711
6	3	51.084	6	-7.086
7	25	49.208	8	-3.476
8	9	48.704	8	-9.261
9	2	47.94	6	-0.054
10	7	46.308	7	-1.054
11	6	44.244	7	-2.226
12	18	40.982	8	-10.293
13	5	39.691	6	-5.868
14	4	38.038	6	-6.014
15	23	36.095	12	-4.681
16	8	35.979	6	14.6
17	14	35.637	11	5.591
18	12	25.007	8	7.958
19	26	20.751	15	9.018
20	16	19.002	13	-7.815
21	10	18.179	5	0.079
22	22	16.835	12	14.516
23	11	14.854	12	9.356
24	27	14.829	15	2.166
25	20	13.001	10	-3.35
26	21	10.034	11	10.544
27	15	7.245	14	8.009

Fig 1: positions of modifications on Phenylquinazoline moiety

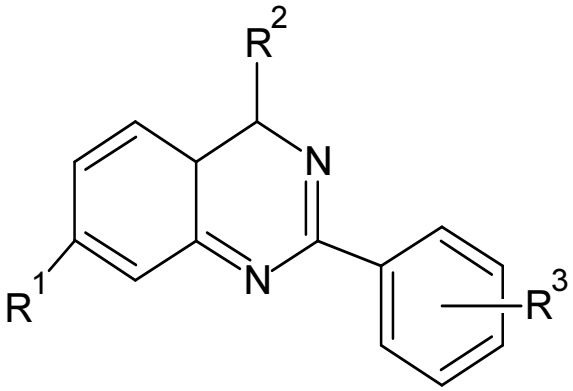


Fig 2: Docking studies of Gefitinib

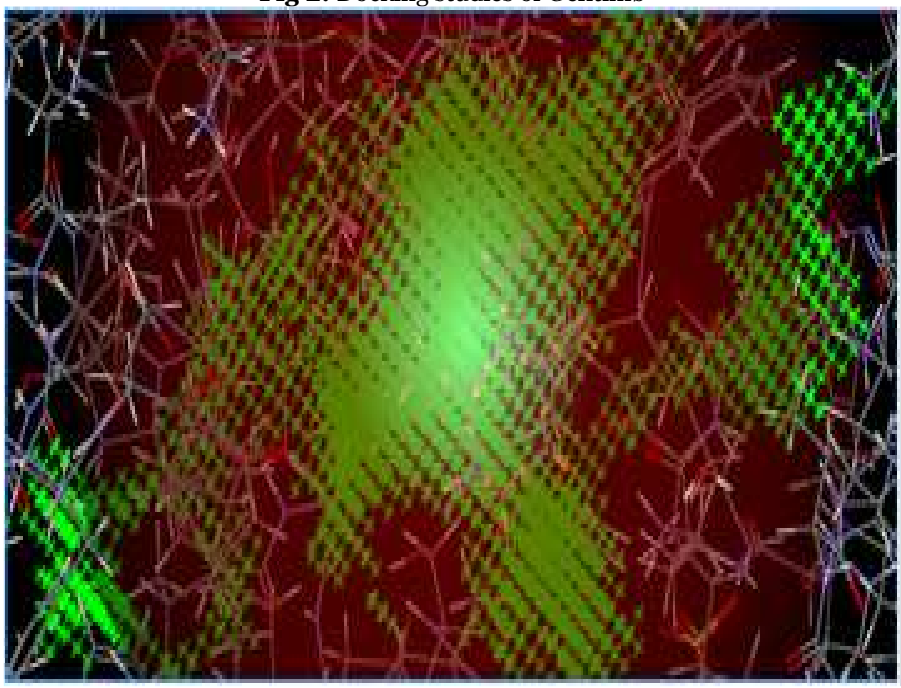


Fig 3: Docking studies of ligand 1

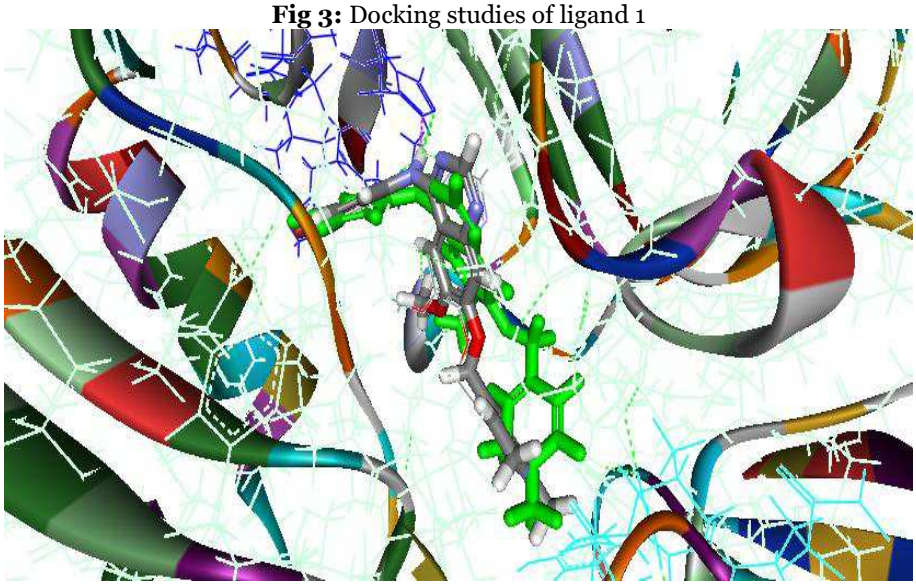


Fig 4: Docking studies of ligand 2

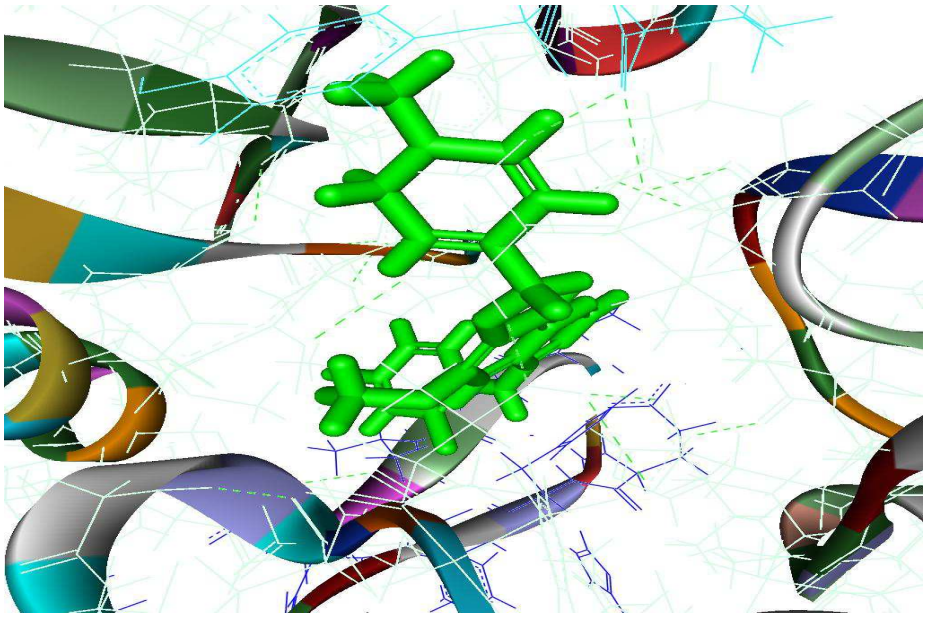
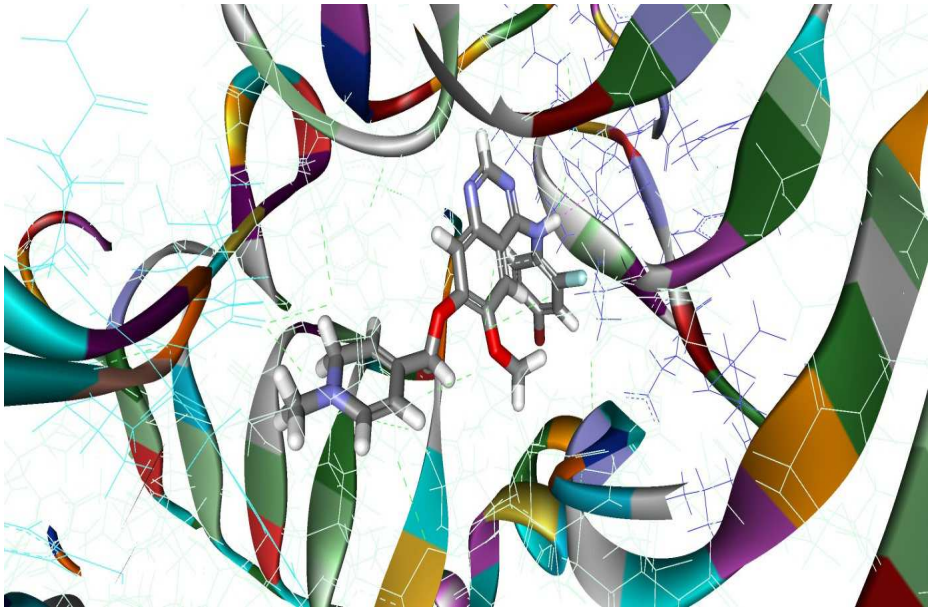


Fig 5: Binding interactions of ligand 3



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