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COMPARATIVE DOCKING AND MUTATIONAL STUDIES CKIT AND PDGFR A INHIBITORS IN GASTROINTESTINAL STROMAL TUMORS

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ABSTRACT

***Background*:** Gastrointestinal stromal tumor (GIST) is a type of sarcoma found in the digestive system, most often in the wall of the stomach. Some GISTs are not cancerous (they are benign.) But they can become cancerous if not treated. Mutually exclusive KIT and PDGFRA mutations are central events in GIST pathogenesis. Mutations in *KIT* can be found in about 80% of GISTs. However, about 10% of GISTs have a normal *KIT* gene (wildtype) but show mutations in the gene for *PDGFRA*. Mutations in the *KIT* gene that are relevant for GISTs are found in exons 9, 11, 13 and 17.Mutations in the *PDGFR* gene that are relevant for GISTs are found in exons 12,14 and 18. Response of GIST patients to tyrosine kinase inhibitors varies by the specific mutation displayed by their tumors. Targeting receptor tyrosine kinases by tyrosine kinase inhibitors (TKIs) thereby blocking kinase domain prevents the phosphorylation of the receptor at TK domain and interferes with cell proliferation, differentiation, migration, and survival and induces cell apoptosis. ***Method*:** Thirty six Tyrosine kinase inhibitors were targeted against the c-KIT protein. The first fifteen TKIs that had good binding affinity were docked with the mutatnts developed (D816V, K642E,V654A) and the results of these TKIs were compared. Twenty Tyrosine kinase inhibitors were targeted against kinase domain of PDGFRA protein. The first seven TKIs that had good binding affinity were docked with the mutants developed(D842V, D842Y) and the TKIs were compared based on the results.

KEYWORDS: Gastrointestinal stromal tumor (GIST),KIT,PDGFRA,Mutation.

INTRODUCTION

Gastrointestinal stromal tumors (GIST)

Gastrointestinal stromal tumors are the most commonly occurring mesenchymaltumors.[1] 70% of GISTs occur in stomach, while 20% in small intestine and less than 10% in esophagus. GISTs are usually benign, but if untreated becomes cancerous.[2] Mutually exclusive cKIT and PDGFRA mutations are responsible for GISTs. 85% of the cases are a result of mutations in cKIT gene, 10% of the cases are associated with mutations in PDGFRA gene and less than 5% are due to wild type which donot harbor either cKIT or PDGFRA mutations.[3] cKIT and PDGFRA are transmembrane receptors whose ligands are stem cell factor(scf) and platelet derived growth factor(PDGF) respectively. Ligand binding leads to dimerisation of the receptors and ultimately results in cell proliferation.[4] Mutations in these receptors lead to their constitutive activation which means ligand binding is not necessary. This results in unlimited cell proliferation, forming tumors and then cancer. The drug responses differ with the exon where the mutations occur. Some of the mutations are resistant to the drugs imatinibmesylate and sunitinib while the other mutations are sensitive to them.

Ckit

85% of GISTs occur as a result of cKIT pathway. Mutations in exon9(10-18%), exon11(50-77%), exon13(1-4%), exon17(1-4%) that occur in GISTs are associated with cKIT gene.[6] Mutations responsible for GIST most often occur in exon 11 which codes for the intracellular domain that acts as the tyrosine kinase domain. cKIT is now an important target in therapy for GIST.[5] The K642E mutation occurring in the juxtamembrane domain results in elevation of kinase activity. The K642E is a primary mutation and is sensitive to imatinibmesylate and sunitinib.[6][7] The V654A mutation is stem cell factor dependent for activation and results in enhanced cell proliferation.[8] The D816V mutation occurring in activation loop of kinase domain results in enhanced mast cell survival and cell proliferation due to constitutive activation.[9] V654A and D816V are secondary mutations acqiured during course of therapy and are resistant to the drugs imatinibmesylate and sunitinib.

PDGFRA

Platelet derived growth factor receptor has two isoforms-alpha and beta. The alpha protein is encoded by PDGFRA gene. It is a transmembrane receptor and upon activation after binding of ligand(PDGF), it regulates cell proliferation. The mutations in exons 12, 14 and 18 are known to cause tumor formation and then cancer.[10] The D842Y is a primary mutation and is

sensitive to imatinibmesylate. The D842V mutation accounts for about 70% of PDGFRA mutations and this occurs in the activation loop of the protein. This is a secondary mutation acquired during the course of therapy and is resistant to some commercially available tyrosine kinase inhibitors.[11]

Tyrosine kinase inhibitors of type 1

Tyrosine kinase inhibitors(TKIs) are classified into type I, type II, typeIII. The typeI TKIs compete with the ligands for ATP-binding site in tyrosine kinase domain of the protein, while type II and type III are non-ATP competitors. Tyrosine kinases are an important target for therapy right now. The inhibitors discussed here are of type I of tyrosine kinase inhibitor. They compete with the ATP binding site of the catalytic domain of cKIT and PDGFRA thereby preventing tyrosine phosphorylation. They are orally active, small molecules that have a favorable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. Several tyrosine kinase inhibitors (TKIs) have been found to have effective antitumor activity and have been approved or are in clinical trials.

Methodology

Previous studies on GIST suggested that certain mutations in cKIT and PDGFRA are resistant to the drugs imatinibmesylate and sunitinib which have been approved by FDA. Primary mutations in cKIT such as K642E, V654A are sensitive to these drugs while the secondary mutation D816V is resistant. Frequently occurring D842V mutation in PDGFRA is also resistant to these drugs. So, we have targeted 36 Tyrosine kinase inhibitors of class 1 against cKIT protein to find ligands other than these drugs to which both primary and secondary mutations are sensitive. Also, we have targeted 24 ligands against PDGFRA to find ligands to which D842V is sensitive. For energy minimization of protein and ligands Accelrys Discovery studio was used. MOE was used for docking studies. These tyrosine kinase inhibitors compete for the ADP binding sites on cKIT and PDGFRA with their ligands.

Protein preparation

For this study, cKIT protein structure was retrieved from the protein databank of NCBI database which is a repository for 3D structural data of large biological molecules such as proteins and nucleic acids. The best protein was selected from the list of proteins in PDB, based on the Ramachandran plot analysis(the number of residues in favourable region). Structure of a cKIT kinase product complex(1PKG) was retrieved from PDB. Since the

structure of PDFRA is not known, homology modeling was performed for building the 3D structure of kinase domain. The sequence of kinase domain was retrieved from Uniprot database which has protein sequence and functional information.Build Homology Models protocol was used which uses MODELLER in Accelrys Discovery studio for building the 3D structure of kinase domain of PDGFRA. DS enables to develop high quality homology models automatically. Using Accelrys Discovery studio protein preparation was done for cKIT and PDGFRA. It generates reports of the loaded protein, identifying the potential problems, automatically fixing, removing water molecules and other complexes bound to the receptor, correcting for missing hydrogens.[12] building missing loops, optimizing side chains of missing residues, managing alternate confirmations and preparing the protein structures . Protein structural models were refined with CHARMmforcefield using steepest descent algorithm.

Ligand preparation

36 and 24 Tyrosine kinase inhibitors were selected to dock with cKIT and PDGFRA respectively. The 2D structure of the ligands were retrieved from Pubchem database which is a free database of chemical molecules. Ligand preparation was done in Discovery studio. It enables to convert 2Dstructure into 3D structure of the protein, filter poor candidates based on Lipinski rules, enumerate ionization states, standardize charges for common groups, add hydrogens, generate tautomers, generate isomers, remove duplicates, receptor ligand interactions and also has multiple conformational generation methods.

Site directed Mutagenesis

Three frequently occurring mutations D816V, K642E, V654A were developed in cKIT protein. Two frequently occurring mutations D842V, D842Y were developed in PDGFRA protein. Confirmational tools on Discovery studio facilities to perform site directed mutations, to obtain mutated receptors. Site-directed mutagenesis was conducted to mutate the selected target residues.

Docking studies

The ligands were docked against the receptors in MOE. MOE provides a collection of applications for visualizing and understanding details of active site regions of protein and the protein ligand interactions. The protein-ligand binding sites were detected and molecular surfaces were built. The site finder in MOE calculates possible active sites in the protein from the 3D atomic coordinates of the protein.The individual sites were visualized and populated

with dummy atoms for docking calculations. The ligand molecules were docked in the binding site of the wild type of cKIT and PDGFRA protein. It gives a list of conformations and the best fit into the active site was found based on various scoring functions such as the interaction energy between protein and ligand. The poses are refined using forcefield based method. The confirmation of the protein that best fits into the binding site is docked with the protein.The residues that are in association and close contact with the ligands were visualized in the ligand interactions window.15 ligands were selected from the 36 ligands with acceptable binding energy when docked with the wild type protein. These ligands were docked with the 3 mutated proteins of cKIT(D816V, K642E, V654A). 7 ligands were selected from the 24 ligands with acceptable binding energy when docked with the wild type protein. These ligands were docked with 2 mutated proteins of PDGFRA(D842V, D842Y).The best drug candidates were selected based on the ligand interactions and their binding energies with the mutated proteins.

RESULTS AND DISCUSSIONS

Protein Preparation

The protein query c-Kit was given in PDB and the search gave 7 protein structures. Out of these, 1PKG was retrieved after analyzing the results of ramachandran plot usingRAPPER protein modeling server performed for all 7 structures. For 1PKG ,amino acids in favorable region were 510 (92.4%), in allowed region were 34(6.2%) and in outliner region were 8(1.4%).Kinase domain sequence submitted to discovery studio has given its homology modeled structure.The energy minimized structure of c-Kit and PDGFRA were obtained from discovery studio.

Ligand Preparation

2D structures of 36 ligands for c-Kit and 24 ligands for PDGFRA were retrieved from Pubchem database. These ligands were submitted to discovery studio which has converted 2D structures to 3D structures and also minimized the energies of these ligands.

Site directed Mutagenesis

These mutants in c-Kit were constructed using discovery studio. In the first mutant, lysine (K) was substituted with glutamic acid (E) at 642 positions. This mutation occurred in the juxtamembrane domain of the protein. In the second mutant generated, valine (V) was substituted with analine(A) at 654 position and in the third mutant generated, glutamic acid (D) was substituted with valine at 816 position and this mutant is reported to be the

secondary mutant. Two mutants were developed using discovery studio. One of which has glutamic acid substituted by valine at 842 position and the other having glutamic acid substituted by tyrosine at 842 position. Energy minimization was done for all the 5 mutated proteins.

Docking Studies

When 36 ligands were docked with wild type c-Kit protein,Quizartinib showed least S score of -15.84, followed by Midostaurin with an S score of-15.82 and Dorsomorphin with an S score of -15.1417. The S score of other ligands are listed in table 1. When24 ligands were docked with wild type PDGFRA protein,PDGFRA rtk inhibitor showed least S score of -12.9986 followed by Pazopanib with an S score of -12.8668 and Tandutinib with an S score of -12.3364. The S score values of other ligands are listed in table 2. The ligands (**in bold**) in table 1 and table 2 have shown considerable S score values and were hence selected for docking with mutated c-KIT and PDGFRA proteins respectively.

Fifteen ligands were selected from 36 c-kit ligands and were docked with the three mutated c-kit proteins .When these ligands were docked with K642E mutated c-kit protein ,Lestaurtinib with an S score of -15.7142 ,Alvocidib with an S score of -15.0841 , Nilotinib with an S score of -14.9227,Crenolanib with an S score of -14.8822, Midostaturin with an S score of -14.3368 and Curcumin with an S score of -14.3549 were observed to have acceptable binding affinities with the mutated protein. The docking results of ligands docked with K642E mutated c-kit protein are mentioned in table 3. Similarly when the ligands were docked with the second mutated c-kit protein V654A ,Quizartinib with an S score of -16.3050, Nilotinib with an S score of -15.6061, Midostaturin with an S score of -14.9696, Crenolanib with an S score of -14.8809 and Curcumin with an S score of -14.3556 were observed to have highest binding affinities with the mutated protein. The docking results of ligands docked with V654A mutated c-kit protein are mentioned in table 4. When these ligands were docked with the third mutated c-kit protein D816V ,the ligands with leading binding affinities are Lestaurtinib with an S score of -15.8336, Nilotinib with an S score of -15.7415, Crenolanib with an S score of -14.8817, Curcumin with -14.7233 and Midostaturin with an S score of -14.0820. The docking results of ligands docked with D816V mutated c-kit protein are mentioned in table 5.These results(table 3,4,5) clearly demonstrate that Nilotinib ,Crenolanib,Curcumin and Midostaturin have most desirable binding affinities and are ,Hence more likely responsive in inhibiting the activity of the mutations K642E,V654A and

D816V.Therefore these four tyrosine kinase inhibitors can be targeted against any of these three mutations.

SUPPLEMENTARY FILES

Docking Results of Ckit with tyrosine kinase inhibitors.

Table1: Dock scores of tyrosine kinase inhibitors with cKIT Protein.

S.no	Ligand	S score	E_conf	E_place	E score
1	Quizartinib	-15.8436	1.8000	-79.3627	-15.8436
2	Midostaurin	-15.8237	0.4435	-105.2870	-15.8237
3	Dorsomorphin	-15.1417	0.0000	-75.0086	-15.1417
4	Imatinib	-14.8840	1.2000	-98.0503	-14.8840
5	Lucitinib	-14.8329	3.4400	-91.3471	-14.8329
6	Imatinib mesylate	-14.7846	1.8000	-113.1296	-14.7846
7	Alvocidib	-14.6099	1.4000	-94.8557	-14.6099
8	Crenolanib	-14.6082	2.1893	-82.4207	-14.6082
9	Lestaurtinib	-14.5634	0.0000	-98.3938	-14.5634
10	Curcumin	-14.3629	1.2000	-95.7735	-14.3629
11	Emodin	-13.9862	0.0000	-89.8201	-13.9862
12	Pazopanib	-13.9579	1.0000	-88.6372	-13.9579
13	Dasatinib	-13.8540	2.6000	-72.7267	-13.8540
14	Tandutanib	-13.6616	3.8736	-77.4687	-13.6616
15	Ki20227	-13.5823	1.8000	-62.8853	-13.5823
16	Genistein	-13.5067	0.0000	-80.5391	-13.5067
17	LCK Inhibitor	-13.4333	1.2000	-100.6055	-13.4333
18	Dovitinib	-13.4308	0.0000	-85.3073	-13.4308
19	Satuosporine	-13.3322	2.6583	-117.0284	-13.3322
20	Masitinib	-13.2757	2.2000	-109.8184	-13.2757
21	Su14813	-13.1862	3.2000	-96.3271	-13.1862
22	Foretinib	-13.0638	3.8000	-92.4512	-13.6038
23	Nilotinib	-13.0426	3.4000	-124.2943	-13.0426
24	Cediranib	-12.8681	1.7938	107.4725	-12.8681
25	JNJ-28312141	-12.7105	1.2000	-86.0061	-12.7105
26	JNJ-10198409	-12.6869	0.8000	-83.6052	-12.6869
27	Apatinib	-12.6852	2.8026	-66.6509	-12.6852
28	Regorafenib mesylate	-12.6225	1.4000	-67.0218	-12.6225
29	linifanib	-12.4748	0.2026	-100.6314	-12.4748
30	Motesanib	-12.4656	2.2000	-96.3142	-12.4656
31	Su1162	-12.2317	1.6000	-80.3390	-12.2317
32	Sunitinib	-12.1825	2.4000	-83.3071	-12.1825
33	NVP-TAE684	-12.1108	0.7999	-123.2018	-12.1108
34	Axitinib	-11.9022	1.9941	-79.1790	-11.9022
35	D65476	-11.6711	2.4010	-109.5911	-11.6711
36	PD-173955	-11.3898	0.0000	-75.4429	-11.3839
37	Gtp 14564	-10.3554	0.0000	-71.8851	-10.3554

Table 2: Dock scores of tyrosine kinase inhibitors against mutant.

S.no	S score				
	Ligand	**Wild**	**D816**	**V654A**	**K642E**
1	Lestaurtinib	-14.5634	-15.8336	-16.3050	-15.7142
2	Nilotinib	-13.0426	-15.7416	-15.6061	-15.0841
3	Crenolanib	-14.6082	-14.8817	-14.9696	-14.9227
4	Curcumin	-14.3629	-14.7233	-14.8809	-14.8822
5	Quizartinib	-15.8436	-14.7172	-14.7106	-14.4689
6	Imatinib mesylate	-14.7846	-14.6401	-14.6373	-14.3894
7	Midostaturin	-15.8237	-14.0820	-14.4862	-14.3549
8	Dasatinib	-13.8540	-13.8267	-14.3556	-14.3368
9	Alvocidib(natural)	-14.6099	-13.7333	-14.1296	-14.2141
10	Masitinib	-13.2757	-13.6545	-13.7924	-14.0569
11	Lucitinib	-14.8329	-13.4721	-13.7050	-14.0166
12	Regorafenib mesylate	-12.6225	-13.4009	-13.7133	-13.9371
13	Imatinib	-14.8840	-13.0031	-13.5630	-13.8157
14	Dorsomorphin	-15.1417	-12.9056	-13.1595	-13.4004
15	sunitinib	-12.1825	-12.1099	-12.6167	-11.1984

structure of cKIT

V654A MUTANT C-KIT.

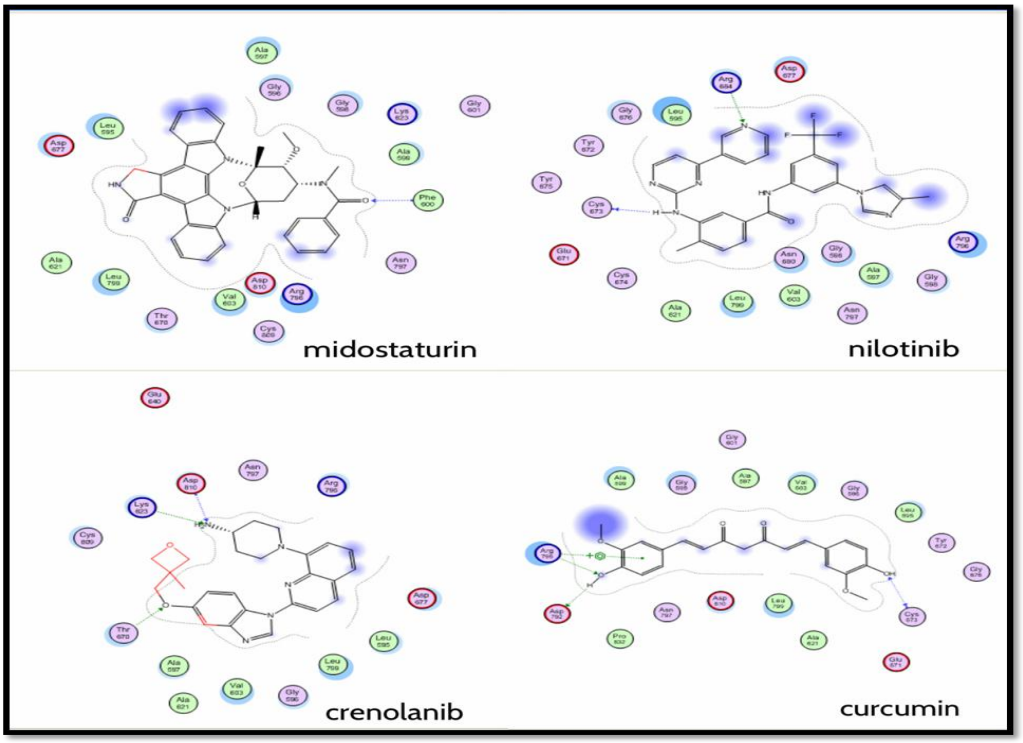

Figure1:Shows receptor -lingand interactions of tyrosine kinase inhibitors with Mutatant V654A mutant c-kit.

D816V MUTANT C-KIT

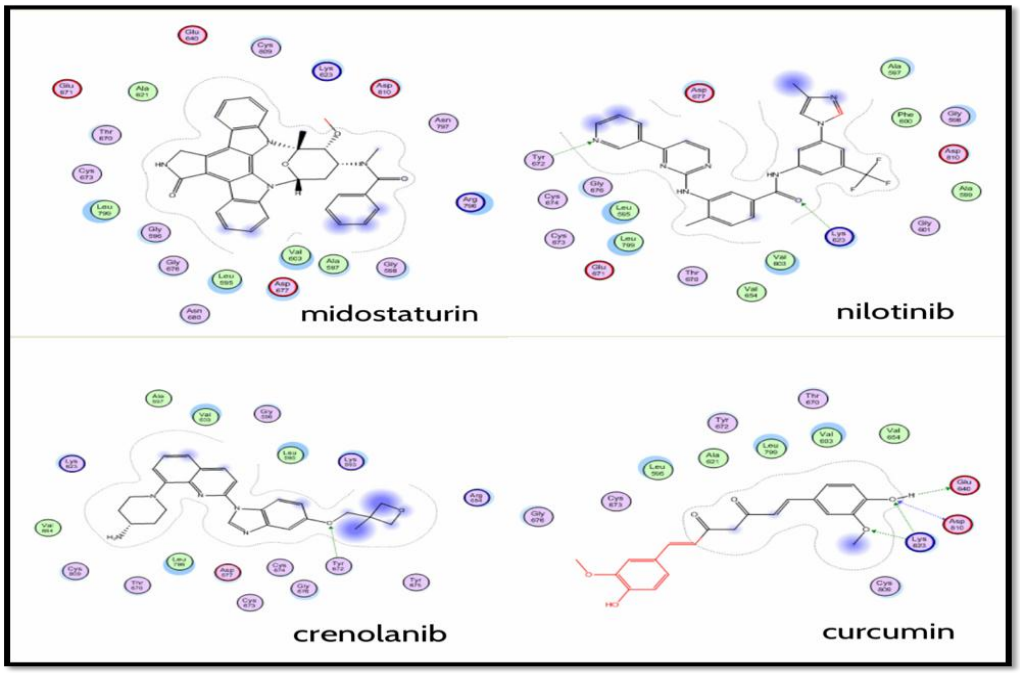

Figure2: Shows receptor -lingand interactions of tyrosine kinase inhibitors with Mutatant D816V mutant c-kit.

K642E MUTANT C-KIT

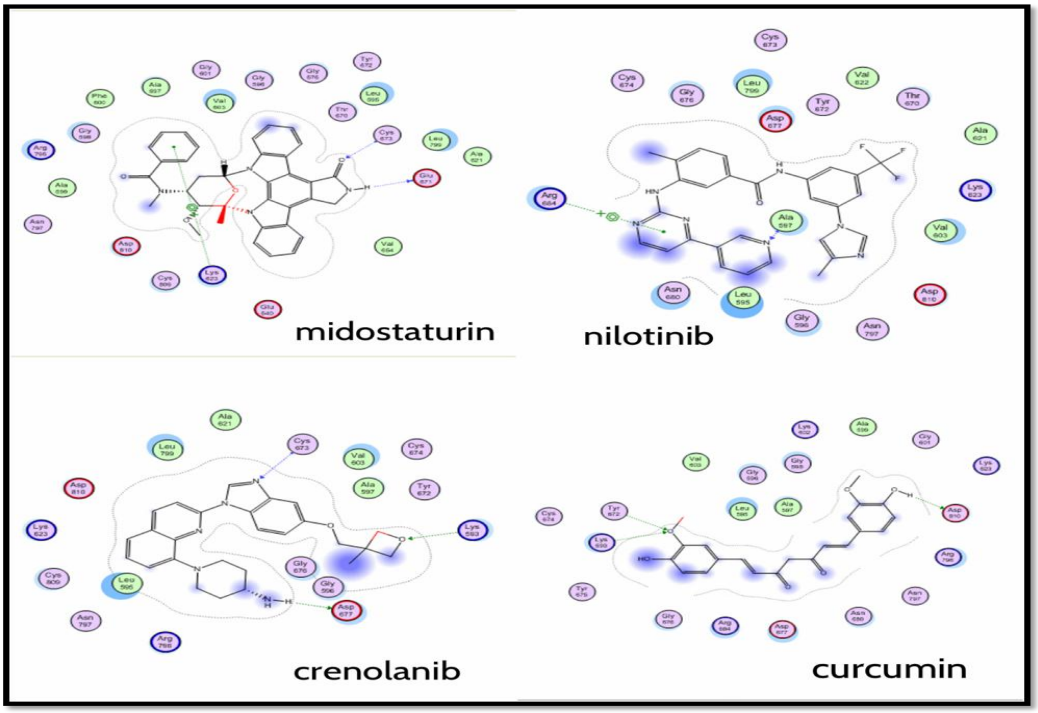

Figure3:Shows receptor -lingand interactions of tyrosine kinase inhibitors with Mutatant K642E mutant c-kit.

PDGFR alpha results

S.no	S score			
	Ligand	**Wild**	**D842Y**	**D842V**
1	PDGFRA r tk inhibitor	-12.9986	-12.5705	-12.5705
2	pazopanib	-12.8668	-11.7753	-11.7753
3	dasatinib	-12.2100	-11.6207	-11.6207
4	cediranib	-12.1670	-11.4522	-11.4522
5	Axitinib	-12.1421	-11.2557	-11.2557
6	motesanib	-11.7099	-11.0616	-11.0616
7	crenolanib	-11.6888	-10.7614	-10.7614

D842V MUTANT PDGFRA.

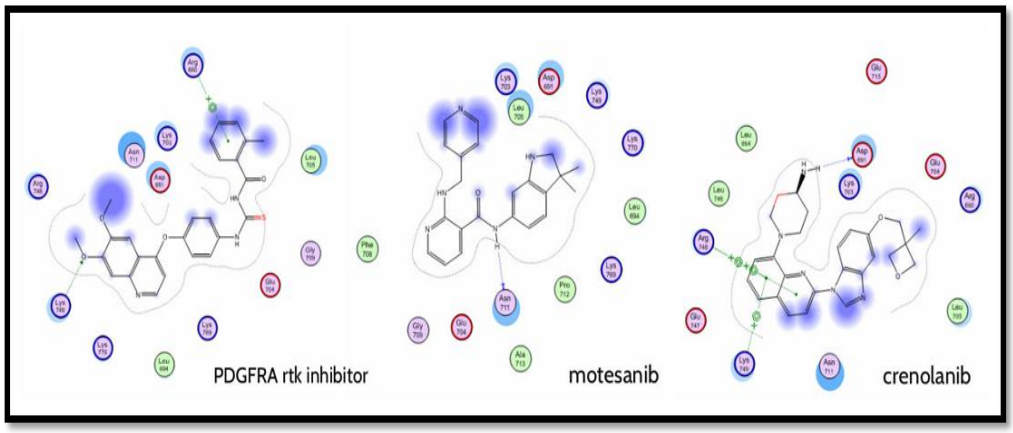

Figure4: Shows receptor -lingand interactions of inhibitors with Mutatant D842V PDGFRA.

D842Y MUTANT PDGFRA.

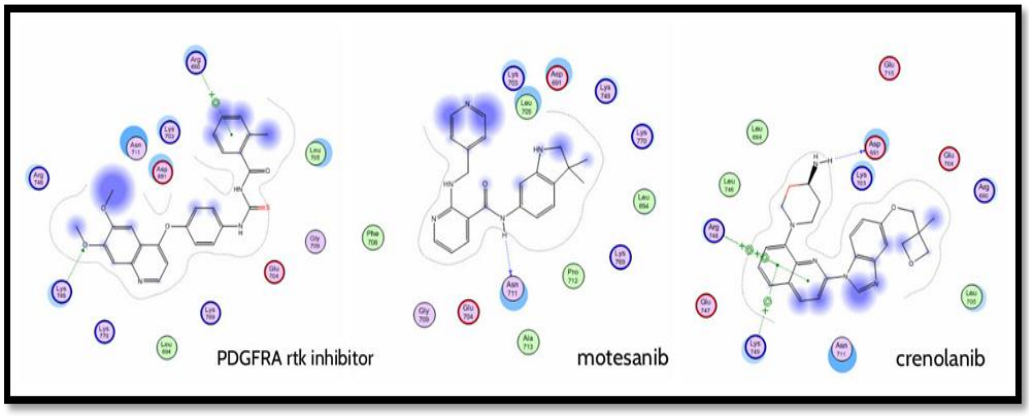

Figure4:Shows receptor -lingand interactions of inhibitors with Mutatant D842Y PDGFRA.

CONCLUSIONS

Tyrosine kinases have been targeted to treat cancer for past few years. A number of Tyrosine kinase inhibitors (small molecules) have been developed which have shown promising results in various types of cancers including GISTs. In GISTs ,Tyrosine kinase inhibitors given as a part of therapy to the patients differ with the type of mutation.Primary mutations in c-KIT can be treated using Imatinibmesylate and Sunitinib ,but the same cannot be used for secondary mutations in c-KIT. The results obtained in the present work suggest that Nilotinib, Crenolanib, Curcumin and Midostaturin can be targeted for both primary and secondary mutations in c-KIT. Curcumin, being a naturally derived compound is one such target that has reduced number of side effects compared to chemically synthesized targets. In PDGFRA, Tyrosine kinase inhibitors like Dasatinib, Pazopanib, Cediranib, PDGFRA rtk inhibitor, Motesanib and Crenolanib can also be used as targets in addition to the presently used drug targets.

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