Scholarly Research Journal for Interdisciplinary Studies,
Online ISSN 2278-8808, SJIF 2016 = 6.17, www.srjis.com
UGC Approved Sr. No.49366, NOV-DEC 2017, VOL- 4/37

DRUG DESIGNING FOR TARGETING FLT3 RECEPTOTTYROSINE KINASE FOR LEUKEMIA DISEASE

ShailjaSingla & Nikunaj Bhardwaj

Noida International University, Greater Noida

Abstract

More than 30% of acute myeloid leukemia (AML) patients possess activating mutations in the receptor tyrosine kinase FMS-like tyrosine kinase 3 or FLT3. A small-molecule inhibitor of FLT3 (tyrosine kinase) that is currently in clinical trials appears promising for the treatment of AML. Here, we report the co-crystal structure of the kinase domain of FLT3 in complex with RTK. FLT3 with quizartinib bound adopts an "Abl-like" inactive conformation with the activation loop stabilized in the "DFG-out" orientation and folded back onto the kinase domain. This conformation is similar to that observed for the uncomplexed intracellular domain of FLT3 as well as for related receptor tyrosine kinases, except for a localized induced fit in the activation loop.The co-crystal structure reveals the interactions between RTK and the active site of FLT3 that are key for achieving its high potency against both wild-type FLT3 as well as aFLT3 variant observed.

Keywords:*myeloid leukemia (AML), tyrosine kinase 3, co-crystal structure*

INTRODUTION

Leukemia is a form of cancer that affects blood-forming tissues such as bone marrow andis associated with uncontrolled growth of white blood cells (Leukocytes).Continuous production of leukemic cells overwhelm the bone marrow, enter the bloodstream, and eventually invade other parts of the body such as the lymph nodes, spleen, liver, central and peripheral nervous systems.Incidence of leukemia ranges between 10-18 per 100,000persons per year worldwide with a little male dominance, while the mortality rate is 5-9per 100,000 per year. Current drugs used in leukemia treatment are Quinoxaline AG1296, Indolinones SU5416 and SU11248, Indolocarbazoles PKC412. Other drugs that are used less offen include Cytarabine, Daunorubicin, Doxorubicin, Idarubicin, Mitoxantrone, Vincristi, Sunitinib, Dasatinib, Nilotinib, Axitinib, Imatinib, Carbozanitinib, Sorafenib, Pazopanib, Tandutinib MLN-518, Vatalanib, Gemtuzumab, CyclophosphamideAnd Cytarabine

MARKERS IN LEUKEMIA OR TARGET OF CURRENT DRUGS AND FLT3 RECEPTOR PROTEIN KINASE

The hematopoietic class III receptor tyrosine kinase (RTK) Flt3 (Flk2, STK1) has recentlyreceived much attention as a potential drug target. Activation of Flt3 by different types ofmutations plays an important role for proliferation, resistance to apoptosis, and prevention of differentiation of leukemic blasts in acute myeloid leukemia (AML). Signal transduction of Flt3 involves activation of several conserved pathways, including the RAS/ MAPKinase and the phosphoinositide3kinase / Akt signaling cascades. Transforming versions of Flt3 exhibit altered signaling, Selective inhibitors of Flt3 tyrosine kinase activity have the potential to suppress aberrant Flt3 signaling.

Flt3 is resistant to the phenylaminopyrimidine STI571 (Gleevec, Imatinib), a potent inhibitor of other RTKs in the family, such as the PDGFreceptor or cKit.STI571 binding to Flt3 is prevented by the phenylalanine 691 sidechain in the ATP binding center and mutating this site to threonine renders the corresponding Flt3 mutant sensitive to STI571.Compounds of several other structural families, including the quinoxaline AG1296, thebis(1H2indolyl) 1methanone D65476, the indolinones SU5416 and SU11248, the indolocarbazoles PKC412 and CEP701, and the piperazonylquinazoline CT53518, arepotent inhibitors of Flt3 kinase.

REVIEW OF LITERATURE

Structural insights into the extracellular assembly of the hematopoietic Flt3 signaling

Flt3 is activated on HSCs and early myeloid and lymphoid progenitors by its cognate ligand (FL), to initiate downstream signaling via phosphatidylinositol 3-kinase/AKT and theRas/Raf/extracellular signal-regulated kinase pathways. Consistent withthe narrow expression profile of Flt3 in the bone marrow environment, signaling via the Flt3 ligand-receptor complex primarily impacts earlyhematopoiesis, particularly the proliferation and development of HSC and Bcellprogenitors. In recent years, Flt3 and FL emerged as potent regulators of dendritic cell (DC) development and homeostasis, and DC-mediated natural killer cell activation, thereby gaining an important role at the interface of innate and acquired immunity and in cancer immunotherapy. Thus, Flt3 has been predicted to display amodular structure featuring an extracellular segment with 5 immunoglobulin(Ig)–like domains (residues 27-543), a single transmembrane (TM) helix(residues 544-563), a cytoplasmic juxtamembrane domain residues572-603), and a split intracellular kinase module (residues 604-958).

Over expression of wild-type or oncogenic forms of Flt3 havebeen implicated in several hematopoietic malignancies, and inflammatory disorders. In particular, internal tandem duplication in the JM region orpoint mutations in the kinase activation loop occurs in 35% of

patients with acute myeloid leukemia (AML), resulting in constitutive activation of thereceptor and uncontrolled proliferation of hematopoietic precursors. Such mutation fingerprints have established Flt3 as the predominant prognostic factor in AML cases and have rationalized the targeting of Flt3in a clinical setting.

Molecular Mechanisms of Human T-cell Leukemia/Lymphotropic VirusType I Infection(Franchini, 1995)

HTLV-I is the cause of both a hematopoietic malignancy, adult T-cell leukemidymphoma (ATLL) as well as a progressive myelopathy called tropical spastic para**paresis/HTLV-I-associated** myelopathy (TSP/HAM). Yet, HTLV-I, like the acutely transforming animal retroviruses, can transform T cells in vitro. Currentspeculations that HTLV-I may lead to neurologic disease by indirect mechanisms, such as autoimmunity. Transinduction of cytokines, come mainly from the lack of demonstration that HTLV-I can infect and replicate in cells of the central nervous system in vivo.

The roles of FLT3 in hematopoiesis and leukemia(Griffin, 2002)

FLT3 (Fms-like tyrosine kinase 3), also known as FLK-2 (fetal liver kinase-2) and STK-1 (human stem cell kinase-1[1]), was cloned independently by 2 groups in 1991. *FLT3* has strong sequence similarities with other members of the class III receptor tyrosine kinase (RTKIII) receptor family. A subset of RTKIII family members that includes FLT3, FMS, platelet-derived growth factor receptor (PDGFR), and KIT are characterized by an extracellular domain comprised of 5 immunoglobulin like (Ig-like) domains and by a cytoplasmic domain with a split tyrosine kinase motif.

Flt3 receptor tyrosine kinase as a drug target in leukemia(Dirk Schmidt-Arras, 2004)

The hematopoietic class III receptor tyrosine kinase (RTK) Flt3 (Flk2, STK1) has recently received much attention as a potential drug target. Activation of Flt3 by different types of mutations plays an important role for proliferation, resistance to apoptosis, and prevention of differentiation of leukemic blasts in acute myeloid leukemia (AML). At least one type of such mutations -internal tandem duplication in the Flt3 juxtamembrane domain (Flt3-ITD) - has been associated with an unfavorable prognosis.

FLT3: ITDoes matter in leukemia(Johns Hopkins University School of Medicine, FLT3: ITDoes matter in leukemia, 2003)

The gene for human FLT3, cloned independently by two groups, was at one point designated STK-1 (stem cell tyrosine kinase-1) by virtue of its having been cloned from a cDNA library derived from CD34-positive cells. It was recognized as having 92% amino-acid homology

with the murine gene, which had been cloned earlier, again from a stem cell-enriched cDNA library. The mouse gene was alternately named FLK-2 (fetal liver kinase-2) or FLT3, and this latter designation has emerged as the term most commonly used to describe both murine and human versions. Human FLT3 maps to chromosome 13 (13q12) and is comprised of 24 exons extending over more than 100 kilobases (kb).FLT3 is a member of the PDGF-R subfamily (the so-called class III) of receptor tyrosine kinases, characterized by an interrupted kinase domain.It has closest homology to FMS, KIT, and the two PDGF receptors, and lesser homology to the vascular endothelial growth factor and nerve growth factor receptor subfamilies. FLT3 ligand (FL) has also been cloned in both mouse and human, and this protein likewise appears to be structurally similar to CSF-1, the ligand for FMS, and to KIT ligand. In human hematopoietic cells, FLT3 expression is restricted to the CD34-positive fraction of bone marrow and a smaller fraction of CD34-negative cells destined to become dendritic cells.Targeted disruption of either FLT3 or FL in mice, while not lethal in embryogenesis, leads to reduced numbers of bone marrow hematopoietic precursors in general and a reduction of lymphoid precursors in particular.

MATERIALS AND METHODS

DATABASES USED

RCSB PROTEIN DATA BANK – RCSB PDB

This resource is powered by the Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and a complex assembly that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

PDBSUM- PICTORIAL DATABASE

The **PDBsum** is a pictorial database that provides an at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (**PDB**). It shows the molecules that make up the structure (*i.e.* protein chains, DNA, ligands and metal ions) and schematic diagrams of their interactions.

Browse

We can also use any of the **Browse** options given on the **PDBsum** home page:

- **Highlights** - which tabulates some of the extreme structures in the database in terms of age, size, etc.
- **List of PDB codes** - which lists all entries by their 4-character code

- **Het Groups** - lists all Het Groups in the PDB with links to the structures that contain them
- **Ligands** - as for Het Groups, but considering each bound molecule as a separate entity (often consisting of several covalently joined Het Groups)
- **Drugs** - lists the drug molecules (and neutraceuticals) found in structures of the PDB.
- **Enzymes** - lists all enzyme structures in the PDB classed by the hierarchical E.C. numbering scheme. Also known as the **Enzyme Structures Database** or **EC->PDB**
- **ProSite** - lists all PROSITE sequence patterns and the PDB entries that contain them
- **Pfam** - lists the PDB entries containing structural information for any Pfam domain
- **Species** - classifies all PDB entries by the source species from which the protein comes

Information presented on the PDBsum pages

The information given on each **PDBsum** entry is spread across several pages, as listed below and accessible from the tabs at the top of the page. Only the relevant tabs will be present on any given page.

- **Top page** - summary information including thumbnail image of structure, molecules in structure, enzyme reaction diagram (where relevant), GO functional assignments, and selected figures from key reference
- **Protein** - wiring diagram, topology diagram(s) by CATH domain, and residue conservation (where available)
- **DNA/RNA** - DNA/RNA sequence and NUCPLOT showing interactions made with protein
- **Ligands** - description of bound molecule and LIGPLOT showing interactions made with protein
- **Prot-prot** - schematic diagrams of any protein-protein interfaces and the residue-residue interactions made across them
- **Clefts** - listing of top ten clefts in the surface of the protein, listed by volume with any bound ligands shown
- **Links** - links to external databases

KEGG PATHWAY DATABSE

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database resource that integrates genomic, chemical and systemic functional information. In particular, gene catalogs from completely sequenced genomes are linked to higher-level systemic functions of the cell.

KEGG is widely used as a reference knowledge base for integration and interpretation of large-scale datasets generated by genome sequencing and other high-throughput experimental technologies. In addition to maintaining the aspects to support basic research, KEGG is being expanded towards more practical applications integrating human diseases, drugs and other health-related substances.

ZINC BANK DATABSE

The ZINC Database contains commercially available compounds for structure based virtual screening. It currently has about 90 million compounds that can simply be purchased. It is provided in ready-to-dock, 3D formats with molecules represented in biologically relevant forms. ZINC15 is in the process of being released as a new version of ZINC. The prior version was ZINC12.

DRUG BANK DATABASE

The DrugBank database is a comprehensive, freely accessible, online database containing information on drugs and drug targets. DrugBank is widely used by the drug industry, medicinal chemists, pharmacists, physicians, students and the general public. Its extensive drug and drug-target data has enabled the discovery and repurposing of a number of existing drugs to treat rare and newly identified illnesses.The latest release of the database (version 4.0) contains 7677 drug entries including 1558 FDA-approved small molecule drugs, 155 FDA-approved biotech (protein/peptide) drugs, 87 nutraceuticals and over 6000 experimental drugs Additionally, 4270 non-redundant protein (i.e. drug target/enzyme/transporter/carrier) sequences are linked to these drug entries. Four additional databases, HMDB, T3DB, SMPD and FDB are also part of a general suite of metaboloic/cheminformatics databases. HMDB contains equivalent information on more than 40,000 human metabolites, T3DB contains information on 3100 common toxins and environmental pollutants, and SMPDB contains pathway diagrams for nearly 700 human metabolic pathways and disease pathways, while FDB contains equivalent information on ~ 28,000 food components and food additives.

BINDING DATABSE

BindingDB is a public, web-accessible database of measured binding affinities, focusing chiefly on the interactions of proteins considered to be candidate drug-targets with ligands that are small, drug-like molecules.BindingDB supports medicinal chemistry and drug discovery via literature awareness and development of structure-activity relations (SAR and QSAR); validation of computational chemistry and molecular modeling approaches such as docking, scoring and free energy methods; chemical biology and chemical genomics; and

basic studies of the physical chemistry of molecular recognition. Data extracted by BindingDB typically includes more details regarding experimental conditions, etc. BindingDB currently contains about 1,136,299 binding data for 7,026 proteins and over 492,007 drug-like molecules.

PUBCHEM

PUBCHEM is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PUBCHEMcontains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PUBCHEMdatabase.

SOFTWARE USED FOR THE VISUALIZATION OF 3-D STRUCTURE OF PROTEIN OR RUNNING MOLECULAR DOCKING

PYMOL

PYMOL is an open-source, user-sponsored, molecular visualization system created by Warren Lyford DeLano and commercialized initially by DeLano Scientific LLC, which was a private Software Company dedicated to creating useful tools that become universally accessible to scientific and educational communities. It is currently commercialized by Schrödinger; Inc. PYMOLcan produce high-quality 3D images of small molecules and biological macromolecules, such as proteins.

AUTO DOCK TOOLS

AUTODOCK Tools, or ADT, is the free GUI for AutoDock developed by the same laboratory that develops AutoDock. We can use it to set up, run and analyze AUTODOCK dockings and is contour AutoGrid affinity maps, as well as compute molecular surfaces, display secondary structure ribbons, compute hydrogen-bonds, and do many more useful things.

PMV TOOLS (PYTHON MOLECULE VIEW)

This viewer has most of the features usually expected in a molecule viewer. In addition to these traditional features it is dynamically extensible, i.e. new commands can be developed independently and placed in libraries. The Viewer inherits from the ViewerFramework the capability to dynamically import these commands as needed. In fact, all commands in that viewer have been developed based on this principle. This provides a way to add features to the application that is incremental and well suited for team development.

AUTODOCK AND AUTODOCK VINA

AUTODOCK is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Current distributions of AUTODOCK consist of two generations of software: AUTODOCK 4 and AUTODOCK VINA.AUTODOCK 4 actually consists of two main programs: AUTODOCK performs the docking of the ligand to a set of grids describing the target protein; auto grid pre-calculates these grids.AUTODOCK VINAdoes not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed, and it does this virtually instantly.We have also developed a graphical user interface called AUTODOCKTools, or ADT for short, which amongst other things helps to set up which bonds will treated as rotatable in the ligand and to analyze dockings.

DOCKING OF FLEXIBLE LIGAND TO THE RECEPTOR

NAME	**LICENSE TERM**	**PLATFORM**	**KEYWORD**
AUTODOCK	COMMERCIAL	UNIX, LINUX,SGI	GA/LGA, MC
AFFINITY	COMMERCIAL	SGI	MONTE CARLO METHOD
DOCK VERSION	COMMERCIAL	LINUX, IRIS	MC, GA
DOT	FREE	UNIX	
FLEX X	COMMERCIAL	UNIX	FRAGMENT BASED
SHAPE	E-MAIL REQUEST	UNIX	STRUCTUREAND CHEMISTRY
LEAPFROG	COMMERCIAL	SGI	LIGAND DESIGN
Q SITE	COMMERCIAL	UNIX, LINUX, SGI	MOLECULAR MECHANICS
HINT	COMMERCIAL	LINUX, SGI	HYDROPHOBIC INTEREACTIONS
GOLD	FREE EVALUATION	UNIX	GA

TABLE 1: LIST OF DOCKING SOFTWARE

PROCEDURE OF DOCKING USING MGL TOOLS AND AUTODOCK VINA

PROCESSING A PDB FILE

- DB→ PMV Molecules→ Right MB→ Read Molecules→**4rjb**→ Open
- In the **dashboard**, click on **the inverted triangle** under **CI** to display color choices.
- MB→ select from string
- Edit→ delete→deleteselected area→ warning→ continue.
- **Edit**→ hydrogen→ add.

PREPARING A LIGAND FOR AUTODOCK

- Ligand→ Input→ Open→ Ok
- Ligand→ Torsion Tree→ Detect Roots
- Ligand→ Torsion Tree→ Choose Torsions
- Ligand→ Torsion Tree→ Set number of torsions→ Enter→ Dismiss
- Ligand→ Output→ Save as PDBQT→ Save
- Ligand→ Torsion Tree→ show/hide root molecules

PREPARING A MACROMOLECULE

- Grid→ Macromolecule→ Choose→ 4rjb→ Select molecule
- Grid→Grid Box
- File→ Close saving current
- Grid→ Set map type→ Choose Ligand→ Select Ligand
- Grid→ Output→ Save GPF

STARTING AUTOGRID

- Run→Runautogrid
- Set the working directory
- Set progress pathname
- Set parameter pathname
- Click launch

STARTING AUTOGRID

- Docking→Macrmolecule→ Set Rigid→ Filename→ Open
- Docking→ Ligand→ Choose→ Select Ligand→ Accept
- Docking→ Search Parameter→ Genetic Algorithm→ Accept
- Docking→Docking Parameters
- Docking→ Output→ Lamarckian OA→ Save

STARTING AUTODOCK AND AUTODOCK VINA

- Run→RunAutodock
- Set a working directory with browse
- Set parameters filename
- Check log filename
- Launch

Here is the list of commands: Vina can read a set of commands from a file. Input:

--receptor arg rigid part of the receptor (PDBQT)

--flex arg flexible side chains, if any (PDBQT)

--ligand arg ligand (PDBQT)

Search space (required):

--center_xarg X coordinate of the center

--center_yarg Y coordinate of the center

--center_zarg Z coordinate of the center

--size_xarg size in the X dimension (Angstroms)

--size_yarg size in the Y dimension (Angstroms)

--size_zarg size in the Z dimension (Angstroms)

Output (optional):

--out arg output models (PDBQT), the default is chosen based on the ligandfile name

--log arg optionally, write log file

Misc (optional):

--CPU arg the number of CPUs to use (the default is to try to detect the number of CPUs or, failing that, use 1)

--seed arg explicit random seed

exhaustivenessarg (=8) exhaustiveness of the global search (roughly proportional totime): 1+

--num_modesarg (=9) maximum number of binding modes to generate

--energy rangearg (=3) maximum energy difference between the best binding (mode and the worst one displayed (kcal/mol)

Configuration file (optional):

--configarg the above options can be put here

Information (optional):

--help print this message

--version print program version

Here are the contents of a sample config.txt file:

Receptor = 14rjb.pdbqt

Ligand = quizatinib.pdbqt

center_x = 2.5

center_y = 6.5

center_z = -7.5

size_x = 22.5

size_y = 22.5

size_z = 22.5

out = quizatinibvina.pdbqt

VISUALIZING AUTODOCK VINA RESULTS

- Analyze ➞ Dockings ➞ Open AutoDockvina result…
- Analyze ➞ Macromolecule ➞ Choose… Choose Macromolecule: 4rjb➞ Select Molecule
- Analyze ➞ Dockings ➞ Show Interactions
- File➞ Exit

Click on ADT icon to restart before going on

VISUALIZING AD4 RESULTS

- Analyze ➞ Dockings ➞Open… Docking Log File: quizatinib.dlg, ➞ Open
- Analyze ➞ Conformations ➞ Load
- Analyze ➞ Conformations ➞Play, ranked by energy…
- Analyze ➞ Macromolecule ➞ Open…
- File name: 4rjb.pdbqt, ➞ Open
- DB➞ hsg1➞MSĐ□ □ □➞Left MB
- DB➞ind➞MSĐ□ □ □➞LeftMB, DB➞DGĐ□ ¯□
- Analyze ➞ Grids ➞ Open… hsg1.OA.map, ➞ Open -0.5,Enter, Sampling➞ 1, Enter Select From String➞Residues: ➞type in ARG8
- Analyze ➞ Dockings ➞ Show as Spheres…
- [Analyze ➞ Dockings ➞ Show Interactions]
- File ➞ Exit

LIPINKI'S RULE OF FIVE

Therule describes molecular properties important for a drug's pharmacokinetics inhumanbody,includingtheir absorption, distribution, metabolism,and excretion ("ADME"). The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are

maintained as described by Lipinski's rule.Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 Daltons
- An octanol-water partition coefficient[5] log *P* not greater than 5

VARITANTS

In an attempt to improve the predictions of drug likeness, the rules have spawned many extensions, for example the following:

- Partition coefficient log *P* in −0.4 to +5.6 range
- Molar refractivity from 40 to 130
- Molecular weight from 180 to 500
- Number of atoms from 20 to 70 (includes H-bond donors [e.g.;OH's and NH's] and H-bond acceptors [e.g.; N's and O's])
- Polar surface area no greater than 140 Å^2

LEAD LIKE

During drug discovery, lipophilicity and molecular weight are often increased in order to improve the affinity and selectivity of the drug candidate. Hence it is often difficult to have been proposed that members of screening libraries from which hits are discovered should be biased toward lower molecular weight and lipophility so that medicinal chemists will have an easier time in delivering optimized drug development candidates that are also drug-like. Hence the rule of five has been extended to the **rule of three** (RO3) for defining **lead-like** compounds.A rule of three compliant compounds is defined as one that has:

- octanol-water partition coefficient log *P* not greater than 3
- molecular mass less than 300 Daltons
- not more than 3 hydrogen bond donors
- not more than 3 hydrogen bond acceptors
- not more than 3 rotatable bonds

RECEPTOR OR LIGAND TABLES (COMPOUNDS FROM ZINC-80% SIMILAR TO QUIZATINIB)

S.NO	ZINC CODE	MWT	XLOGP	HBD	HBA	PSA
1	66352023	424.482	4.08	2	8	97
2	66352026	394.50	3.20	2	6	75
3	151513	339.376	4.58	1	6	56
4	1390343	339.376	4.56	1	6	56
5	65319362	410.543	4.69	1	6	56
6	2454926	325.349	4.20	1	6	65
7	409643302	294.379	4.62	1	7	75
8	12555231	324.405	4.68	3	5	73
9	40964261	324.405	4.68	1	5	56
10	1493837	464.831	4.76	3	8	104
11	3817152	480.63	3.35	3	7	92
12	6745272	482.821	4.85	3	7	92
13	59182076	415.371	3.82	2	6	71
14	48786914	312.247	2.62	2	5	71
15	3776052	316.666	3.28	2	4	62
16	3834201	238.290	2.88	2	6	49
17	12410091	238.290	2.88	2	5	59
18	31356634	272.735	3.54	1	7	96
19	3875026	272.35	3.54	1	5	56
20	3874586	210.235	2.29	1	6	80
21	31262374	210.23	2.29	1	5	56
22	3834032	309.345	2.75	1	5	56
23	12404521	309.34	2.76	1	6	73
24	719733626	271.295	1.46	4	6	76
25	12832834	222.247	1.96	1	6	65
26	71863783	345.358	4.19	2	6	94
27	36758429	385.443	4.53	2	5	88
28	6927939	286.718	2.34	2	4	65
29	8330942	286.716	2.34	2	4	66
30	65748723	324.38	3.22	2	5	75
31	13538893	287.322	2.99	2	4	62

TABLE 2: LIST OF RECEPTOR OR LIGAND COMPOUND (QUIZATINIB) FROM ZINC DATABASE. 80% SIMILAR TO SUNITINIB

S.NO	ZINC ID	MWT	XLOGP	HBD	HBA	PSA
1	35052139	287.322	2.99	2	4	62
2	574970	252.317	2.48	1	3	37
3	33168191	244.681	2.48	1	8	89
4	68711929	299.305	4.01	4	5	92
5	68603371	396.249	4.92	1	5	56

TABLE 3: LIST OF RECEPTOR OR LIGAND COMPOUNDS (SUNITINIB) FROM ZINC DATABASE 70% SIMILAR TO SORAFENIB

S.NO	ZINC ID	MWT	XLOGP	HBD	HBA	PSA
1	21992390	395.527	3.21	4	6	95
2	33168189	286.334	3.63	1	6	84
3	479304	376.391	3.99	2	6	80
4	6730025	300.361	4.03	3	6	83
5	12671487	238.29	3.88	2	7	80
6	4270707	362.364	3.77	2	6	80
7	5572379	474.944	3.32	3	8	90
8	5447418	446.910	4.19	1	7	69
9	5736637	396.249	4.92	1	5	56
10	23653640	328.735	4.20	1	6	87
11	20877765	357.772	4.25	3	5	74
12	35429769	372.428	4.28	2	6	80
13	83295042	390.468	4.89	2	6	80
14	35430062	386.455	4.74	2	6	80
15	4302693	353.809	4.51	3	5	74
16	72319624	395.825	4.73	1	6	73
17	12154180	360.417	4.92	2	6	72
18	44460331	361.760	3.54	1	6	73
19	38349197	444.919	3.81	2	8	89
20	04973805	323.327	3.65	3	5	74
21	04973804	323.327	3.60	3	5	74
22	35430199	416.456	4.95	2	6	76
23	02933064	438.94	4.83	2	6	80

TABLE 4: LIST OF RECEPTOR OR LIGAND COMPOUNDS (SORAFENIB) FROM ZINC DATABASE- 80% SIMILAR TO IMATINIB

S.NO	ZINC ID	MWT	XLOGP	HBD	HBA	PSA
1	22059475	299.281	1.55	2	5	89
2	4302693	253.305	2.44	2	4	61
3	33384040	254.567	2.24	2	4	62
4	3989259	415.945	3.44	4	6	82
5	33847714	272.307	3.21	1	3	46
6	90609819	343.382	5.01	6	6	105
7	22057143	343.780	4.78	6	6	102

TABLE 5: LIST OF RECEPTOR OR LIGAND COMPOUNDS (IMATINIB) FROM ZINC DATABASE

RESULT

PROTEIN – RECEPTOR DOCKING, DOCKING ENERGY (ORIGINAL COMPOUNDS)

S.NO	ZINC NAME	ESTIMATED G	FULL FITNESS
1	QUIZATINIB	-9.10	-1677.38
2	SUNITINIB	-9.09	-1679.11
3	SORAFENIB	-9.43	-1731.49
4	IMATINIB	-12.67	-1761.23

TABLE 6: LIST OF DRUG COMPOUNDS AND ITS BINDING ENERGY- 80% SIMILAR TO QUIZATINIB

S.NO	ZINC CODE	ESTIMATED G (KCAL/MOL)	FULL FITNESS (KCAL/MOL)
1	66352023	-9.40	-1684.77
2	66352026	-8.60	-1656.67
3	151513	-8.72	-1676.65
4	1390343	-8.04	-1695.22
5	65319362	-8.37	-1697.54
6	2454926	-9.03	-1681.98
7	40964302	-8.32	-1686.47
8	12555231	-8.92	-1679.54
9	40964261	-7.91	-1689.46
10	1493878	-8.53	-1704.56
11	3817152	-9.46	-1672.37
12	59182076	-8.71	-1704.56
13	48786914	-8.64	-1704.01
14	3776052	-8.36	-1687.79
15	3834201	-8.50	-1697.43
16	12410091	-8.56	-1765.63
17	31356634	-8.75	-1706.99
18	3875026	-7.86	-1698.76
19	3874586	-7.72	-1683.66
20	31262374	-8.09	-1688.36
21	3834032	-8.53	-1696.78
22	12404521	-9.23	-1754.32
23	71973626	-8.32	-1686.42
24	12832834	-8.21	-1705.19
25	3815485	-8.21	-1694.18
26	6927939	-8.25	-1694.65
27	71863783	-8.76	-1698.34
28	36758429	-7.98	-1698.98
29	83309242	-9.54	-1726.76
30	65748723	-8.55	-1699.38
31	13538893	-8.35	-1680.16

TABLE 7: DRUG (QUIZATINIB) WITH ZINC ID AND ITS BINDING ENERGY FOR DIFFERENT COMPOUNDS.

BINDING OF MOLECULES WITH FLT3

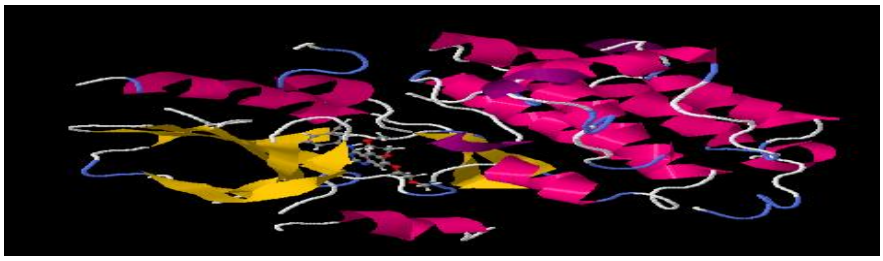


FIG 1: BINDING OF QUIZATINIB WITH FLT3 RECEPTOR

80% SIMILAR TO SUNITINIB

S.NO	ZINC ID	ESTIMATED G (KCAL.MOL)	FULL FITNESS (KCAL/MOL)
1	35052139	-8.99	-1679.72
2	574970	-9.85	-1694.29
3	33168191	-9.73	-1683.70
4	68711929	-9.73	-1687.16
5	68603371	-9.87	-1682.43

TABLE 8: DRUG (SUNITINIB) BINDING ENERGY WITH DIFFERENT COMPOUNDS

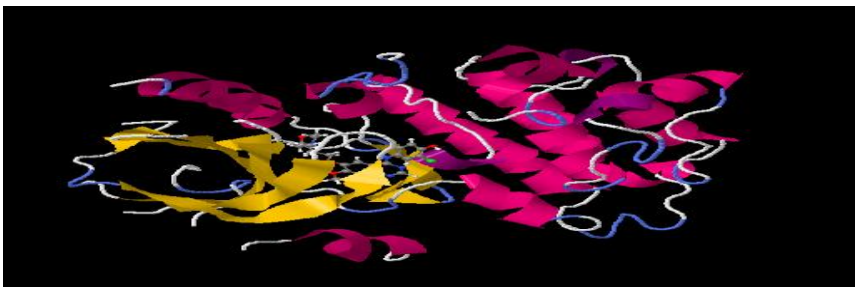


FIG 2: BINDING SUNITINIB WITH FLT3 RECEPTOR

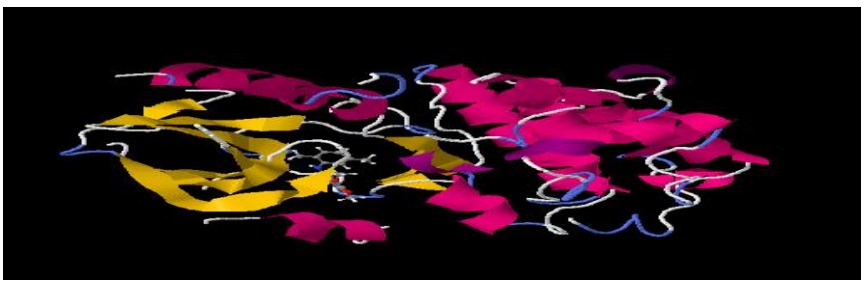


FIG3: BINDING OF FLT3 WITH ZINC ID- 68603371

70% SIMILAR TO SORAFENIB

S.NO	ZINC ID	ESTIMATED G(KCAL/MOL)	FULL FITNESS (KCAL/MOL)
1	21992390	-10.54	-1771.43
2	33168189	-12.98	-1832.65
3	479304	-9.13	-1675.45
4	67300025	-8.89	-1712.28

5	**12671487**	**-11.89**	**-1786.21**
6	**4270707**	**-9.06**	**-1674.91**
7	**5572379**	**-8.97**	**-1691.65**
8	**5447418**	**-8.90**	**-1678.98**
9	**573637**	**-12.75**	**-1798.04**
10	**23653640**	**-9.07**	**-1711.09**
11	**20877765**	**-8.67**	**-1721.33**
12	**35429769**	**-9.29**	**-1675.01**
13	**83295042**	**-9.13**	**-1740.45**
14	**35430062**	**-9.38**	**-1676.82**
15	**4302693**	**-8.59**	**-1721.74**
16	**72319624**	**-10.76**	**-1765.98**
17	**01259865**	**-13.22**	**-1865.41**
18	**44460331**	**-8.95**	**-1696.80**
19	**38349197**	**-12.11**	**-1796.78**
20	**04973805**	**-8.52**	**-1724.03**
21	**04973804**	**-8.68**	**-1723.58**
22	**35430199**	**-8.83**	**-1658.95**
23	**02933064**	**-12.76**	**-1799.04**

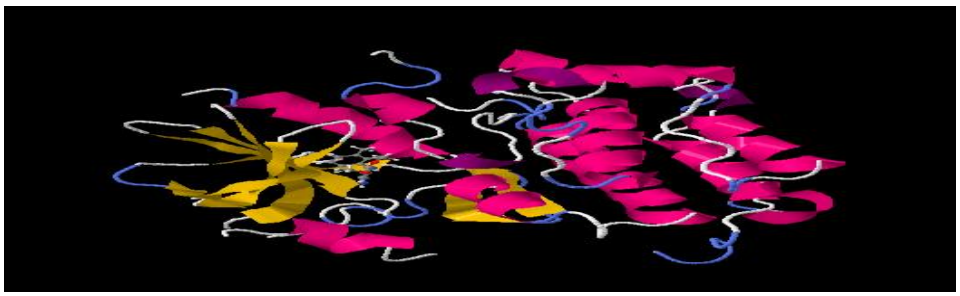


FIG 4: BINDING OF FLT3 WITH ZINC ID- 01259865

80% SIMILAR TO IMATINIB

S.NO	ZINC ID	ESTIMATED G (KCAL/MOL)	FULL FITNESS (KCAL/MOL)
1	**22059475**	**-8.57**	**-1729.27**
2	**4302693**	**-9.31**	**-1786.65**
3	**33384040**	**-8.76**	**-1711.08**
4	**3989259**	**-9.66**	**-1713.54**
5	**33847714**	**-9.62**	**-1671.54**
6	**90609819**	**-9.87**	**-1734.22**

TABLE 10: DRUG(IMATINIB) BINDING ENERGY WITH DIFFERENT COMPOUNDS

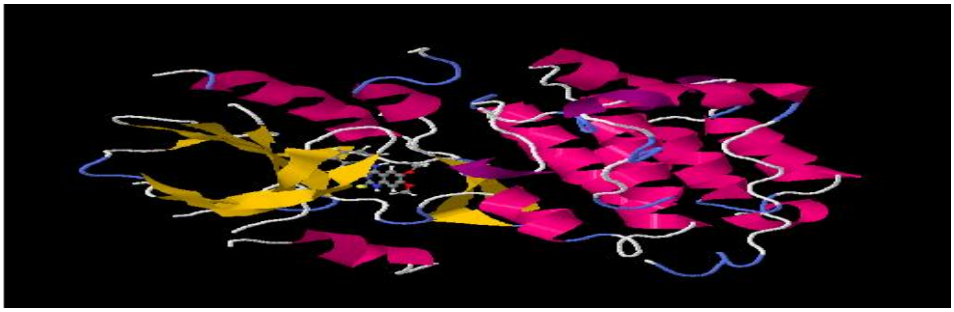
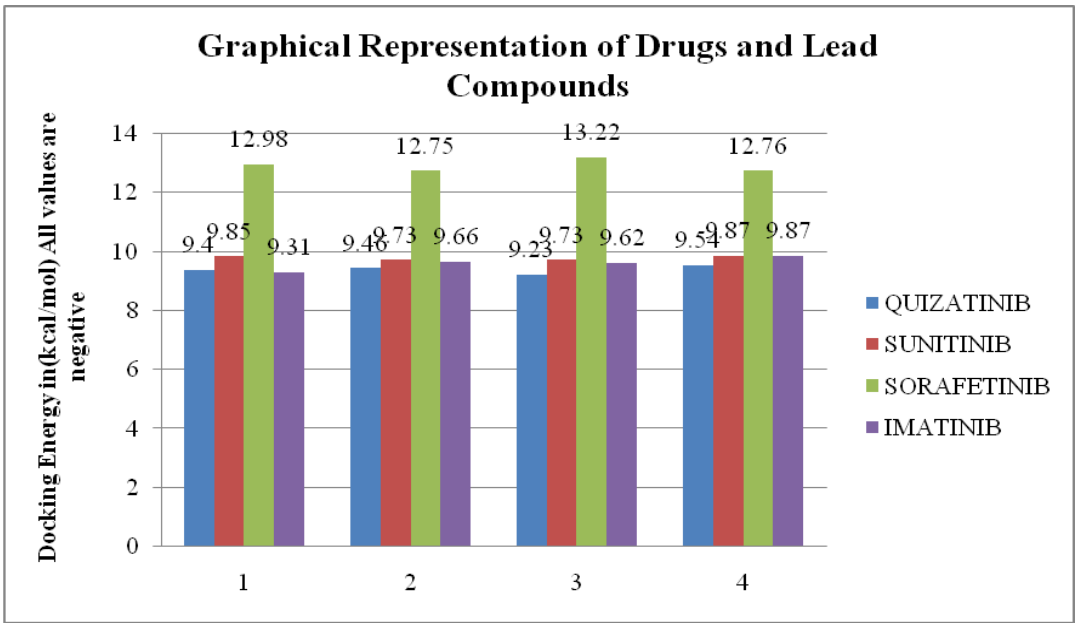


FIG 5: BINDING OF FLT3 WITH ZINC ID- 9060819

GRAPHICAL REPRESENTATION OF FLT3 WITH DIFFERENT MOLECULES



GRAPHICAL REPRESENTATION OF RESULTS

DISCUSSION: Rational structure approaches are increasingly being used in pharmaceutical industry because of the potential decrease of cost and time of drug discovery. Over recent years, the computational or mathematical modeling of biological system has become increasingly valuable and can provide useful information to understand their behaviors. Coupled with the increase in the number of techniques and software tools available to simulate and analyze them. In this project "Docking studies of FLT3 Receptor protein kinase with optimized potent Inhibitors to recuperate Leukemia Cancer in Human" structure based drug designing has been mainly used for getting drug molecule. FLT3 Protein kinase Receptor plays a vital role in Leukemia cancer. Here FLT3 protein has been taken as the target protein. The known inhibitors of protein were identified using binding database and zinc database. Interaction of the inhibitors with the protein was examined through Insilco Docking Approach. Then the results were compared with the docking results of the present

going on drugs. It was found that the docking energy of "ZINC01259865" (-13.22 kcal/mol) was greater than the docking energy of Quizatinib,Sunitinib and Imatinib.

SUMMARY AND CONCLUSION

FLT3 tyrosine Protein Kinase is a nuclear hormone receptor. It can activate the transcriptional activity; therefore it can be used as a drug target. Rational drug designing strategies helps to find out the best inhibitor for any disease and also reduces the cost of the drugs for different diseases; therefore this area provides vast opportunities in research and designing of drugs. In this work "ZINC01259865" was found to be the best selective inhibitor of FLT3 protein among different types of inhibitors because it shows maximum docking energy (-13.22 kcal/mol).The docking work suggests virtual derivatives of the predicted inhibitor that can improve bond interaction between inhibitor and protein. As is evident from Auto dock docking program in this study, "ZINC01259865" proves to be the best inhibitor among other inhibitors to inhibit FLT3 protein because it has low docked energy and forms two H-bonds, which signifies better interaction of ligand with the protein.The rationale of the reported work is amply justified by the presented results. After the project work on rational drug design for FLT3 tyrosine kinase protein, the conclusion is that out of all the inhibitors chosen for docking, "ZINC01259865" inhibitor emerged to be the best inhibitor for FLT3 tyrosine Protein kinase.

REFERENCES

Dirk Schmidt-Arras, J. S.-D. (2004). Flt3 Receptor Tyrosine Kinase as a Drug Target in Leukemia. Bentham Science , 1867-1883.

Franchini, G. (1995). Molecular Mechanisms of Human T-cell Leukemia/Lymphotropic Virus. American Socitey of Hematology,Blood.Griffin, D. G. (2002). The roles of FLT3 in hematopoiesis and leukemia. Blood , 100.Kenneth Verstraete, G. V. (2011). Structural insights into the extracellular assembly of the hematopoietic Flt3 signaling compliex. Blood Journal , 118.