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A Comprehensive Virtual Drug Screening & Molecular Docking Approach to Target VEGFR-2 & C-Met Receptors

Student: Baraya Saleem

Student ID: 3334933

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Supervisor: Dr. Mohammed Mansour

This research was carried out with in collaboration with Lutimba Stuart (National Tsing Hua University, Taiwan)

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Contents

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Abbreviations

- **VEGFR-2:** Vascular Endothelial Growth Factor Receptor-2
- **C-MET:** Mesenchymal-Epithelial Transition Factor
- **VEGF:** Vascular Endothelial Growth Factor
- **HGF:** Hepatocyte Growth Factor
- **STD:** Standard Deviation
- **SEM:** Standard Error Mean
- **FDA:** U.S. Food and Drug Administration
- **FASTA:** FAST-All
- **PDB:** Protein Data Bank
- **pKa:** Acidity of a particular compound
- **RMSF:** Root Mean Square Fluctuation
- **NAMD:** Nanoscale Molecular Dynamics
- **CHARMM:** Chemistry at Harvard Macromolecular Mechanics
- **OpenMM:** Molecular Mechanics
- **VMD:** Visual Molecular Dynamics
- **Å:** Angstrom
- **NS:** Nanoseconds
- **kD:** Kilodalton
- **VTN:** Vitronectin
- **EMT:** Epithelial Mesenchymal Transition
- **PCR:** Pathologic Complete Response
- **RT-PCR:** Real Time – Pathologic Complete Response
- **TRAMP:** Transgenic Adenocarcinoma of the Mouse Prostate
- **VEGF-C:** Vascular Endothelial Growth Factor - C
- **CRC:** Colorectal Cancer
- **NSCLC:** Non-small Cell Lung Cancer
- **OS:** Overall Survival
- **RFS:** Relapse Free Survival
- **HRs:** Hazard Ratios
- **PC3:** Prostate Cancer Cell Line
- **BMDCs:** Bone Marrow Derived Cells
- **KDR:** Kinase Domain Receptor
- **TSAD:** T- Cell Specific Adaptor Molecule
- **SEMA:** Sema homology region
- **PTK:** Protein Tyrosine Kinase
- **ATP:** Adenosine Triphosphate

Abstract

Cancer remains a major health concern worldwide despite the continuous efforts by the scientific community to eradicate the disease. Several strategies have been devised to treat various cancers via different targeted therapies however, there are some limitations to these treatments. One of the drawbacks observed in the already existing treatments of cancer is that at times either it is not fully cure and can reoccur in patients. Most cancer therapies to date are solely dependent on single targeted inhibitors which are profound of increasing drug resistance among cancer patients. The aim of this study is to find a novel dual inhibitor out of the FDA approved drugs that would be used to target the receptors; VEGFR-2 and c-Met and aid in treating cancer. The two tyrosine kinase receptors VEGFR-2 and c-Met play vital roles in the progression of cancer, the group of deadly diseases. The two receptors are efficiently able to conduct the processes such as cell proliferation, cell migration, metastasis, and progression in cancer. The Two computational analysis techniques: molecular docking and virtual screening approach were used to find a novel dual inhibitor for the two tyrosine kinase receptors. Among 2016 FDA approved drugs, 11 were selected for their inhibition and drug ability properties. Computational and biophysical approaches were utilized, thus screening for 3 approved drugs with the highest binding affinities and close interaction distance (Two single inhibitors and one dual). For c-Met one FDA approved drug that proved to be a single inhibitor was Entacapone. Another FDA approved drug that was a single inhibitor, for VEGFR-2 receptor was Telmisartan. Out of 11 shortlisted FDA approved drugs only one drug Triamterene was a novel inhibitor for both c-Met and VEGFR-2.

Observed findings in this study will complement on existing strategies in cancer therapies and thus this approach can be used in the identification and validation (*in vitro*) of novel dual inhibitors and drug targets to annihilate multidrug resistance in cancer treatment.

Keywords: C-met, VEGFR-2, Receptors, Tyrosine kinase, Telmisartan, Triamterene, Entacapone, Single Inhibitor, Dual Inhibitor, Cell Proliferation, Metastasis, Cell Migration.

Chapter 1 - Introduction

Cancer is a disease in which cell division becomes unregulated. The uncontrollable, abnormal growth of body's cells lead to cancer (Cancer Research UK, 2017). There are various characteristics of the cancer cells that make them different from the normal cells. Cancer cells lack ability for apoptosis, unlike the normal cells they ignore the cell signals to stop growth and continue to proliferate. Cancer cells grow rapidly and some of them are highly invasive also the process of differentiation is not as profound among them as in normal healthy cells (National Cancer Institute, 2021). The metastatic nature of cancer cells is one of the leading cause of deaths due to cancer (World Health Organization, 2019). Cancer is a disorder that can be caused due to combination of multiple factors including external and internal changes in genes. The genetic complications with cells that can lead to cancer include errors that arise while cell grows, malfunctioning of a tumour suppressing gene, damage that occurs to DNA due to environmental factors and the inheritance of disease from parents (National Cancer Institute, 2021). The changes in DNA can be identified as oncogene.

Cancer incidence unfortunately keeps on increasing. From 2016 to 2018 around 375,000 new cases of cancer occurred every year only in UK, which comes around to one thousand cases very single day (Cancer Research UK, 2015). 182,000 new cases of cancer in women and 193,000 in men during years of 2016 to 2018 were found (Cancer Research UK, 2015). If these stats are looked a bit closely it can be noticed that every two minutes someone is diagnosed with cancer in United Kingdom. Unfortunately, the mortality rates of cancer are not low. From 2017 to 2019, 460 deaths occurred because of cancer every day and 167,000 deaths every year (Cancer Research UK, 2015). 78,000 women and 89,200 men deaths were caused by cancer from 2017 to 2019. Every 4 minutes someone died due to cancer in the years of 2017 to 2019. Almost 50% of the patients that are diagnosed with cancer in UK survive for 10 or more than 10 years. The survival rate of cancer is higher in women than men (Cancer Research UK, 2015). The survival rate is normally higher in patients who are under the age of 40 years compared to prostate, bowel, and breast cancer where survival age is middle age (Cancer Research UK, 2015). However, one of the things that needs to be addressed here is that the survival rate does not mean that the patient was fully cured. Prostate cancer occurs in the prostate which is a small gland in the reproductive system of men (NHS Choices, 2019). Prostate cancer caused 12,039 deaths only in UK from 2017-2019 (Cancer Research UK, 2015). Prostate cancer is the 4th most common cancer in general and the 2nd most common cancer that occurs in men. 1.4 million cases of cancer were found in 2020 (WCRF International, n.d.).

The ability of cancer cells to proliferate uncontrollably is one of the major causes of concern in relation to the cancer. The genetic alterations which lead to uncontrollable proliferation of cells is the first step in the development of cancer cells. This step is also known as the tumour initiation (Cooper, 2016). As mutations continue to occur in the cells the tumour progression also seems to continue. The rate of mutations in the cells is directly

proportional to the progression of the cancerous tumour. Cancer cells motility not only depends on the expression of the gene A and gene Ba but also on the microenvironment of the tumours. When the gene A and gene B are expressed separately it is observed that the motility rate of the cancer cells is really low as compared to when the gene A and gene B are expressed in the same cell with the same microenvironment (Sahai, 2005). The drugs used to treat cancer mainly hinder not only with the DNA formation and its repair but also mitosis. Different drugs target various mechanisms.

The drug resistance in the treatment of cancer has proven to be a major complication. Drug resistance can not only be intrinsic but also extrinsic in terms of the characteristics and properties. In both intracellular and extracellular drug resistance ATP plays a crucial role in cancer cell proliferation, metastasis, and survival (Wang, Zhang and Chen, 2019). The level of ATP is observed to be elevated in the cancer cells when compared to the ATP levels in the normal healthy cell due to a procedure known as Warburg effect. A study where the levels of intracellular ATP in colon cancer cells was observed found that the ATP levels was not only elevated, but it was doubled the level in the cell lines which were chemo resistant when contrasted with the cell lines that were drug sensitive (Wang, Zhang and Chen, 2019). The one of extracellular drug resistance mechanisms include the competition between the intracellular ATP molecules with the tyrosine kinase inhibitors to ATP binding site on receptor tyrosine kinases which can be observed to lead to the phosphorylation and the downstream signalling pathways activation (Wang, Zhang and Chen, 2019). The current studies involve the treatments including some sort of combination between both chemotherapy and drugs while targeting different proteins. One of the causes of drug resistance is that protein tyrosine kinases are able to form secondary mutations with the tyrosine kinase inhibitors while the treatment is carried out (Yang et al., 2022).

The release of the drugs outside the cells takes place via the ABC transporters present outside the cells. When the drug has binded to the phosphate group from ATP is released and ATP is hydrolysed, the energy from this hydrolysed ATP changes the ABC conformational due to which the drugs are released outside the cell in the extracellular matrix (Mansoori et al., 2017). Another reason for the complication of drug resistance is when the absorption of the drugs is decreased in the tumour cells. The three ABC molecules are responsible for the transport of cytotoxic agents into the cells, but the drugs are absorbed into the cells with the help of active transport. The complication of decreased absorption of drugs in the cells can be classified in to two reasons. The hype in the decreased drug absorption by the cell is believed to be influenced by two factors which includes drug affinity and concentration. One of the reasons is that the ability of binding drugs has significantly decreased, and the second reason would be that the transporters that help in transporting and absorption of drugs have significantly decreased too (Mansoori et al., 2017). Mutation plays a vital role in creating these complications.

The targeted therapy can prove to be beneficial as the treatment of cancer as it not only controls how cancer cells grow but also it controls their spread and division. Targeted therapies can play a vital role in treating cancer due to their many functions. One of the ways targeted therapies help in treating cancer is that it can assist the immune system to

destroy cancer cells as cancer cells have the ability to hide from immune system (National Cancer Institute, 2018). Another reason is that targeted therapies can prevent proliferation. Further reasons include the termination of the signals form the blood vessels, transportation of antibodies to specific cells, apoptosis and starvation of the tumour cells of hormones system (National Cancer Institute, 2018). Tyrosine kinase inhibitor can be classified into six different types. Type I kinase inhibitors are the inhibitors that bind to the active ATP pocket binding site as they compete with the substrate. The type II kinase inhibitors involve inhibitors that bind to the inactive sites of proteins. Type III and IV do not include ATP bindings. A different quality of type IV and type V kinase inhibitors is that they are able to form covalent bonds at the binding sites which leads to the changes in the activity of the target, this process of covalent bond formation among the kinases and the inhibitors is permanent and not reversible (Yang et al., 2022).

Protein tyrosine kinases (PTKs) as the name indicates belong to the group of proteins. The tyrosine kinase phosphorylates the residues of tyrosine to carry out the signalling pathways. Tyrosine kinase receptors play a crucial role in the normal and healthy cell growth, development, and cell differentiation (Yang et al., 2022). The abnormal growth of cells can lead to carcinogenesis. Tyrosine kinase receptors are not only able to activate the T cell and B cell signalling pathways but also, they initiate numerous other functions such as cell proliferation, cell differentiation, migration, adhesion, and apoptosis in the cells (Yang et al., 2022). The overexpression of protein tyrosine kinase in terms of their mutation can be split into four different categories, category one is the mutation where function gain occurs. These types of mutations elevate the signalling and increases the responsiveness and sensitivity. The second category includes the mutation that amplifies and overexpresses genomes. The third and second last category involves the rearrangement of the chromosomes. Fourth and the last mutation category is autocrine ligand (Yang et al., 2022).

VEGFR-2 (Vascular Endothelial Growth Factor Receptor-2) is a receptor that belongs to the family of tyrosine kinase receptors. VEGFR-2 promotes cell proliferation, migration, and metastasis in cancer (Lian et al., 2019). This tyrosine kinase receptor VEGFR-2 is an important element that plays a crucial role in the signalling of VEGF. The extracellular ligand binding domain of VEGFR-2 includes seven immunoglobulin domains and a transmembrane domain. The intracellular segment of VEGFR-2 consists of two tyrosine kinase domains, one of the kinase domains split into two tyrosine kinase domains. VEGFR-2 KDR gene (human) is based on a chromosome locus 4q11-12 which encodes 1356 amino acids. Among the two forms of non- glycosylated of VEGFR-2 weighing 150 kD and 200 kD only the matured glycosylated form can carry out the intracellular signal cascade. When VEGFR-2 ligand VEGF (Vascular Endothelial Growth Factor), activates the VEGFR-2 a phosphorylation signal cascade starts which leads to the enhanced endothelial proliferation and migration in cancer (Miettinen et al., 2012). When VEGFA binds to the active site of VEGFR-2 the receptor dimerization is initiated. During tumour growth when T-cell specific adaptor molecule (TSAD) bind to the phosphorylation site Y951 the site phosphorylated by VEGFA (Modi and Kulkarni, 2019).

Hypoxia also known as low oxygen tension in cancer cells is one of the reasons for VEGF expression in cancer. Factors derived by hypoxia and transcriptional factors raise the gene transcription ability and stabilizes the strengthening of VEGF mRNA. As VEGFR-2 is related with the vascular permeability when the growth factor receptor is activated it leads to endothelial sprouting, expression of tissues and an elevated vascular permeability which then leads to not only extensions of vessels but also establishment of a vascular network (Modi and Kulkarni, 2019). The general observation is that the healthy tissues and cells have low expression of VEGFR-2, and the overexpression of VEGFR-2 was observed to be in various types of cancer.

The inhibition of VEGFR-2 can help with not only decreased angiogenesis but also lymph angiogenesis. The bi-lobed structure includes two lobes, one small N- lobe and the other one a large C- lobe both of which were connected by a linker which has a hinge region. On VEGFR-2 the binding of ATP relies on these lobes. These lobes consist of two sides front and back. The front cleft includes the binding site of ATP consisting of sugars, adenine, and phosphate. Whereas the back cleft performs as an extra binding site.

VEGFR-2 inhibitors are divided into three types. ATP inhibitors are considered to be the Type I inhibitors. They bind where adenine ring of ATP region is and they have three hydrogen bonds at the active site. Type II inhibitors encourage the inactive activation of DFG of activation loop. The third type of inhibitors Type III, are inhibitors that consist of covalent bonds. They bind with cysteine amino acid residues which leads to the prevention of the ATP binding at the binding site (Modi and Kulkarni, 2019). An antibody – inhibitor which offsets the signal cascade that is initiated by VEGF would have antiangiogenic effects in cancer.

C-Met (Mesenchymal-Epithelial Transition Factor) is a type of tyrosine kinase receptor. The ligand for c-Met is HGF (Hepatocyte Growth Factor). Hepatocyte growth factor is single chain. When HGF/ c-Met signalling is activated, normally it can initiate tissue regeneration, wound healing, and embryogenesis. But if the activation of c-Met is not normal it can lead to the onset of diverse types of cancer (Zhang et al., 2018). The interaction of c-Met and other tyrosine kinase receptors initiates different downstream signal cascades that help in progression of invasion, tumour proliferation, metastasis, and anti-apoptosis (Zhang et al., 2018). The abnormal activation of c-Met can prove to be a resistance in treating cancer. There are three types of the interventions in relation to pharmacology of c-met signalling such as creating an aggressive interference in regard to the c-met and HGF signalling second type would be blocking of the activation of the downstream signalling pathways that occur the third and the type explored in this study is inhibiting the activity and signalling cascade of the tyrosine kinase receptor c-met (Hass et al., 2017).

The HGF binding with c-met leads in receptor homodimerization and phosphorylation of two residues which are Y1234 and Y1235. A network of an upstream signalling pathway co -receptor has a corelation with c-met triggering (Organ and Tsao, 2011). The MET gene is located on chromosome 7 (7q21-q31) which consist of 21 exons and 20 introns. The protein size of the met is 120 kDa (Zhang et al., 2018). The mature form of c-met consist of alpha chain and beta subunits. The alpha subunits are the size of 32 kDa and the beta subunits

have the size of 120 kDa. The binding of HGF with c-met takes place at Sema homology region (SEMA) domain. The autophosphorylation of Tyr-1234 and Tyr-1235 is initiated in the intracellular tyrosine kinase domain when the HGF binds to the c-met. The HGF gene in total has 728 amino acid proteins (Zhang et al., 2018). When the autophosphorylation of intracellular protein tyrosine kinase (PTK) is initiated the downstream guanine nucleotide exchange factors (GEFs). When HGF binds with the c-met various downstream signalling pathways are instigated and c-met go through with some structural alterations. When the intracellular domain with protein tyrosine kinase activates the docking site i.e., multisubstrate docking site is revealed. As the autophosphorylation of the protein tyrosine kinase is over, the binding of SH2 and SH3 can take place (Zhang et al., 2018).

The transcriptional upregulation of cytokines such as interleukin 1,6, transforming growth factor beta for HGF among the fibroblasts. Pro-HGF gets activated when expression of proteases is high which leads to the target cell activation of MET (Zhang et al., 2018). The binding of HGF to c-met tyrosine kinase receptor leads to the autophosphorylation of some residues in the intracellular matrix. This activation involves the phosphorylation of three residues, Y1230, Y1234 and Y1235. One of the residues that is a crucial residue and is often related with the phosphoinositide 3 kinase Akt (Zhang et al., 2018). Two tyrosine residues Y1349, Y1356 that are at the terminal c in c-met receptor are involved in the activation of not only multisubstrate docking site but also eventually leads to activation of the signalling pathways. The domains on the docking sites such as phosphotyrosine binding domain (PTB), Src homology-2 domain and the MET binding domains (MBD) can bind with their specific receptors which leads to the activation of the cellular cascades (Zhang et al., 2018). The fact that the signalling pathways between c-met and VEGFR-2 directly corelate with the deadly disease various types of cancers has been observed and explored in numerous studies. The mutation in EGFR, T790 and MET proto-oncogene can lead to the downstream ERBB3 PI3K/AKT pathway activation (Zhang et al., 2018).

Virtual screening approach is a computational technique that assists in screening the potential molecules from the chemical databases. Virtual screening approach can be divided into two types. Type one is known as ligand based virtual screening approach (LBVS) and the type two is known as the structure based virtual screening (SBVS) (Kumar, Krishna and Siddiqi, 2015). One of the benefits of the virtual screening approach is that hundreds and thousands of the molecules can be analysed during a short period of time. Not only fast but virtual screening technique has also been proven to be cost effective.

Molecular docking is another computational analysis technique that is used to analyse the orientation of small molecules based in the binding site of a target molecule. Computational algorithms like AutoDock, AutoDock vina and virtual docker are among some of the softwares that can be used to analyse the molecules via molecular docking approach. Obtaining the 3D structures of the target from the protein data bank (PDB) is a crucial step in molecular docking approach. This also highlights one of the limitations of molecular docking approach which is the availability of the 3D structures in the protein data bank (Torres et al., 2019) (see appendix no. 5). The first and utmost important step in molecular docking is acquiring the 3D structures of the targets and ligands from the protein

data bank. After this step hydrogen i.e., a proton is added to the ion or molecule this step is known as the protonation state. This leads to the next step where the charges are assigned hence the name partial charges. In case the target binding site is unknown then the blind docking simulation would be carried out. The two steps that are used to help with molecular docking approaches are posing and scoring (Torres et al., 2019).

The aim of this study is to find an inhibitor that can inhibit the signal cascades that take place in result of abnormal activation of both (VEGFR-2 & c-Met) of these receptors, which would lead to treating cancer. Molecular docking and virtual drug screening are the methods that were used in this study to accomplish the aim. Molecular docking is a technique that is used to analyse and predict of how molecular structures interact with each other (Roy, Kar and Das, 2015). Virtual screening is a computational technique that uses wide-ranging libraries of drugs to screen with potential targets in order to find the structures that bind well together (Roy, Kar and Das, 2015). After the interaction of FDA approved drugs and c-met and VEGFR-2 the dual inhibitor drugs for both the receptors was Triamterene and the single inhibitor drug for c-met was Entacapone and for VEGFR-2 the single inhibitor was Telmisartan (For 2D chemical structures of single and dual inhibitors see appendix 4).

Chapter 2 - Methods

2.1 Structure Acquisition and preparation:

For the structure acquisition the 3D structure and FASTA sequences (nucleotide or protein sequences) of c-met (P08581) and VEGFR-2 (P35968) were accumulated (For FASTA sequences of c-met and VEGFR-2 see appendix no 1 & 2). The protein structures of the receptors VEGFR-2 and C-Met were obtained from the pdb database online. After the acquisition of the PDB structures of the two receptors the structures were cleaned of all unwanted residues and only the protein structure was saved for further modelling and identification of terminal N-/-C terminals, loops among the structure, transmembrane helics and the kinase domain. FASTA sequence is a single line description that is crucial as it represents the nucleotide sequences or the protein (amino acid) sequences. FASTA sequences start from sign '>' followed by the data of sequences. After finding the missing residues and the nonstandard residues, replace the nonstandard residues using homology modelling MODELLER v.9.3. Modeller is a tool that is used to find the three-dimensional structures of proteins.

2.2 Molecular Dynamics Simulation:

Interaction energy of ligand and receptor is calculated. In order to explore the structural conformation Gromacs/ OpenMM / NAMD was used. NAMD is a software tool which is used when a high-performance simulation is required for a substantial amount of bimolecular system. The composition of the complex membrane system for molecular simulation was done using CHARMM GUI. The properties of this software involves the representations of the interactions of two charges and the born radius. The reorganisation of PDB files in relation to the atoms. For the assignment and pKa determination the software PROPPKA and chemicalize are used. To correctly validate the structure PROCHECK Ramchandran plot analysis software was used. The ramachandran plot consists of alpha helics and beta strands. It is a 2D dimensional plot that consists of amino acids (phi and psi) in the protein sequence. PyMol and VMD were used to inspect any structural irregularities. Root mean square deviation and the root mean square fluctuations the structures were analysed (see appendix no.3). The radius of gyration and solvent accessible area (SASA). CHARMM36 FF to use the force field. The system composed of three things: water (TIP3P water model), Ions (KCI ions) and Protein (VEGFR-2 and C-Met).

2.3 Molecular Docking and Virtual Screening:

2.3.1 Receptor Preparation:

To identify the transient of binding pockets EPOS (Ensemble of pockets on protein surfaces) tool was used. As the name suggests EPOS was used in investigating the flexibility, nature, sequence, and other relevant details of the pockets such as how open the pocket is in a conformation analysis. Then the pockets were ranked in all the conformations from the trajectory. After that pockets were chosen on the basis of drug ability features which would provide information whether the drug would be able to temper a target or not. The docking input files such as SDF, mo2 and the pdbqt file were prepared using the Autodock tools.

Autodock shows the binding of novel drug candidates with small molecular novel targets which in this study were the FDA approved drugs with the receptors VEGFR-2 and c-met. The water and all other molecules that are not required are removed and hydrogen atoms are added to the receptors. Virtual screening is done using the FDA approved drugs using Autodock/Vina (MGL) tools in-house based scripts.

2.3.2 Ligand Preparation:

To access the drug libraries the sources like Drug Bank, Zinc database, PubChem and in-house drug libraires of London south bank university and NTHU (National Tsing Hua University in Thailand) were used. The structure obtained had many missing atoms. To prepare the ligand hydrogen is added using the Discovery studio, Avogadro. To determine the pKa and to assign Chemicalize was used. Chemicalize is a software that is used to provide not only the chemical calculations but also search and provides other features required. Avogadro helps with the minimization of ligands and generate force fields.

2.4 Clustering of Docked Conformations:

The clustering of poses was based on the free energy and RMSD (root mean square deviation) distance for which the algorithm extension in PyMol (PyRA) was used. By the help of PyMol software the 3D macromolecules visualization was done. A large surface area for conformation search of the ligand during the docking process. The observation of the conformation from all the trajectories was crucial. All Catalytic and ligand binding residues were included. The aim was to find an equitable perfect grid box for the docking which would not only avoid the missing out of the crucial residues for ligand binding but also would be computationally efficient as well. The computational efficiency is crucial when the large number of drug library samples are assessed.

2.5 Supervised Molecular Dynamics:

For the exploration of the ligand and receptor recognition pathway that is the extension of the supervision algorithm to standard simulation protocol SuMD was used. Supervised molecular dynamics is an approach that assists in recognizing the pathways of ligand and receptor in less time. It is essential to be careful in the supervised molecular dynamics as there is a risk of rejection and simulation again using old coordinates. Using supervised molecular dynamics, a computational approach the pathways with ligand and receptor in nano seconds was observed. This approach was not only used to observe the supervised ligand and receptor approaching distance but also to speed up the ligand and receptor trajectory. The Conformation of the space search via energetically favourably.

2.6 Steered Molecular Dynamics:

The characterization of the binding and the unbinding events in protein and ligand complex the SMD in Gromacs (GROningen Machine for chemical simulations) and the visualization of the applied energy terms in xmgrace was used. Steered molecular dynamics is a technique that requires the external forces that are time dependent after which the system is analysed. That quantifies the genetics in the transport pathways. Umbrella sampling is

an approach that improves the sampling or either one or various systems. The umbrella sampling can improve the calculation of free energy. Protein and ligand interactions energy was looked at using Umbrella sampling and MM/PBSA energy quantification. All 11 shortlisted FDA approved drugs were used to test if there is a dual inhibitor for VEGFR-2 and C-met was available.

Chapter 3 - Results

Out of 2016 FDA approved drugs 11 drugs (Cabozantinib, Crizotininb, Entacapone, Eltrombopag, Mizolastine, Salsalate, Telmisartan, Triamterene, Fludarabine phosphate, Dihydroergotamine, Oxandrolone) were shortlisted for the interaction with two tyrosine kinase receptors c-Met and VEGFR-2. After performing the molecular docking and virtual screening one of the eleven shortlisted FDA approved drugs was found as a dual inhibitor for both of the receptors (VEGFR-2 & C-Met), the drug is triamterene. Whereas the two drugs apart form the one that was recognized as a dual inhibitor was observed as a single inhibitor. The single inhibitor for the receptor c-met out of 11 shortlisted FDA drugs was Entacapone and for the receptor VEGFR-2 was Telmisartan. The details of how the result was obtained would be discussed in this section.

3.1 Obtaining and Preparing the Structure:

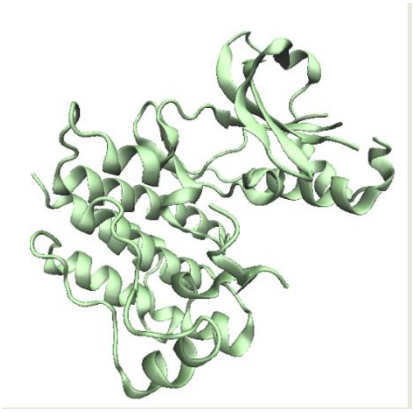


Figure 1: 3D Cleaned C-Met Image occupied from PDB (PDB ID: 3LQ8).

The structures were obtained using the PDB databank. Above figure shows the cleaned image of c-met, without the water molecules. The PDB ID for c-met is 3LQ8. Only the protein structure was saved for modelling of missing regions.

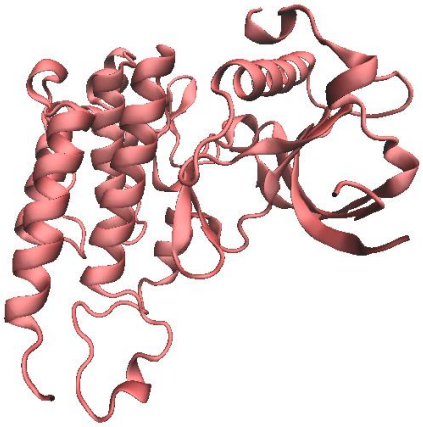


Figure 2: 3D Cleaned VEGFR-2 Image occupied from PDB (PDB ID: 3U6J).

The PDB ID for VEGFR-2 is 3U6J. The red in the figure above shows VEGFR-2 receptor. In order to make it easier for modelling of the missing regions the water molecules and all other unwanted molecules were removed.

3.2 Modelling To Build a Reliable Structural:

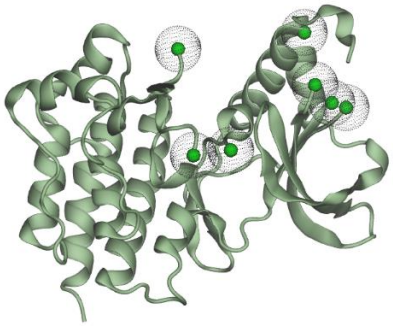


Figure 3: 3D Structure of C-Met with missing loops marked in green with transparent sphere around it.

The figure 3 represents the 3D structure of c-met which has the missing loops marked with dots in green with black sphere around to make it visible for understanding. In the figure two terminals, C-Terminal and N-Terminal are marked as well.

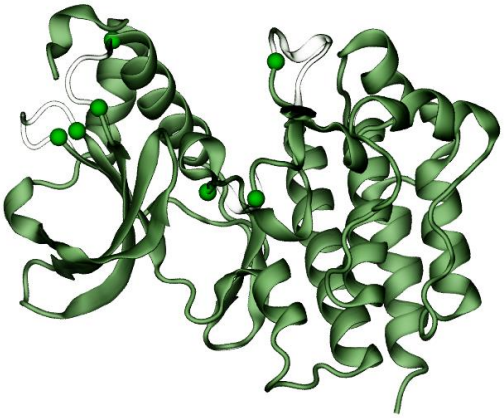


Figure 4: Combination of wildtype structure and modelled structure with missing residues in transparent for C-Met.

The figure 4 shows the combination of the superimposed wildtype structure which was obtained from the database and the modelled structure from swiss-model which was filled with the missing residues that are represented in the transparent colour in the figure. RMSD is 0.06 Å.

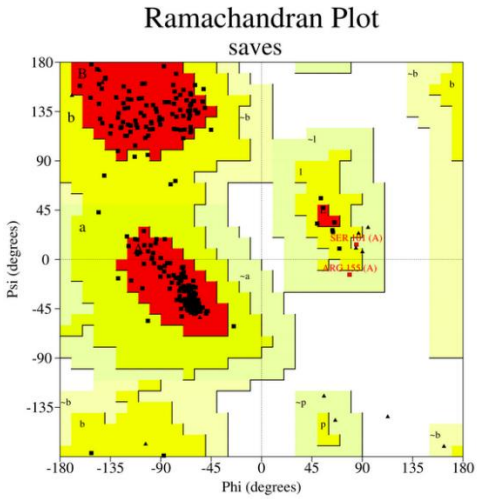


Figure 5: Ramchandran plot for C-Met, validated protein structures in red (core) and yellow represents allowed region.

The graph (Figure 5) is the Ramachandran plot represents the evaluation of the protein model. Around 92.6% of residues are in the region that is electronically favoured which shows that it is an ideal model.

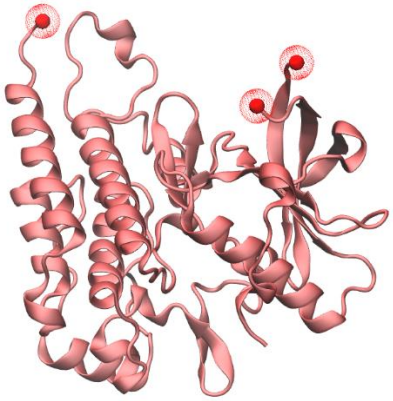


Figure 6: 3D Structure of VEGFR-2 with missing loops marked with red dot and a red sphere around it.

Representation of 3D structure of VEGFR-2 with the missing loops that are marked in red with a red sphere around it. The c-terminal and N-terminal are marked.

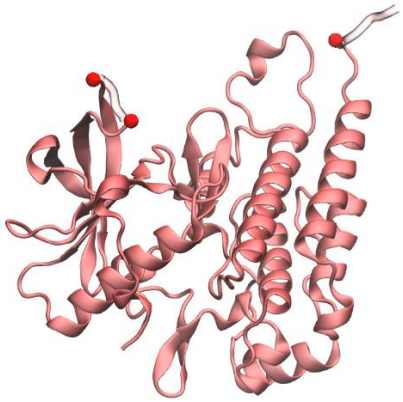


Figure 7: Combination of wildtype structure and modelled structure with missing residues in transparent for VEGFR-2.

In the figure 7 the transparent part shows the missing residues filled in modelled structure and superimposed wild type structure.

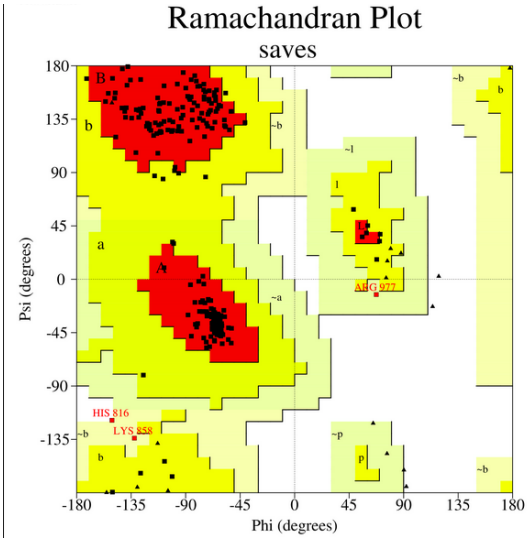


Figure 8: Ramchandran plot for C-Met, validated protein structures in red (core) and yellow represents allowed region.

Figure 8 graph represents the protein model evaluation and the 92.8% of residues that are in electronically favoured region which makes it an ideal model.

3.3 Formation of Maps of Catalytic Site and Ligand Binding Site:

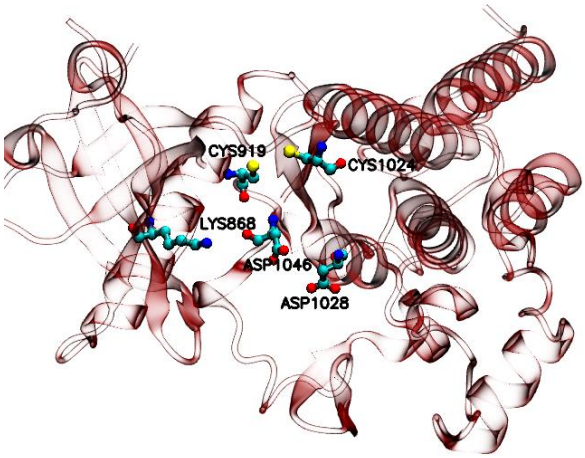


Figure 9: Amino acid residues Asp 1028, Lys868, Cys919 and Cys1024 in VEGFR-2 interactions via hydrogen bond.

Figure 9 shows the interaction of the amino acid residues in the 3U6J_VEGFR-2 through hydrogen bonds interactions (Asp1028, Lys868, Cys919 and Cys1024). The pKa values for amino acid residues of VEGFR-2 such as Asp1028, Lys868, Cys919 and Cys1024 are mentioned in the table (Table 1: The pKa of the active Site residues).

Table 1: The pKa value of the active Site residues for Asp1028, Lys868, Cys919, Cys1024 and Asp1046.

Amino Acid Residues	**pKa Value by PROPKA**
Asp1028	3.72
Asp1046	5.36
Csy919	12.29
Cys1024	10.37
Lys868	10.05

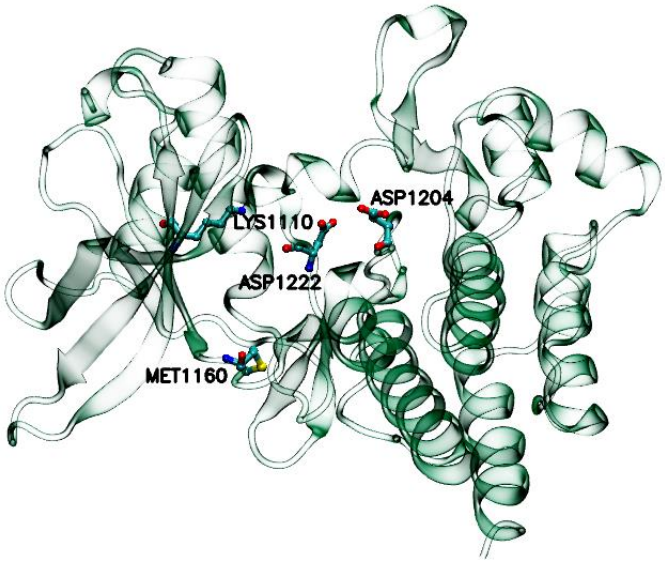


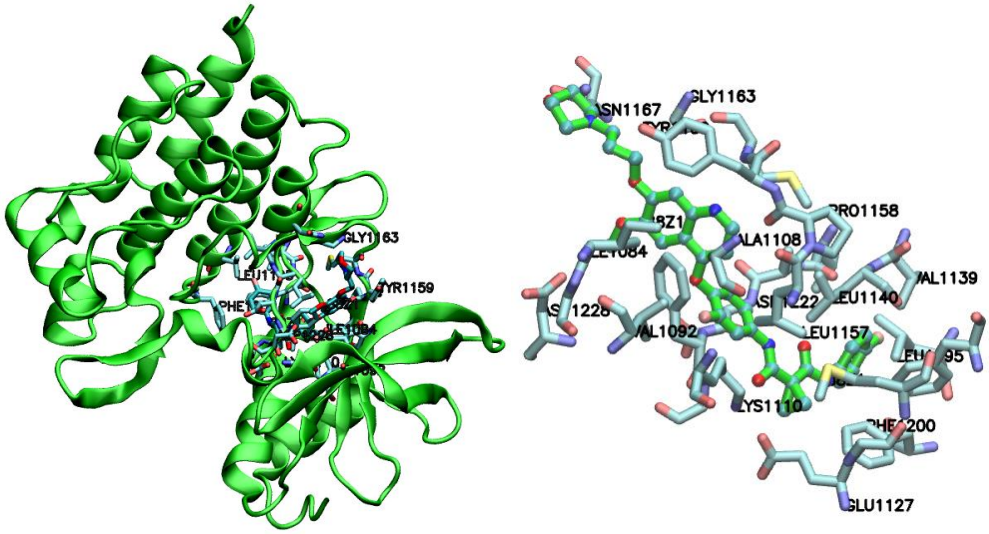
Figure 10: Amino acid residues Asp 1222, Lys110 and Asp1024 in c-Met interactions via hydrogen bond.

Figure 10 shows the interaction of the amino acid residues in the 3LQ8_c-Met through hydrogen bonds interactions (Asp1222, Lys1110 and Asp1204). The pKa values for C-MET amino acid residues such as Asp1222, Lys1110 and Asp1204 are mentioned in the table (Table 2: The pKa of the active Site residues).

Table 2: The pKa value of the active site residues Asp1222, Lys1110 and Asp1204

Amino Acid Residues	**pKa Value by PROPKA**
Asp1204	3.66
Asp1222	5.92
Lys1110	9.50

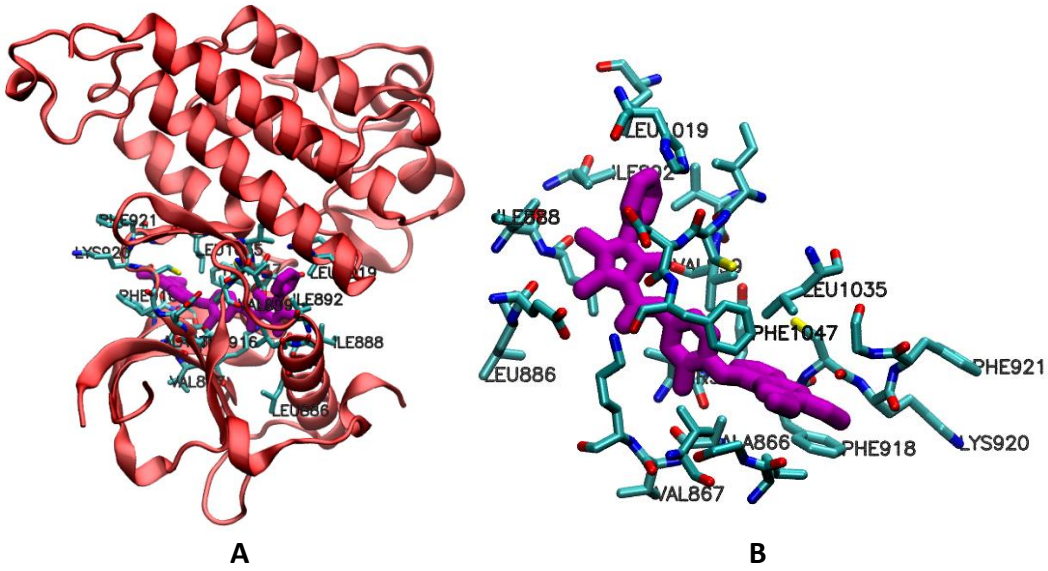
3.4 Ranking the pockets & Virtual Screening:



A **B**

Figure 11: Native C-Met complex superimposed with Model Structure (A), Zoomed image of the interaction for easy understanding (B).

In the figure11 the pockets in c-met are shown. Image B is the zoomed image of A. The residues that are selected are within the 5 Å distance of the ligand.



A **B**

Figure 12: Native VEGFR-2 complex superimposed with Model Structure (A), Zoomed image of the interaction(B).

In the figures 12 the pockets in VEGFR-2 are shown. The residues that are selected are within the 5 Å of the ligand.

Table 3: C-Met Interaction with Shortlisted FDA approved drugs Cabozantininb, Crizotininb, Oxandrolone, Fudarabine phosphate, Entacapone, Salsalate and triamterene to find the Log odds ranking, drug binding affinity and distance to catalytic residues.

Candidate Drugs	Log odds Score Ranking Method	Drug Binding Binding Affinity (kcal/mol)	Distance To Catalytic Residues (Å)
Cabozantinib[381]	-37.18	-7.7	3.05
Crizotinib[520]	-19.46	-7.9	2.4
Oxandrolone[1]	-0.64	-7.7	3.3
Fludarabine phosphate[2]	-0.64	-7.4	2.3
Entacapone[3]	-19.32	-7.5	2.15
Salsalate[4]	-19.32	-7.1	2.2
Triamterene[5]	-0.64	-7.2	2.8

As table 3 shows virtual screening results for c-Met. The ranking of the ligand was based on the distance of the ligand to the target catalytic residues, binding affinity, and log odds score function. Based on the ranking first five posed drugs would be Oxandrolone, Fludarabine phosphate, Entacapone, Salsalate and Triamterene.

Table 4: VEGFR-2 Interaction with Shortlisted FDA approved drugs Cabozatininb, Crizotinib, Dihydroergotamine, Eltrombopag, Mizolastine and telmisartan to find the Log odds ranking, drug binding affinity and distance to catalytic residues

Candidate Drugs	Log odds Score Ranking Method	Drug Binding Binding Affinity (kcal/mol)	Distance To Catalytic Residues (Å)
Cabozantinib[1]	-12.24	-7.6	7.7
Crizotinib[853]	-37.14	-7.2	7.3
aDihydroergotamine[2]	-12.78	-8.0	7.6
Eltrombopag[3]	-12.78	-9.5	8.8

Mizolastine[4]	-13.33	-9.4	8.8
Telmisartan[5]	-36.12	-8.3	7.8

The table 4 shows virtual screening results for VEGFR-2. Ligand ranking is based on ligand distance to the target catalytic residues, binding affinity, and log odds score ranking. According to the first drug posed ranking the drug ranks are Cabozantinib, Dihydroergotamine, Eltrombopag, Mizolastine, Telmisartan.

3.5 Protein & Drug Interactions For c-Met & VEGFR-2 With Shortlisted FDA Approved Drugs:

3.5.1 c-Met & Shortlisted FDA Approved Drug Interaction:

The catalytic domains such as 1110 Leucine, 1204 Aspartic Acid (OD2), Histidine (NE2) 1202 and Aspartic Acid 1222 (N) for HGF (c-met) interacted with the shortlisted FDA approved drugs. After molecular dynamics (10 ns) the interaction was in accordance with the average distance between the interacting residues and drug inhibitor. The FDA approved drug that was the single inhibitor for c-Met was entacapone. The dual inhibitor for c-met was Triamterene.

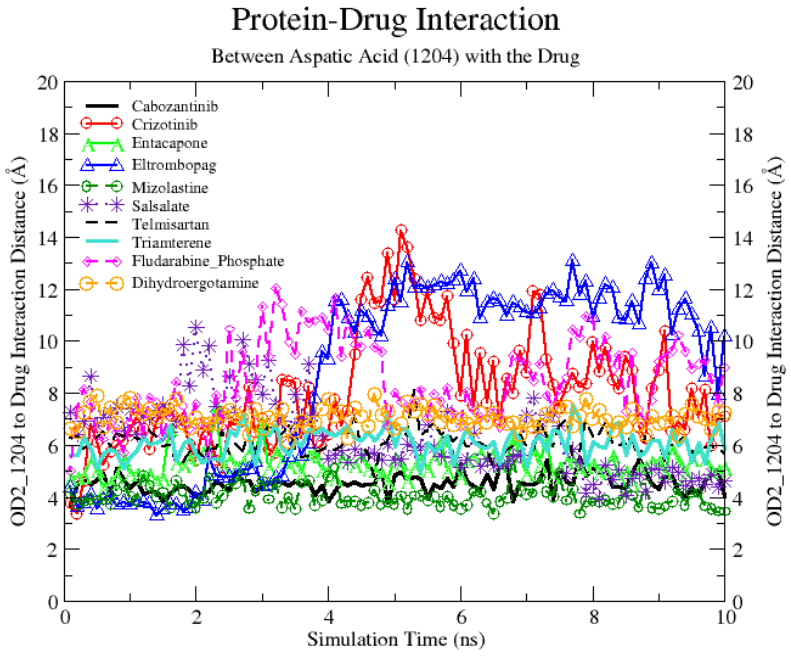


Figure 14: Protein-Drug Interaction Between Aspartic Acid (1204) & Shortlisted FDA Approved Drugs.

Figure 14 shows the protein drug interaction between Aspartic Acid (1204) which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents OD2_1204 to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). FDA approved drugs Cabozantinib, Crizotaninb, Entacapone, Eltrombopag, Mizolastine, Salsalate, Telmisartan, Triamterne, Fludarbine phosphate, Dihydrodotamine are represented by different colours for easy visibility.

The table (Table 5) show the basis of distance for the interactions of c-met with FDA approved drugs. The FDA approved drugs that interacted with c-met are Cabozatinib, Crizotaninb, Entacapone, Elthrombopag, Mizolastine, Salsalate, Telmisartan, Trimaterene, Fludarabine_phosphate and Dihydrodotamine. The table 5 includes the mean, standard deviation and standard error mean for the FDA approved drugs.

Table 5: Explanatory Statistics of the distance (Å) interactions between Aspartic Acid (1204) & Shortlisted FDA Approved Drugs.

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Cabozantinib	4.54	0.38	0.04
Crizotinib	9.69	4.10	0.41
Entacapone	5.30	0.48	0.05
Eltrombopag	8.75	3.49	0.35
Mizolastine	4.02	0.41	0.04
Salsalate	6.34	1.47	0.10
Telmisartan	6.11	0.47	0.04
Triamterene	6.07	0.52	0.05
Fludarabine_phosphate	8.49	1.53	0.15
Dihydroergotamine	6.75	1.91	0.19

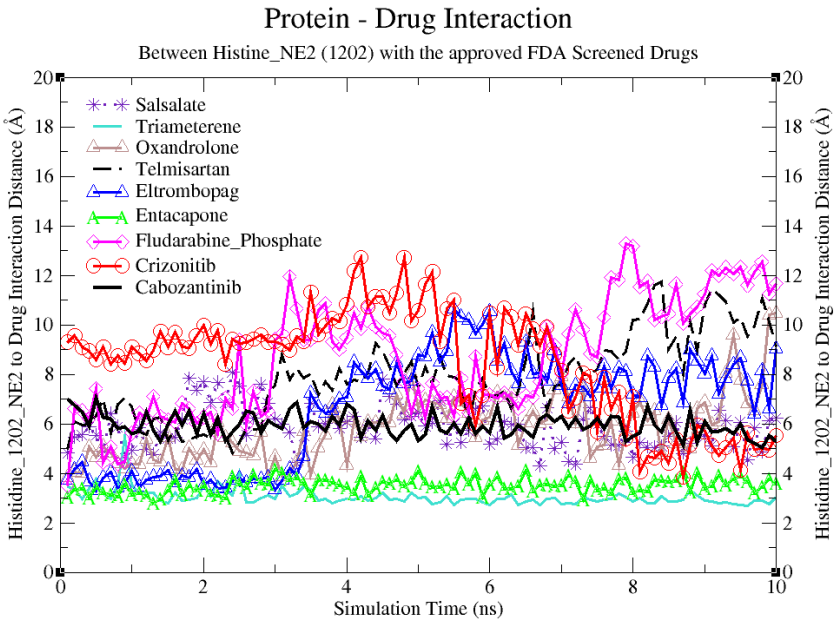


Figure 15: Protein-Drug Interaction Between Histidine_NE2 (1202) & Shortlisted FDA Approved Drugs.

Figure 15 shows the protein drug interaction between Histidine_NE2 (1202) which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents Histidine_NE2 (1202) to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Cabozantinib, Crizotaninb, Entacapone, Eltrombopag, Mizolastine, Salsalate, Telmisartan, Triamterne and Fludarbine phosphate are FDA approved drugs.

The distance for the interactions of c-met with FDA approved drugs table (Table 6) was drawn. The FDA approved drugs that interacted with c-met are Cabozatinib, Crizotaninb, Entacapone, Elthrombopag, Mizolastine, Salsalate, Telmisartan, Trimaterene and Fludarabine_phosphate. The table includes the mean, standard deviation, and standard error mean for the FDA approved drugs.

Table 6: Descriptive Statistics for the distance (Å) interactions (CMET) Between Histidine_NE2 (1202) & Shortlisted FDA Approved Drugs.

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Triamterene	3.05	0.35	0.03
Oxandrolone	5.84	1.33	0.13
Telmisartan	7.85	1.71	0.17

Eltrombopag	6.65	2.21	0.22
Entacapone	3.51	0.30	0.03
Fludarabine_Phosphate	13.18	1.43	0.14
crizotinib	8.43	2.21	0.22
Cabozantinib	5.95	0.45	0.05

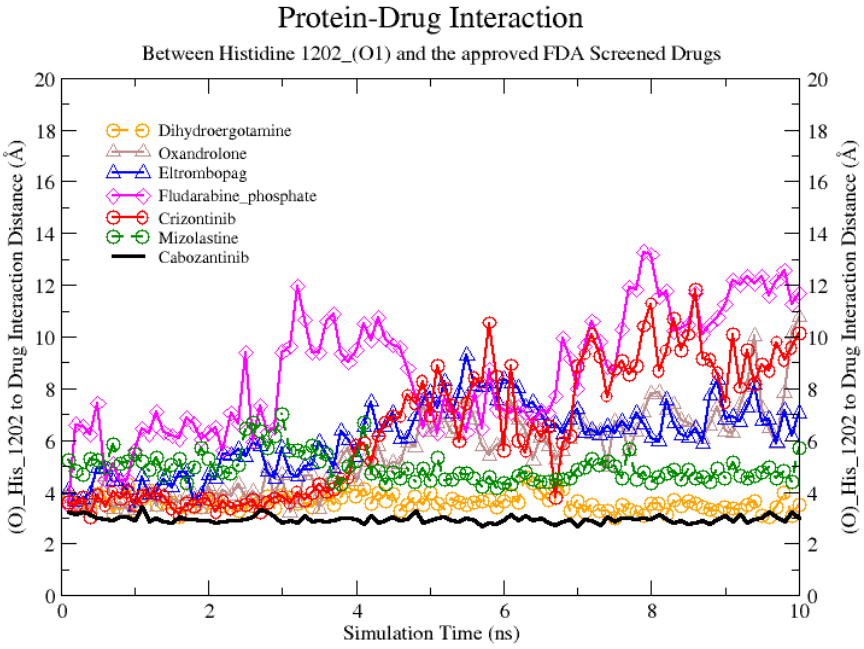


Figure 16: Protein-Drug Interaction Between Histidine_1202 (O1) & Shortlisted FDA Approved Drugs.

Figure 16 shows the protein drug interaction between Histidine 1202_O1 which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents (O)_His_1202 to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Dihydroergotamine, Oxandrolone, Cabozantinib, Crizotaninb, Eltrombopag, Mizolastine and Fludarbine phosphate are FDA approved drugs.

The distance for the interactions of c-met with FDA approved drugs following table was drawn. The FDA approved drugs that interacted with c-met are Dihydroergotamine,

Oxandrolone, Cabozantinib, Crizotaninb, Eltrombopag, Mizolastine and Fludarbine phosphate.

Table 7: Descriptive Statistics for the distance (Å) interactions Between Histidine_1202 (O1) (CMET) & Shortlisted FDA Approved Drugs.

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Dihydroergotamine	3.58	0.30	0.03
Oxandrolone	5.53	1.61	0.16
Eltrombopag	6.12	1.22	0.13
Fludarabine_Phosphate	8.66	2.32	0.23
Crizotinib	6.00	2.48	0.25
Mizolastine	5.00	0.44	0.04
Cabozantinib	2.95	0.14	0.01

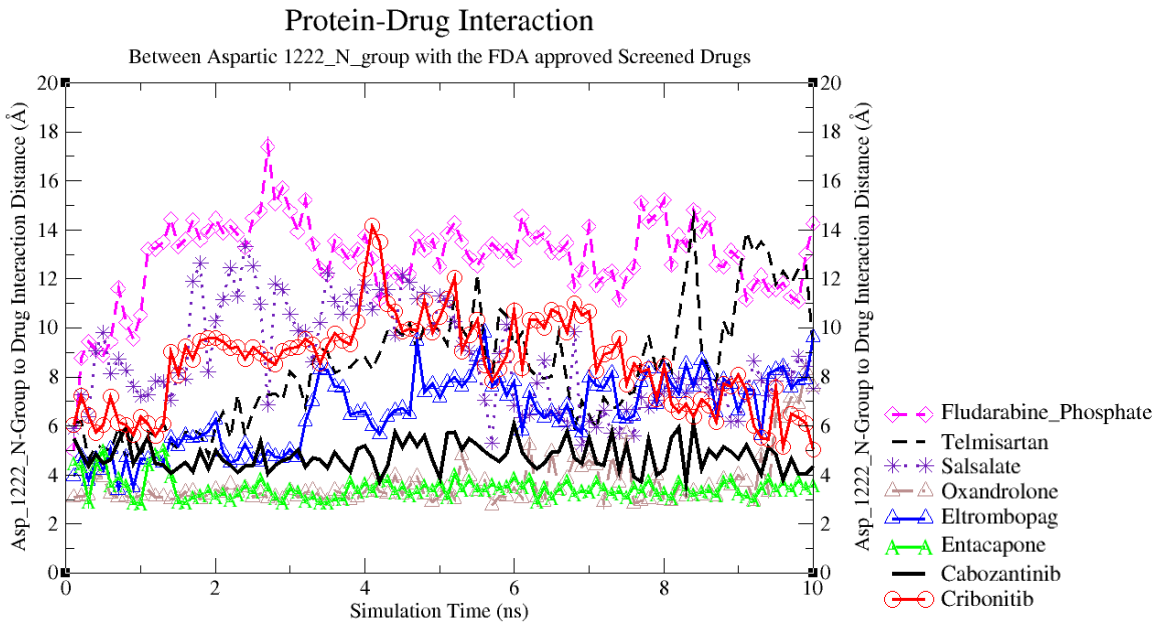


Figure 17: Protein-Drug Interaction Between Aspartic 1222_N_group & shortlisted FDA Approved Drugs.

Figure 17 shows the protein drug interaction between Aspartic 1222_N_group which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents Asp_1222_N group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Telmisartan, Oxandrolone, Cabozantinib, Crizotaninb, Eltrombopag, Salsalate, Entacapone and Fludarbine phosphate are FDA approved drugs.

The distance for the interactions of c-met with FDA approved drugs table (Table 8) was drawn. Telmisartan, Oxandrolone, Cabozantinib, Crizotaninb, Eltrombopag, Salsalate, Entacapone and Fludarbine phosphate are FDA approved drugs.

Table 8: Descriptive Statistics for the distance (Å) interactions (CMET) Between Aspartic 1222_N_group (CMET) & FDA Approved Drugs.

Drugs	**Mean distance (Å)**	**STD**	**SEM**
Fludarabine_Phosphate	12.88	1.70	0.171
Eltrombopag	6.58	1.47	0.14

Telmisartan	8.39	2.35	0.24
Salsalate	8.72	2.06	0.21
Oxandrolone	3.67	1.05	0.11
Entacapone	3.47	0.44	0.04
Crizotinib	8.57	1.87	0.19
Cabozantinib	4.77	0.58	0.06

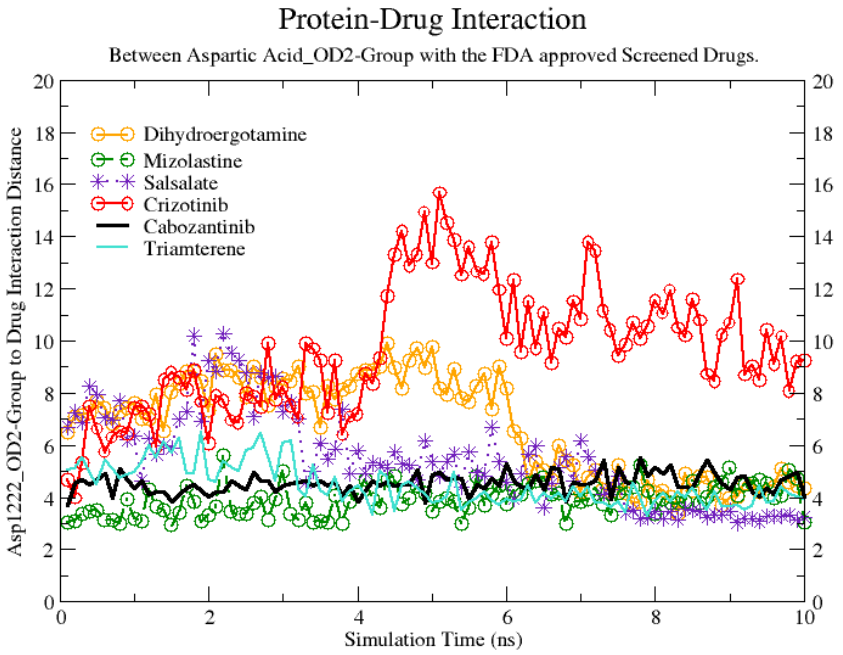


Figure 18: Protein-Drug Interaction Between Aspartic Acid_OD2_group & shortlisted FDA Approved Drugs.

Figure 18 shows the protein drug interaction between Aspartic Acid_OD2_group which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents Asp_1222_OD2 group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Mizolastine and Salsalate are FDA approved drugs.

The distance for the interactions of c-met with FDA approved drugs table (Table 9)_ was drawn. Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Mizolastine and Salsalate are FDA approved drugs.

Table 9: Descriptive Statistics for the distance (Å) interactions Between Aspartic Acid_OD2_group (CMET) & shortlisted FDA Approved Drugs.

Drugs	Mean Distance (Å)	STD	SEM
Dihydroergotamine	6.75	1.91	0.19
Mizolastine	3.89	0.59	0.06
Crizotinib	8.09	2.19	0.22
Triamterene	4.46	0.77	0.077
Cabozantinib	4.54	0.38	0.04

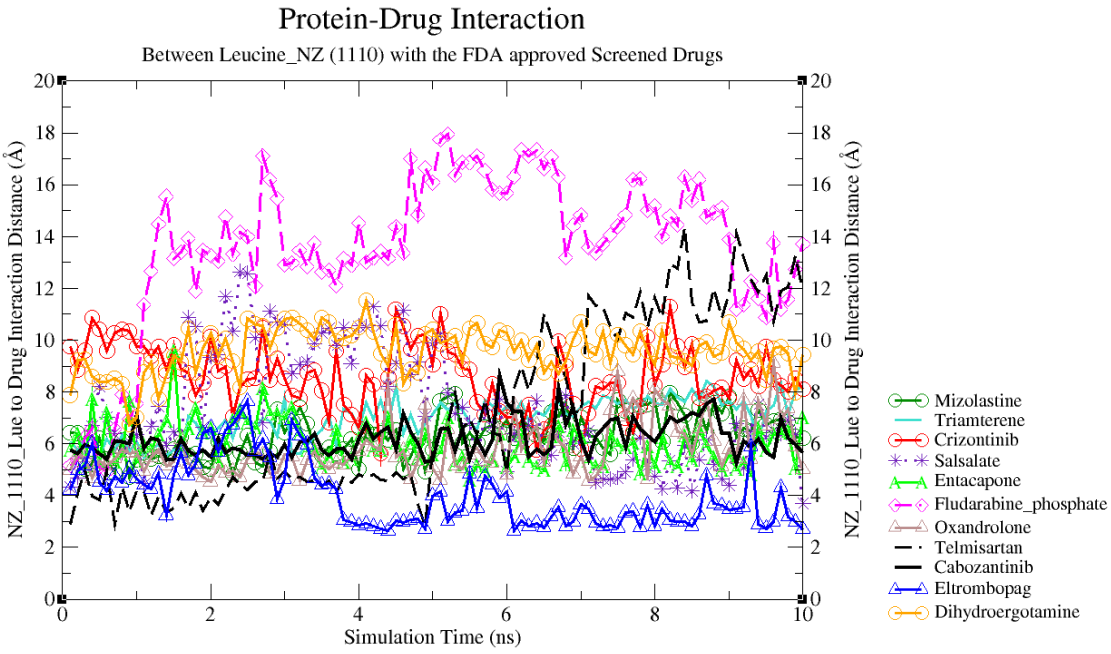


Figure 19: Protein-Drug Interaction Between Leucine_NZ (1110) group & shortlisted FDA Approved Drugs

Figure 19 shows the protein drug interaction between Leucine_NZ (110) group which is a catalytic domain for HGF (c-met) and screened FDA approved drugs. In the graph the x-axis represents NZ (110)_Lue_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Mizolastine and Salsalate, Entacapone, Oxandrolone, Telmisartan and Eltrombopag are FDA approved drugs.

On the basis of distance for the interactions of c-met with FDA approved drugs table (Table 10) was drawn. Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Mizolastine and Salsalate, Entacapone, Oxandrolone, Telmisartan and Eltrombopag are FDA approved drugs.

Table 10: Descriptive Statistics for the distance (Å) interactions Between Leucine_NZ (1110) group (C-Met) & shortlisted FDA Approved Drugs

Drugs	**Mean Distance (Å)**	**Standard Deviation (STD)**	**SEM**
Cabozantinib	6.19	0.68	0.07
Crizotinib	8.60	1.25	0.13
Entacapone	6.71	0.82	0.08
Eltrombopag	3.86	1.27	0.12
Mizolastine	6.32	0.81	0.08
Oxandrolone	5.83	1.01	0.1
Salsalate	7.33	2.24	0.22
Telmisartan	7.21	3.40	0.22
Triamterene	6.99	0.74	0.07
Fludarabine_phosphate	13.59	2.98	0.29

Dihydroergotamine	9.58	0.86	0.09

3.5.2 VEGFR-2 & Shortlisted FDA Approved Drug Interaction:

The catalytic domains such as Cystine 919, Cystine 1024, Lys868, Asp 1046, and Asp 1028 for VEGF (VEGFR-2) interacted with the shortlisted FDA approved drugs. After molecular dynamics (10 ns) the interaction was in accordance with the average distance between the interacting residues and drug inhibitor. The FDA approved drug that was the single inhibitor for VEGFR-2 was Telmisartan. The dual inhibitor for VEGFR-2 was Triamterene.

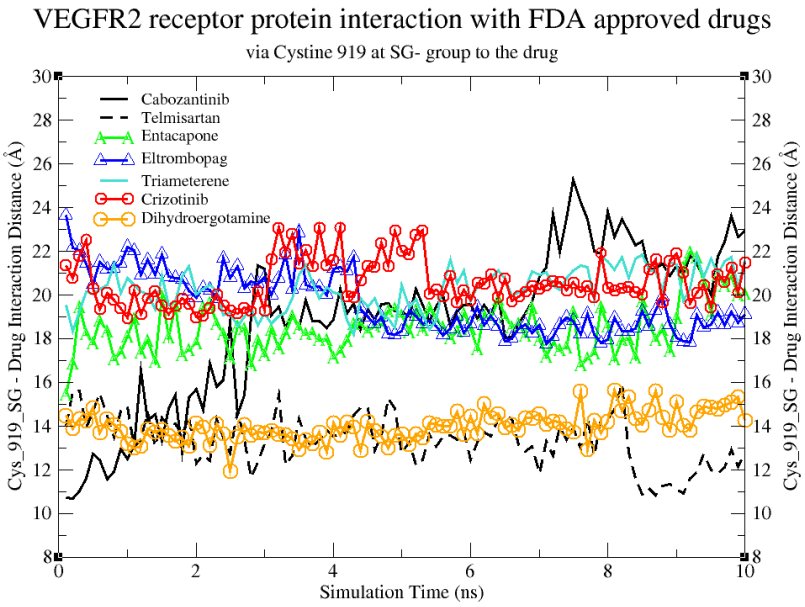


Figure 20: Protein-Drug Interaction Between Cystine 919 SG group & shortlisted FDA Approved Drugs.

Figure 20 shows the protein drug interaction between Cystine 919 at SG _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Cys 919 at SG Lue_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Entacapone, Telmisartan, Dihydroergotamine and Eltrombopag are FDA approved drugs.

On the basis of distance for the interactions of c-met with FDA approved drugs table (Table 11) was drawn. Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Entacapone, Telmisartan, Dihydroergotamine and Eltrombopag are FDA approved drugs.

Table 11: Descriptive Statistics for the distance (Å) interactions Between Cystine 919 SG group (VEGFR-2) & shortlisted FDA Approved Drugs.

Statistics for distance interactions (VEGFR2 - Cys_919_SG)			
Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Cabozantinib	18.76	3.46	0.35
Telmisartan	13.35	1.15	0.12
Entacapone	18.50	1.12	0.11
Eltrombopag	19.68	1.39	0.13
Triamterene	20.27	0.93	0.09
Crizotinib	20.59	1.07	0.11
Dihydroergotamine	13.99	0.66	0.07

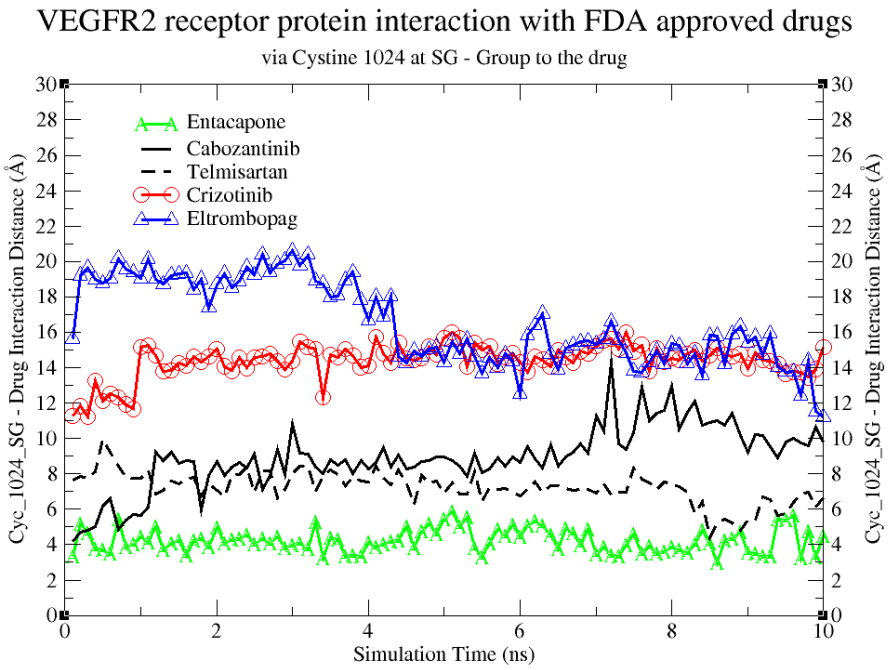


Figure 21: Protein-Drug Interaction Between Cystine 1024 SG group & Shortlisted FDA Approved Drugs.

Figure 21 shows the protein drug interaction between Cystine 1024 at SG _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Cys 1024 at SG Lue_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Cabozantinib, Crizotaninb, Entacapone, Telmisartan, and Eltrombopag are FDA approved drugs. On the basis of distance for the interactions of c-met with FDA approved drugs table (Table 12) was drawn. Cabozantinib, Crizotaninb, Entacapone, Telmisartan, and Eltrombopag are FDA approved drugs.

Table 12: Descriptive Statistics for the distance (Å) interactions Between Cystine 1024 SG group (VEGFR-2) & Shortlisted FDA Approved Drugs

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Entacapone	4.22	0.66	0.07
Cabozantinib	8.94	1.77	0.17
Telmisartan	7.22	0.89	0.09
Crizotinib	14.39	0.96	0.1
Eltrombopag	16.54	2.34	0.24

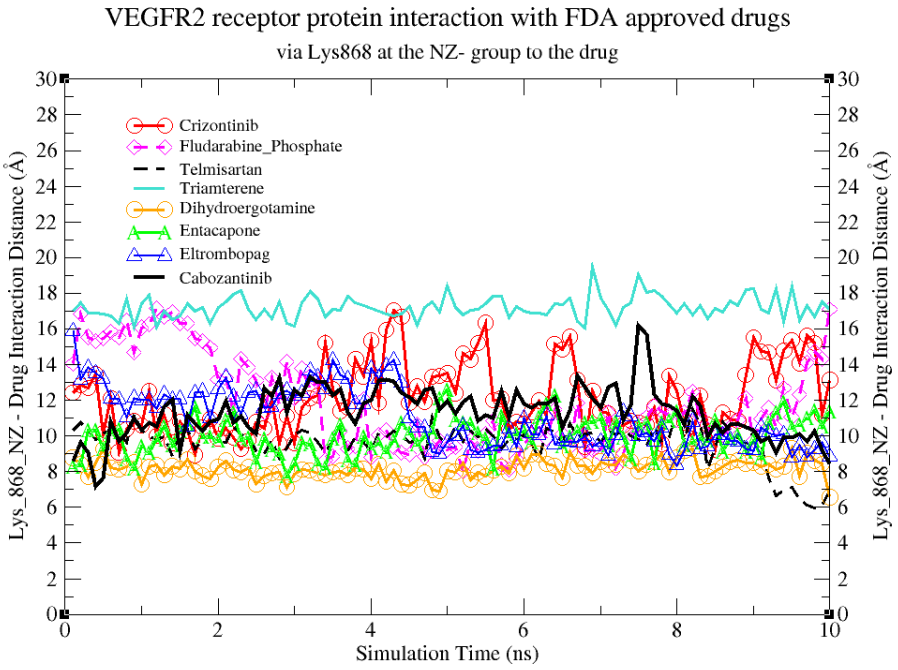


Figure 22: Protein-Drug Interaction Between Lys868 NZ group & Shortlisted FDA Approved Drugs.

Figure 22 shows the protein drug interaction between Lys 868 at SG _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Lys 868at SG Lue_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Entacapone, Telmisartan, Dihydroergotamine, Fludarabine_phosphate and Eltrombopag are FDA approved drugs. Triamterene, Dihydroergotamine, Cabozantinib, Crizotaninb, Entacapone, Telmisartan, Dihydroergotamine, Fludarabine_phosphate and Eltrombopag are FDA approved drugs, and their Mean, Standard deviation and standard error mean is shown in table (Table 13).

Table 13: Descriptive Statistics for the distance (Å) interactions Between Lys868 NZ group (VEGFR-2) & Shortlisted FDA Approved Drugs

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Crizotinib	12.14	1.98	0.20
Fludarabine_phosphate	11.81	2.57	0.26
Telmisartan	9.64	1.09	0.11

Triamterene	17.20	0.62	0.06
Dihydroergotamine	8.17	0.53	0.05
Entacapone	10.02	0.99	0.10
Eltrombopag	11.02	1.63	0.16
Cabozantinib	11.32	1.42	0.14

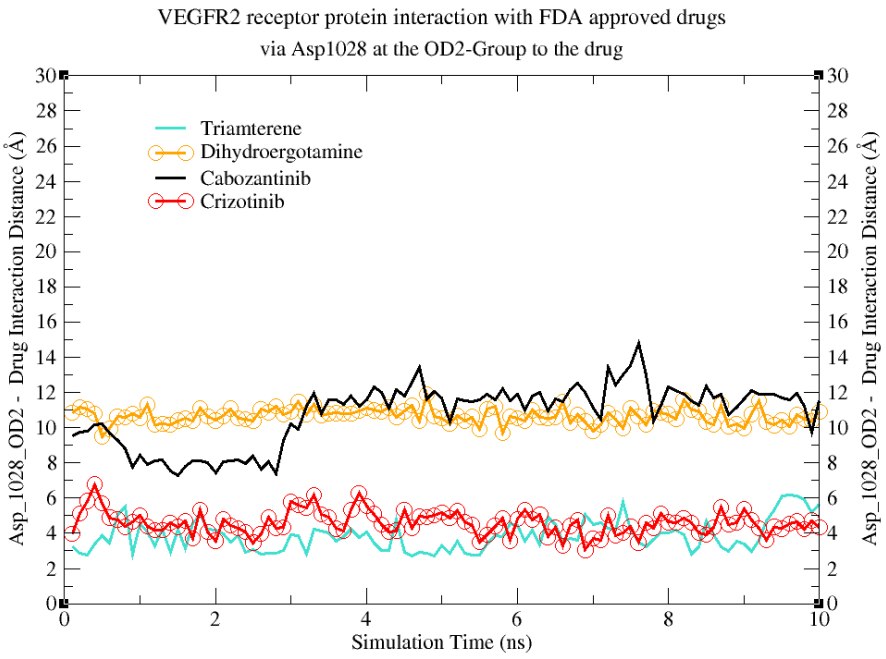


Figure 23: Protein-Drug Interaction Between Asp 1028 OD2 group & Shortlisted FDA Approved Drugs

Figure 23 shows the protein drug interaction between Asp 1028 at OD2 _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Asp 1028 OD2_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Triamterene, Dihydroergotamine, Cabozantinib and Crizotaninb are FDA approved drugs. Table (Table 14) shows the descriptive statistics for the distance interaction of vegfr-2 and Triamterene, Dihydroergotamine, Cabozantinib and Crizotaninb are FDA approved drugs.

Table 14: Descriptive Statistics for the distance (Å) interactions Between Asp 1028 OD2 group (VEGFR-2) & Shortlisted FDA Approved Drugs

Drugs	Mean Distance (Å)	STD	SEM
Triamterene	3.85	0.87	0.09
Dihydroergotamine	10.66	0.43	0.04
Cabozantinib	10.78	1.70	0.17
Crizotinib	4.59	0.66	0.07

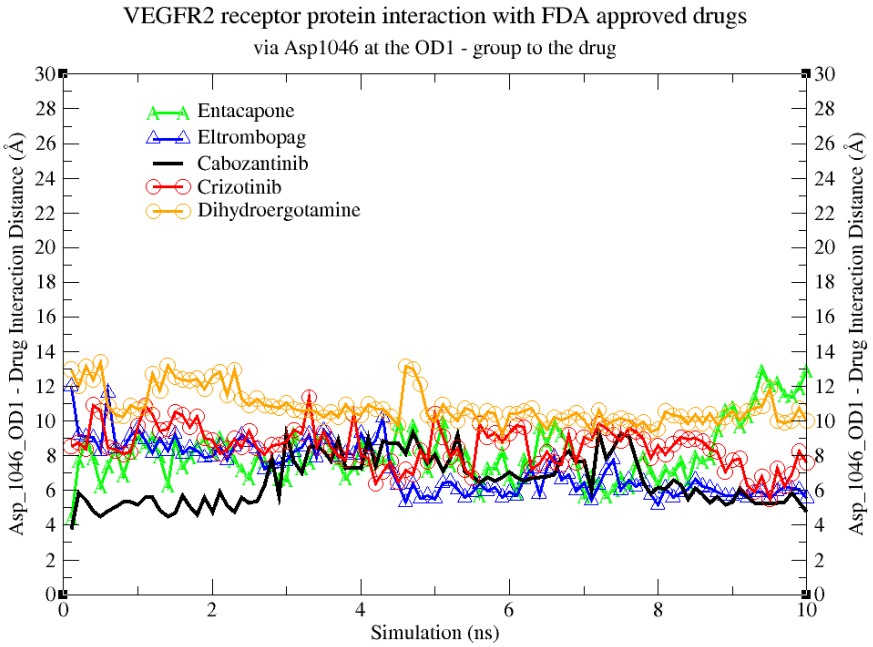


Figure 24: Protein-Drug Interaction Between Asp 1046 OD1 group & Shortlisted FDA Approved Drugs.

Figure 24 shows the protein drug interaction between Asp 1046 at OD1 _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Asp 1046 OD1_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Entacapone, Eltrombopag, Dihydroergotamine, Cabozantinib and Crizotaninb are FDA approved drugs.

The Table (Table 15) shows the descriptive statistics for the distance interaction of vegfr-2 and Entacapone, Eltrombopag, Dihydroergotamine, Cabozantinib and Crizotaninb are FDA approved drugs.

Table 15: Descriptive Statistics for the distance (Å) interactions Between Asp 1046 OD1 group (VEGFR-2) & Shortlisted FDA Approved Drugs.

Drugs	Mean Distance (Å)	STD	SEM
Entacapone	8.16	1.64	0.16
Eltrombopag	7.24	1.46	0.10
Cabozantinib	6.57	1.41	0.14
Crizotinib	8.54	1.16	0.12
Dihydroergotamine	10.81	1.00	0.10

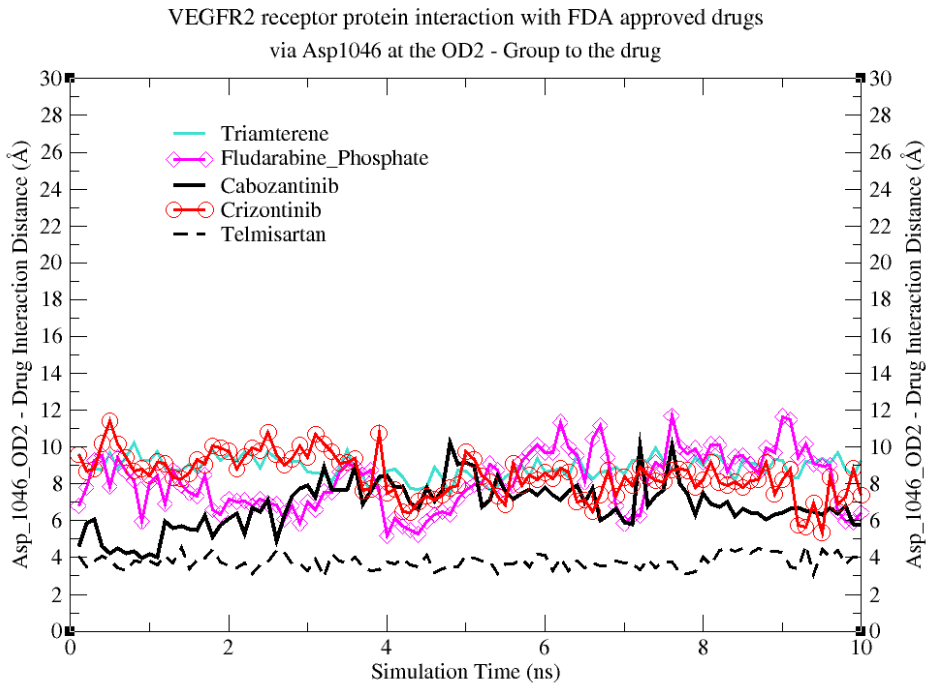


Figure 25: Protein-Drug Interaction Between Asp 1046 OD2 group & Shortlisted FDA Approved Drugs.

Figure 25 shows the protein drug interaction between Asp 1046 at OD2 _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Asp 1046 OD2_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Trimaterene, Fludarabine_phosphate, Telmisartan, Cabozantinib and Crizotaninb are FDA approved drugs. Table (Table 16) shows the descriptive statistics for the distance interaction of vegfr-2 and Trimaterene, Fludarabine_phosphate, Telmisartan, Cabozantinib and Crizotaninb are FDA approved drugs.

Table 16: Descriptive Statistics for the distance (Å) interactions Between Asp 1046 OD2 group (VEGFR-2) & Shortlisted FDA Approved Drugs.

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Triamterene	8.89	0.56	0.06
Fludarabine_phosphate	8.15	1.57	0.16
Cabozantinib	6.81	1.29	0.13
Crizotinib	8.47	1.15	0.12
Telmisartan	3.78	0.39	0.04

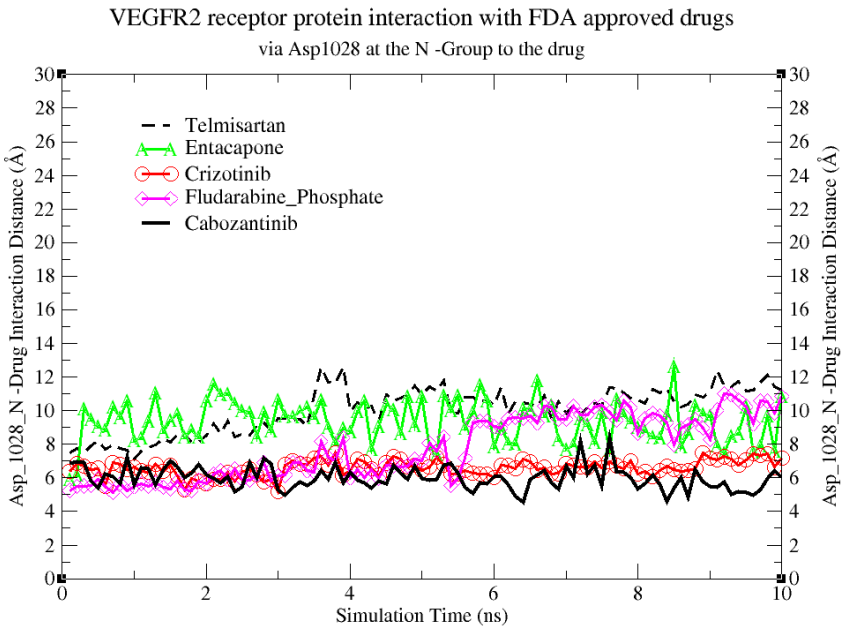


Figure 26: Protein-Drug Interaction Between Asp 1028 N group & Shortlisted FDA Approved Drugs.

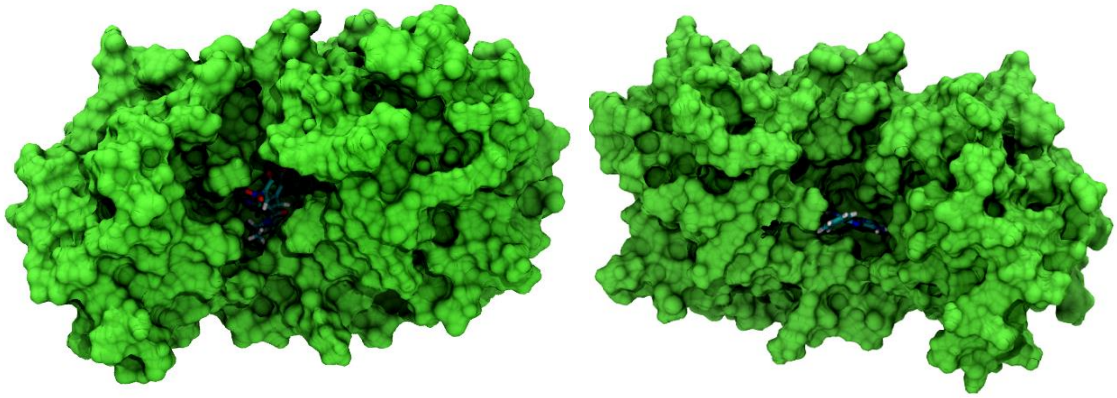
Figure 26 shows the protein drug interaction between Asp 1046 at OD1 _group which is a catalytic domain for VEGFR-2 and screened FDA approved drugs. In the graph the x-axis represents Asp 1046 OD1_ group to drug interaction distance in angstrom (Å) and the y-axis represents the simulation time in nanoseconds (ns). Fludarabine_phosphate, Telmisartan, Entacapone, Cabozantinib and Crizotaninb are FDA approved drugs. Table (Table 17) shows the descriptive statistics for the distance interaction of vegfr-2 and Fludarabine_phosphate, Telmisartan, Entacapone, Cabozantinib and Crizotaninb are FDA approved drugs.

Table 17: Descriptive Statistics for the distance (Å) interactions Between Asp 1028 N group (VEGFR-2) & Shortlisted FDA Approved Drugs.

Drugs	**Mean Distance (Å)**	**STD**	**SEM**
Telmisartan	8.63	1.03	0.10
Entacapone	9.35	1.21	0.12
Crizotinib	6.58	0.47	0.05
Fludarabine_phosphate	7.78	1.87	0.19

Cabozantinib	6.00	0.68	0.07

3.6 Single & Dual Inhibitor with Binding sites In c-Met:

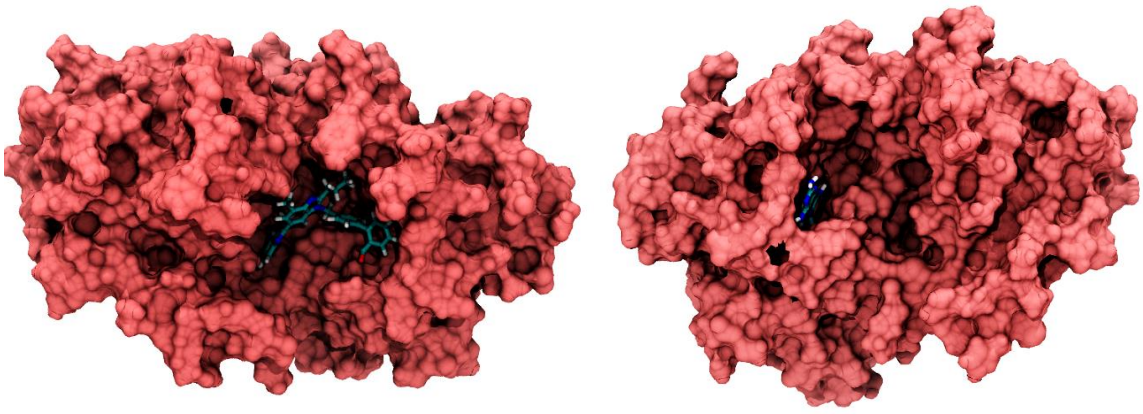


A B

Figure 27: Front side image of binding of Entacapone in c-met pocket (A), Front side image of binding of Triamterene in c-met pocket (B).

Figure 27 show the FDA approved drugs i.e, single and dual inhibitors binding with the c-met pocket. The figure shows the front binding face of the receptor. Image A shows the single inhibitor Entacapone binding with the c-met pocket and the image B shows the dual inhibitor Triamterene binding with the c-met pocket.

3.7 Single & Dual Inhibitor with Binding Sites In VEGFR-2:



A B

Figure 28: Front side image of binding of Telmisartan in VEGFR-2 pocket (A), Front side image of binding of Triamterene in VEGFR-2 pocket (B)

Figures 28 show the FDA approved drugs i.e., single, and dual inhibitors binding with the VEGFR-2 pocket. The figure shows the front binding face of the receptor. Image A shows the single inhibitor Telmisartan binding with the VEGFR-2 pocket and the image B shows the dual inhibitor Triamterene binding with the VEGFR-2 pocket.

Chapter 4 - Discussion

The aim of this study is to find an FDA approved drug that is a novel dual inhibitor for the tyrosine kinase receptors c-Met and VEGFR-2 in cancer. The study used computational analysis methods such as pocketome analysis, virtual drug screening and molecular docking to find the multikinase inhibitors for cancer from the shortlisted FDA approved drugs. Out of 2016 FDA approved drugs 11 drugs were shortlisted. These shortlisted 11 drugs were tested separately with both tyrosine kinase receptors c-met and VEGFR-2 to find the drug that would co-inhibit both of the receptors. While trying to find a co-inhibitor it was found that these two receptors have separate single inhibitors for example, c-met's single inhibitor is entacapone and VEGFR-2 single inhibitor is telmisartan. While they both have different single inhibitors there is one common drug that inhibits both of the receptors in cancer that is triamterene. This section will in-depth clarify not only the steps taken in study to achieve this outcome but also expand on the research around the topic.

The relationship between VEGFR-2 expression and an invasive breast cancer, one of many cancer types was analysed and it was found that out of 100 samples taken of breast carcinoma 86% showed positive VEGFR-2. The high expression of VEGFR-2 was linked to positive lymph node and substandard prognosis which led to the conclusion that there is a relationship between VEGFR-2 expression and high metastasis in cancer (Yan et al., 2015). In membrane, cytoplasm and in the endothelial VEGFR-2 was detected. The mechanisms involving VEGFR-2 that possibly regulate EMT to encourage breast cancer metastasis and progression (Yan et al., 2015). Like breast cancer the relationship between VEGFR-2 and gastric cancer also exists (Lian et al., 2019). A study focusing on the tumorigenesis, metastasis, and pro-angiogenesis of VEGFR-2 in cancer used immunohistochemistry, Real time PCR, western blots to identify the expression of VEGFR-2 & VTN among specimens collected and cell counting to find out cell proliferation. It was concluded that the overexpression of VEGFR-2 in gastric cancer cells not only increases the cell proliferation but also invasiveness in vitro and formation of tumour cells. It was also found that VTN was expressed simultaneously with VEGFR-2 and was consistent in regulating cell growth and poor survival rate in vitro and as well as in vivo (Lian et al., 2019.

In a study in which how crucial the signalling of VEGFR-2 is in pancreatic cancer was investigated using RT-PCR and western blots. In addition, invasion assay and wound healing assay were used to observe the effects of antibodies such as sunitinib and bevacizumab on VEGFR-2 in pancreatic cancer cells (Doi et al., 2011). It was found that not only pancreatic cells expressed VEGFR-2 but also the inhibitors sunitinib and bevacizumab readily reduced the rate of motility in pancreatic cancer cells. Thus, proving that VEGF -A / VEGFR-2 play a crucial role and can greatly influence the invasion and migration of pancreas cancer cells (Doi et al., 2011). A recent study observed the importance of VEGFR-1, VEGFR-2 expression in non-tumour cells in oesophageal cancer involving bone marrow derived cells effects on cancer cells (Xu et al., 2014). In vivo it was observed that with the structural and comprehensive hindrance of VEGFR-1 and VEGFR-2 using the antibodies can remarkably

suppress not only angiogenesis, metastasis but also the growth of oesophageal tumour in mice (Xu et al., 2014). It was also observed that the two receptors, VEGFR-1 and VEGFR-2 can prove to be a valid target for the therapeutic measures for cancer. Anaplastic thyroid cancer is one of the most fatal types of cancer among human cancers (Gule et al., 2011). In a study where whether VEGFR-2 and EGFR targeted therapy can inhibit the growth of tumour in thyroid cancer was investigated it was observed that vandetanib, an inhibitory drug can inhibit the EGFR and VEGFR-2 in vivo. Making it an effective approach for anaplastic thyroid cancer therapy (Gule et al., 2011). The expression of VEGFR-2 in colorectal cancer cells is high.

To investigate the molecular mechanisms that are initiated with the angiogenesis and progression in prostate cancer immunohistochemical and the analysis of prostate tissue specimens from the place of their origin of mouse was tested (Huss et al., 2001). As the prostate cancer progressed it was observed that the prediction characteristics of TRAMP model were verified and that the delay in some of the molecular mechanisms can provide a foundation for the 'progression switch' model. which would be able to explain changes that might occur in result of an antiangiogenic therapy for the progression of tumour in prostate cancer (Huss et al., 2001). Studies have shown that the novel inhibitors such as VEGFR-2 have direct corelation with the tumour angiogenesis. A recent study investigated and observed that the higher the expression of VEGFR-2 is the aggressive cancer would be making VEGFR-2 a fundamental mediator in angiogenesis of cancer (Fontanella et al., 2014). Therefore, finding drugs that would target VEGFR and VEGFR-2 specifically can prove to be a reliable treatment for cancer.

The tyrosine kinase receptor, C-Met which belongs to hepatocyte growth factor (HGF) is involved in wide range of cellular mechanisms such as oncogenesis, tumour progression and cellular invasiveness in human cancer (Peruzzi, 2006). Three main signalling pathways are involved that help c-Met oncogenic behaviour ligand and receptor interaction, inhibition of the catalytic mechanisms of tyrosine kinase receptor and obstruction of the receptor intracellular interactions (Peruzzi, 2006). Powerful and strong inhibitory novel drugs that can help with cancer treatment are formed by keeping all of these three properties in consideration. In a study where the aim was to investigate that whether c-met is a marker for pancreatic cancer stem cells and if c-met can be used as a targeted therapy NOD-SCID mice were used. The ability of pancreatic cancer cells to rejuvenate was tested with the help of in vitro assays and was studied with high expression of c-met and low expression of c-met (Li et al., 2011). It was observed that c-met proves to be a marker for pancreatic cancer and that the both growth and metastasis of pancreatic cancer cells in mice are dependent on c-met signalling pathways and c-met expression therefore, c-met targeted drug therapy would be functional in terms of treating cancer (Li et al., 2011). Among the reviews that in depth focus on c-met signalling pathways from the molecular level to the clinical evidence one of them observed that even targeted therapy approach for c-met (HGF) has contributed to cancer the single inhibitor therapy is not as efficient as the dual inhibitor targeted therapy in treating cancer (Fu et al., 2021).

The expression of c-met (HGF) and VEGF-C play crucial role in the progression of primary tumour. According to a study that investigated whether the high expression of mRNA in c-met and VEGF-C would be able to predict the invasiveness and metastasis of tumour in primary colorectal cancer or not (Takeuchi et al., 2003). Using RT-PCR assay and immunohistochemistry analysis the study detected that the elevated expression of mRNA c-met shows that its expression is a crucial marker to indicate the early onset for the metastasis and invasive traits in primary colorectal cancer (Takeuchi et al., 2003). A study that explored the expression of c-met with liver metastasis in relation to gastric cancer examined stage IV gastric cancer cases with C-met (HGF) expression (Amemiya et al., 2002). Cases were divided in to two groups one group with liver metastasis and the second group with no liver metastasis. It was observed that the group with liver metastasis significantly had lower rate of survival than the group with no liver metastasis. Therefore, it was concluded that the elevated expression of c-met in the carcinoma cells may be related to liver metastasis pathways in gastric cancer and that the overexpression of c-met can work as a crucial indicator of the liver metastasis signalling pathways in gastric cancer cells (Amemiya et al., 2002).

The mutation characteristics of c-met were investigated in a study in relation to non-small cell lung cancer (NSCLC). In all the samples with NSCLC tumour the c-met expression was observed, 61% of the tumour tissues showed strong expression of c-met. The in-depth study of the alterations in c-met within semaphoring domain and juxta membrane domains in NSCLS cell lines demonstrated that the targeted therapy for small RNA interfering signalling pathways inhibition of c-met plays a crucial role in NSCLC (Ma et al., 2005). The expression of c-met and HGF and their role in breast carcinoma was investigated in a study using immunohistochemistry and in situ hybridization. The staining technique used to study the 88 cases of breast carcinoma was 'front accentuation pattern'. After corelating the expression of c-met and HGF with the rate of patient survival, high ki-67 labelling index and histologic grade it was concluded that the signalling pathways of c-met / HGF can operate as an agent that can trigger mitosis in the cells in breast cancer, which can lead to some clinical complications with patient survival. The prognostic characteristics and values of c-met in breast cancer were investigated in a study using overall survival (OS) and relapse free survival (RFS) rates. The measures for overall survival rate and relapse free survival included hazards ratios (HRs) in relation to the expression of c-met. The findings of the study were that c-met high expression was correlated with less OS and RFS in the western patients and c-met expression was not corelated with RFS or OS in Asian patients (Yan et al., 2015).

A recent study investigated the expression of c-met and its corelation with metastasis and inhibition of tumour growth in prostate cancer cells. When an adenovirus decreased the expression of c-met in an extremely metastatic human prostate cancer cell line PC3-LN4 (Kim et al., 2003). Along with c-met the expression of extracellular signal regulated kinase phosphorylation and VEGFR expressions were also investigated in vitro. It was concluded that the decreased expression of c-met can considerably inhibit not only the growth of tumour but also decreases the lymph node metastasis in prostate cancer cell lines among the mouse models (Kim et al., 2003). For that reason, it is considered that the c-met

targeted therapy for cancer would play a crucial role in controlling and lowering tumour growth and metastasis in prostate cancer. One of the studies explored the function, role, and reaction of neutrophils in relation with the receptor c-met in cancer. It was observed that not only the c-met / HGF signalling induce a response from neutrophils in relation to cancer immunotherapies but also the immunosuppressive characteristics of neutrophils provoke the expansion and the functions of T cells. Therefore, the c-met inhibition can prove to be a reliable course of action in treating cancer (Glodde et al., 2017).

In order to explore the potency of the inhibition of c-met in prostate cancer cells in a study researchers used MTS assay and cell proliferation assay with prostate cancer cell lines for humans (Tu et al., 2010). In addition to the MTS and cell proliferation assay renal subcapsular and orthotopic xenograft mouse models were also used. It was found that both c-met inhibitory molecules (PHA-665752 and PF-2341066) had an effect on the prostate tumour cells and that PF-23410066 significantly decreased the tumour growth of prostate cancer cells (Tu et al., 2010). The cell proliferation decreased significantly when c-met inhibitors were combined with androgen ablation therapy (Tu et al., 2010). In a similar study in vitro where the focal point was BMS-777607, C-Met inhibitor expression in prostate cancer cells (PC3) and DU145 cells found that the BMS-777607 can play a crucial role and influence the downregulation of c-met. A dose of BMS-777607 in certain concentration not only vigorously obstructed the HGF induced autophosphorylation of c-met but also the Akt downstream activation. Which led to the conclusion that the approach where c-met is targeted and inhibited can prove to be a reliable plan of action in treating prostate cancer (Dai and Siemann, 2010). Therefore, it is especially important to inhibit c-met receptor as a way to treat cancer.

Various studies demonstrate that the escalated levels of c-met activation have a corelation with drug resistance in VEGFR-2 inhibitors (Lai et al., 2018). Which leads to the observation that inhibition of both the receptors VEGFR-2 & c-met could possibly assist in overcoming the drug resistance complications for VEGFR-2. A study focusing on targeted dual inhibition of signalling pathways of VEGFR-2 and c-met with the help of foretininb and its effects on tumours in gastric cancer conducted animal studies using mice (Grojean et al., 2021). Immunohistochemical and immunoblot analysis approaches were used. The patients with high expression of c-met, NPT and foretininb showed the inhibition of the tumour growth. The survival rate of patients with foretininb was 100% compared to NTP which was 83%. This study showed that the targeted anticancer therapy, the suppression of receptors vegfr-2 and c-met at the same time can be beneficial in cancer treatment. The suppression and dual inhibition approach of vegfr-2 and c-met was conducted in a study using the FDA approved drug cabozantinib in prostate cancer bone metastasis (Lee et al., 2018). Prostate cancer cell line (PC3) were used to observe the inhibition of VEGFR-2 and c-met in vivo, they were cabozantinib resistant. It was concluded that the simultaneous inhibition of c-met and VEGFR-2 are associated with decreased tumour in prostate cancer bone metastasis (Lee et al., 2018). In another study that focused on cabozantinib as an inhibitor of Met, Ret and VEGFR-2, tyrosine kinase receptors conducted clinical phase I and clinical phase II research among various invasive types of cancer. The type of cancers among which test were carried out included thyroid cancer, NSCLS, breast cancer, ovarian cancer,

pancreatic cancer, and prostate cancer tumours. The early stages showed that lymph node metastasis and progression of bone has a corelation with high expression of c-met. It was concluded that in phase I and II of the trials cabozatininb exhibited prominent results in patients with prostate cancer and continuation of trials III are ongoing to confirm if prostate cancer patients can be pre-treated with the help of cabzatininb (Grüllich, 2014).

One of many cancer types is heads and neck squamous cell carcinoma (HNSCC) with the incidence increasing rapidly and it is predicted to be risen by 30% by the year 2030 (Zhang et al., 2018). A study exploring the regulations of c-met and HGF signalling pathways in the head and neck cancer found that the apoptosis led by the FDA approved drug crizotinib in vivo decreased the rapid of growth of tumour cells. As the activation of the pathways P13K plays a crucial role in the development of the tumour therefore the inhibition of this pathway would be able to assist in the treatment of cancer but there are few limitations that have been observed with this approach for the treatment of cancer. One of the limitations is the resistance in the intercellular matrix which leads to the decrease in efficacy of the inhibitors for PI3K pathways.

Several studies have investigated the small molecular kinase inhibitors which have been approved by the FDA. Few of the characteristics of the FDA approved drugs that were observed in a study that explored the small molecule kinase inhibitors approved by the US food and drug administration were that all of the small molecule kinase receptors have no more than five rings, the rings range from 3 to 5. Not only that but also most of the small molecule kinase inhibitors were within the range of 400 to 600 in terms of their molecular weight. With the gradual increase of small molecular kinase inhibitors approval by FDA it can be observed that the increasingly new studies are being done (Wu, Nielsen and Clausen, 2016).

In a study the effects of triamterene on HCT116 and CT26 in colon rectal cancer were studied in vitro. This study used molecular docking approach as well as cell culture and HAS binding experiments to examine triamterene effects on cancer (Moghadam et al., 2019). The cytotoxicity level of triamterene opposite HCT116 and CT26 cells exhibited that triamterene can possibly have a positive effect on the cancer therapy. Therefore, triamterene can be used as an inhibitor and can help in treating cancer. A study which investigates the effects of not only the management of bombesin but also triamterene in relation to the incidence of peritoneal metastasis in intestinal cancer in males. It was observed that the two doses of triamterene (10 mg/kg body weight and 20 mg/kg body weight) with the dose of bombesin (40 microliter/ kg body weight) not only had less or significantly no effect on carcinogenesis but also it decreased incidence of cancer metastasis (Iishi et al., 1996).

Cancer is a major public health concern not only in the UK but also around the world. There have been some treatments discovered for cancer but there are still some challenges and limitations that exist in treating cancer entirely. Over the years numerous studies have explored the mechanisms, treatments and limitations that come along with those treatments of cancer. One of the studies exploring the challenges in cancer treatment

showed that targeting small molecules or selected pathways in cancer can increase the survival rate among the patients of cancer (Zugazagoitia et al., 2016).

Among the many markers of cancer one of the markers that plays a vital role in the cancer is the ion channels. Various studies have explored and observed the relation between oncogenesis and ion channels in relation to the progression of cancer. Therefore, targeting ion channels only seems logical. Repurposing and repositioning of the FDA approves drugs will not only prove to be beneficial and safe for the patients with cancer but also it would assist in treating cancer (Kale, Amin and Pandey, 2015). This targeting ion channels to treat the major complication that is cancer can be done by using the drug repurposing and repositioning approach.

Drug repositioning also known as drug repurposing or drug profiling involves identifying the new uses of the medicines than already existing uses of drugs. The drug repurposing can involve the different dose or form of the drug. Computational technique molecular docking and virtual drug screening are two of the approaches that are involved in drug repositioning (Pushpakom et al., 2018). Molecular docking predicts the correct binding site for the interaction between the receptor and ligand. Theres no limit set on how many drugs can be used. A study that analysed the molecular fits for 3671 FDA approved drugs with 2335 human crystal structures observed that the mebendazole which is an anti-parasitic drug can be used to inhibits VEGFR-2 as VEGFR-2 plays crucial role in angiogenesis. This conclusion was formed by using molecular docking approach which showed that the drug mebendazole is structurally capable of inhibiting the receptor VEGFR-2 (Pushpakom et al., 2018). As much reliable as molecular docking approach is it does come with certain limitations. There are three main limitations that were observed in relation to molecular docking approach. One of the limitations is that the required 3D structures of certain target proteins may possibly be not available. The other limitation is that there may not be proper databases for macromolecular targets, and they might have lack of structural information of the macromolecules. The third limitation that was observed in molecular docking approach was that whether docking algorithms are predicting the binding affinity correctly or not and that whether different software were providing different results (Pushpakom et al., 2018).

Molecular docking is one of the crucial silico approaches for the discovery of the potential drug targeted therapies. This approach molecular docking was used for discovery of a suitable therapy for breast cancer. The drugs which demonstrated the characteristics such as good affinity targets were explored in depth. It was found that not only the in silico approach molecular docking but also other studies done in vitro can prove to be extremely beneficial for the discovery of new potent drugs that can treat the cancer (Cava and Castiglioni, 2020). A study explored the molecular docking in the target protein HIF-1alpha and genistein angiogenesis in the breast cancer cells. Some of the techniques used in the study included the molecular docking simulation, western blot analysis. The tests were done using the human breast cancer cells lines such as MDA-MB231 and T-47D (Mukund et al., 2019). It was found that genistein is not only able to bind to the HIF-1alpha protein, but it can also be used to decrease the pursuit in the breast cancer cells. This can further

lead to the conclusion that inhibitory approach by genistein can avert the activation of the downstream signalling of HIF-1 alpha which also includes vascular endothelial growth factor (Mukund et al., 2019).

The Molecular docking analysis for the biochemicals involved in cancer is a useful approach to find a potent treatment for the cancer. One of the studies used molecular docking approach to investigate the cytotoxic lonchocarpus flavoinds. The molecular docking was performed using molegro virtual docker version 2.3 and to check whether the docking analysis was accurate and valid or not argus lab 4.0.1 version was used (Cassidy and Setzer, 2009). The binding affinity for the cytotoxic lonchocarpus flavoinds is very strong in the cancer. It was concluded that due to the ability of flavaniods binding with some important target it has exhibited some signs of being an anti-cancer agent (Cassidy and Setzer, 2009). A study that investigated and explored the cytotoxic activities

Virtual drug screening, a computational approach has been used in various studies to find the novel inhibitors for numerous diseases including cancer. Virtual screening can be divided into two types. Type one is the ligand based approach and the type two is the structural based techniques. This computer aided drug discovery (CADD) approach has not only been widely studied but also well noticed and used in order to screen the various compounds and can eventually lead to assisting in treating harmful diseases (Kumar, Krishna and Siddiqi, 2015). In one of the studies where p53-mortalin complex inhibitor was being looked at for cancer virtual drug screening approach was used (Utomo, Widodo and Rifa'i, 2012). Along with the virtual screening approach to check the compounds that are similar to the structure of the drug and their inhibition of p53- mortalin the study also used molecular docking to analyse and find the binding site for p53- mortalin (Utomo, Widodo and Rifa'i, 2012). After using the virtual screening tools such as a software program Auto Dock Vina and analysing 9000 compounds similar to drug structures using ZINC database it was concluded that 3 drug like compounds proved to be good inhibitors for cancer. The three drug like compounds that can be anticancer agents and inhibitors for p53-mortalin were ZINC01019934, ZINC00664532 and ZINC00624418 (Utomo, Widodo and Rifa'i, 2012). It was also found that the substrate binding site of p53- mortalin domain was from 423 to 450 residues (Utomo, Widodo and Rifa'i, 2012). Drug resistance is a crucial complication that immediately requires a solution.

Discovery of new potent inhibitors for targeted pathways to treat cancer has recently been a major topic of interest for the researchers. One of the studies that investigated the inhibitors for mTOR (mammalian target of rapamycin) to treat cancer using the virtual drug screening approach. Other techniques that were used with the virtual drug screening approach was the in vitro mTOR kinase assay and western blots. In silico screening models were used and it was discovered that the inhibitory concentration of the fifteen inhibitors of mammalian target rapamycin was 10 microM (Wang et al., 2016). The seventeen of the derivatives showed the inhibitory properties against four of the tumour cells, the four tumour cells include MCF-3, MGC-803, Hela and C6 (Wang et al., 2016). Currently most of the known target therapies either involve the monoclonal antibodies (mAb) or the small

molecules. Either of these two can bind and initiate the redirect the signalling cascades of the target (Kumar, Krishna and Siddiqi, 2015).

The inhibition of the pathways that lead to the angiogenesis in the patients with hepatocellular carcinoma (HCC) was explored. It was noticed that sorafenib shows elevated survival in the patients with the hepatocellular carcinoma due to the inhibition characteristics for the angiogenic pathways (Berretta et al., 2016). A promising approach has yet to be found to anti-angiogenesis. Targeted therapies have been observed to play a significant role as they assist in treating cancer. Angiogenesis, cell proliferation, invasion and metastasis are some of the complications that have been observed in cancer.

The metabolic reprogramming of the tumour cell has some effects on the drug resistance with the tyrosine kinase inhibitors. During the treatment tumour cells reprogram in an attempt to adjust to the changed environment that is a result of the tyrosine kinase inhibiting treatment. These reprogramming characteristics that are observed include the cells that are resistant to elevate and make changes to the glycolytic pathways in order to elevate the process of glycolysis. Another characteristic of the drug resistant is that the lactate production elevates which is a result of Warburg effect that can eventually lead to the activation of the signalling cascade pathways that initiate the met (Yang et al., 2022). Inhibition of the apoptosis i.e., cell death process is one of the leading causes of uncontrollable cell proliferation in the cancer. The cell survival and the cell death both features depend on either the overexpression of the molecules that exhibit anti apoptotic properties or the low expression of the molecules that exhibit pro apoptotic properties (Yang et al., 2022). The cancer cells have been observed to have over expression of the molecules with the properties against apoptosis, so in order to treat cancer only targeting the expression and initiation of apoptosis would not solve the problem.

The inhibition of VEGFR-2 and c-met signalling is an important approach that can be used for treating cancer. A study in which the dual tumour inhibition and signalling of VEGFR-2 and C-Met was studied in hepatocellular carcinoma used NZ001 to check its inhibitory effects. The mice model that was chosen to investigate the effects of NZ001 on the receptors VEGFR-2 and C-met was immunocompetent orthotopic in vivo. It was observed that not only NZ001 has some positive effects on inhibition of VEGFR-2 and c-met receptor but also it can prove to be really beneficial for the patients that have overexpression of Met (Zhang et al., 2018). Aldeflour assays, side population assays, HMLER assays, Tumorsphere assays, apoptosis assays, siRNA transfection, cell dissociations, limiting dilution assays and in vivo tumour xenograft studies were some of the methods used in a study which investigated the dual inhibition of PI3K/mTOR and VS-5584 in cancer stem cells. It was observed that VS-5584 had positive effect and delayed the re growth of tumour after chemotherapy in small cell lung cancer. It was also observed and suggested that when VS-5584 is added into the treatment with already existing chemotherapy it can have more stronger response to delaying tumour growth and it can prove to be a better approach in treating cancer (Kolev et al., 2015). The virtual screening of compounds among the Poly pharmacological to pin down an inhibitor for the three tyrosine kinase receptors such as VEGFR-2, C-Met and EGFR was investigated in a study recently. EGFR is a tyrosine kinase

receptor that is also responsible for not only the tumour growth but also the metastasis and invasion in cancer cells just like the other two tyrosine kinase receptors, VEGFR-2, and c-Met. The investigation in a study was related to whether the molecular tyrosine kinase inhibitor such as anlotinib delays the growth of the VEGFR-2 and c-met in osteosarcoma or not.

Various studies have aimed to not only to investigate the roles VEGFR-2 and C-Met receptors play in the progression of diseases like cancer but also the search for finding a novel inhibitor for the two receptors in an attempt to treat the deadly disease cancer. One of those studies investigated the efficacy of derivatives of pyrrolo [2,1-f][1,2,4] triazine as a novel dual inhibitor for the receptors VEGFR-2 and c-met receptors in cancer (Shi et al., 2018). In the study most targets showed less proliferation against c-met in the cancer cell lines with the inhibitory concentration (IC50). The ideal value of the inhibitory concentration that was tested by inspecting inhibitory and anticancer activity among the targeted compounds for the receptors VEGFR-2 and c-met is 27a (Shi et al., 2018). The pharmokinetic and physicochemical factors support the findings that 27a can be used in anticancer therapies (Shi et al., 2018). In another similar study quinazolin-4-amines derivatives were observed to be dual inhibitors for VEGFR-2 and c-met receptors (Shi et al., 2014). The compound that was observed to be a better potent inhibitor than other derivatives of quinazolin-4-amines against the receptors VEGFR-2 and c-met was compound 7j. Not only that it was observed that 7j compound also exhibits the elevated anticancer activity against the cancer cell lines that were tested (Shi et al., 2014). After the docking simulation it was concluded that the there is a common interaction approach at the binding site ATP for c-met and VEGFR-2 receptors and not only that but also the derivative compound 7j of quinazolin-4-amines is a potent inhibitor for VEGFR-2 and c-met receptors therefore it can be as a potential factor in the upcoming therapies for cancer (Shi et al., 2014). Cancer can occur in various parts of the body including neck and head. The squamous neck and head cell carcinoma (HNSCC) have an elevated expression of the tyrosine kinase receptor c-met which not only plays role in the progression of the tumour, but it also reacts to the anti-cancer therapy (Arnold, Enders and Thomas, 2017). The study investigated the signalling relationship between the HGF/c-met and the survival of cancer cells.

Numerous studies have investigated and looked into the receptor c-met for its prognostic marker properties and its potential as a potent target and to be used in the treatment of cancer. Among those studies one of the studies investigated the whether c-met can be used as a potential target due to it being a prognostic marker in renal carcinoma cell or not. The existence of the signalling pathways of c-met receptor in renal cell carcinoma (RCC) is not very clear. The study included the exploration of the c-met expression and its inhibition in the renal cell carcinoma tumours among different cell lines (Gibney et al., 2013). To analyse the expression of receptor c-met automated quantitative analysis approach was used in the study with a tissue microarray (TMA). It was observed that the c-met expression was elevated in all renal cell carcinoma derivatives. It was found that the elevated expression of c-met was directly corelated with terrible disease survival rate. Therefore, the conclusion can be drawn that the among renal cell carcinoma c-met relates to poor pathological

properties (Gibney et al., 2013). The potency of C-met as an inhibitor in relation to the phenylbenzamide derivatives, benzamide derivatives and malonaminde derivatives was investigated in a recent study (Jiang et al., 2016). Among the derivatives 11c, 11i, 13b and 13h showed not only the inhibition characteristics against the receptor c-met but also elevated anticancer properties in vitro. This led to the conclusion that the derivatives with 13b and 13h can be used as an inhibitor for the c-met receptor in cancer (Jiang et al., 2016). The molecular docking approach in the study demonstrated that the derivatives not only bind well with the receptor c-met but also with the tyrosine kinase receptor VEGFR-2 (Jiang et al., 2016).

The requirement for the therapeutic interventions for cancer involving the receptors c-met and VEGFR-2 have researchers studying the potential compounds that can inhibit these tyrosine kinase receptors in depth. Among these studies one study explores the compound E7050 as a potential inhibitor for c-met and VEGFR-2 receptors. It was observed that in vitro the compound E7050 not only inhibits the receptors c-met and VEGFR-2 but also decreases the progression of tumour cells. The xenograft models in vivo showed very strong inhibition of tumour angiogenesis proving that E7050 does have a potential for anticancer therapies (Nakagawa et al., 2010). Computational molecular docking, molecular dynamics simulation and binding energy were the approaches that were used in a study to explore the activity of the c-met receptor with type II inhibitors (Li et al., 2017). In the study the focus was on the certain parameters such as the effects of various force fields, methods used for calculating the binding energy and binding sites. The findings showed that these parameters can have effects on the corelation coefficient. These findings can be used as the building blocks for computational analysis in the future studies on inhibition (Li et al., 2017).

The potential of the tyrosine kinase receptor, vascular endothelial growth factor receptor 2 has been explored in some studies. In a recent study the investigation of the potential of vascular endothelial growth receptor in order to treat prostate cancer and leukaemia with the human antibodies was done. VEGF binding assays along with tube formation assay, human tumour vasculature staining was some of the techniques used in this study. As the angiogenesis play a crucial role in the growth of the tumour, metastasis, and invasion of tumour cells it is extremely important to either decrease or eliminate the process of vasculature in the tumour this would not only assist in stopping metastasis but also in treating cancer (Lu et al., 2019). It was concluded that vascular endothelial growth factor receptor exhibits the ability to assist in treating cancer using targeted therapies (Lu et al., 2019).

The inhibition of receptor c-met to use as an anti-cancer therapy has been a topic of interest for researchers for quite some time. In a recent study the dual inhibition of c-met / HDAC was investigated. MCF-7 and A549, the two human cancer cell lines were observed to have anti proliferative characteristics due to the targeted lead derivative 11j with the inhibitory concentration of 21.44 and 45.22 nM (Dong et al., 2020). Therefore, one of the conclusions drawn was that c-met and HDAC can be used in a targeted approach for cancer therapy (Dong et al., 2020). Both the tyrosine kinase receptors c-met and VEGFR-2 have

been an interest of the researchers for a while now. One of the studies that explored the compounds derivatives such as [1,2,4] triazolo [4,3-*a*] pyrazine in relation to the tyrosine kinase inhibitors c-met and VEGFR-2 in vitro used kinase selectivity assay, cell cycle assay, cell apoptosis assay, western blot approach, fluorescence quantitative PCR, haemolytic test, Molecular dynamics simulation and molecular docking study (Liu et al., 2022). Among all the compounds one compound that was observed to stand out and showed prominent antiproliferative properties was the compound 17I against cancer cell lines. The inhibitory concentration (IC50) for the receptor c-met were observed as 26.00 nM whereas the inhibitor concentration for the receptor VEGFR-2 (IC50) was noticed to be 2.6 microM (Liu et al., 2022). It was observed that 17I compound that could be same as the FDA approved drug forteninb can bind to the tyrosine kinase receptors c-met and VEGFR-2 making VEGFR-2 and c-met receptors possibly a good potent target for treating cancer (Liu et al., 2022). To improve the treatments of cancer multiple approaches are not only being considered but also studied. One of the famous and most researched upon procedures for an anti-cancer therapy is the inhibition of selective pathways that prove to be very crucial in progression of the disease. The pathways selected for a study that explored the above approach were c-met and VEGFR-2 receptors. Some FDA approved drugs such as cabozatininb and foretinib has been observed to inhibit the tyrosine kinase receptors such as VEGFR-2 and c-met. It was concluded that the tumour progression and metastasis were decreased as XL 880 and XL 184 showed less invasive tumours and metastasis (You et al., 2011).

As the tyrosine kinase receptor c-Met plays a crucial role in the metastasis, invasiveness, and progression of tumour cells in cancer it is mandatory to explore whether c-met inhibitors are safe and are tolerable or not (Puccini et al., 2019). A recent study investigated the safety of the c-met inhibitors and found that the already existing FDA approved drugs i.e., inhibitors of c-met such as crizotinib and cabozatinib have been observed to be not only safe but also have proved to play an important role in the anti-cancer therapies (Puccini et al., 2019). A study that explored the inhibition pathways, growth of tumours and lymph angiogenesis among pancreatic cancer cells in corelation with the FDA approved drug foretinib which is a multikinase inhibitor used VEGFR-2 /3 and TIE-2 (Chen, Tsai and Hung, 2015). It was observed that foretinib not only reduced the primary c-met activity but also it decreased proliferation, angiogenesis, and lymph angiogenesis by inhibiting pathways that cause tumour growth in vivo (Chen, Tsai and Hung, 2015). It was observed that at higher concentrations foretininb can decrease the cell proliferation in pancreatic cancer cells. It was concluded that foretininb, an FDA approved drug can not only inhibit but also suppress the lymph angiogenesis among the VEGFR-2, VEGFR-3 and TIE-2 signalling pathways (Chen, Tsai and Hung, 2015). Studies have shown that the two tyrosine kinase receptors i.e., VEGFR-2 and c-met play a crucial role in the progression and metastasis of cancer cells making the two receptors and their signalling pathways a good target for anti-cancer therapies. One such study which explored the dual inhibition of c-met and VEGFR-2 receptors approach by investigating the [1,4] dixino [2,3-f] quinazoline derivative compounds (Wei et al., 2019). Enzyme assay, proliferation assay, xenograft mouse model was used. It was observed that compounds with the inhibitory concentration (IC50) of 7m

and 7k were potentially reliable targets for inhibition. It was concluded that these two compounds have potential and properties to inhibit the receptors VEGFR-2 and c-met and can aid in treating cancer efficiently (Wei et al., 2019).

When the expression of c-met in the patients with epithelial ovarian cancer cells was investigated with the assistance of a meta-analysis approach. Along with the meta-analysis publication searching strategy, data extraction and statistical analysis. It was found that the patients who had elevated rate of expression of c-met had worse survival rates as compared to the patients that had less expression of c-met (Kim et al., 2018). Therefore, a conclusion that can be drawn is that the expression of c-met can be used as a prognostic marker for epithelial ovarian cancer patients (Kim et al., 2018). C-met can be seen expressed in the normal healthy human cells but a high expression is observed in the mutated tumour cells in various types of cancer. A recent study investigated the effects that inhibition has on the tyrosine kinase receptor VEGFR-2 derivatives among the anti-breast cancer cells (Ahmed et al., 2020). Among the approaches used was in vitro cell proliferation, In vitro VEGFR-2 kinase activity assay and docking were used. It was observed that out of twenty-five derivative compounds the 4a derivative not only interacts with the amino acids of VEGFR-2 as proved by molecular docking but also it presents extremely good properties and characteristics similar to the drugs that can be used in treating cancer (Ahmed et al., 2020).

Various studies have proven that VEGFR-2 plays a crucial role in the progression, metastasis and migration in the cancer cells making it a crucial target for treating the cancer. One of the studies explored in both in vivo and in vitro the derivatives of quinazolin-4 (3H) – ones for the inhibition of the receptor VEGFR-2 and their signalling pathways and cascades in the hepatocellular carcinoma (Eissa et al., 2021). Molecular docking was the approach used. It was observed that in vivo the derivatives 29b and 29c show the inhibition of the tumours and in vivo after using molecular docking technique it was concluded that the interactions showed the VEGFR-2 inhibition (Eissa et al., 2021). The investigation for finding a potent inhibitor for EGFR among the compound derivatives of chalcone shown some promising results in regard to anti-cancer agents. The twenty five derivative compounds of chalcone after the molecular docking analysis were observed to have the binding energy from -6.10 to -9.25 Kcal/mol (Rao et al., 2015). Out of all the twenty five derivative compounds only one compound i.e. the compound twenty one was observed to not only have the -9.25 Kcal/mol binding energy but also the inhibitory concentration of 164.66 nano molar. The conclusion can be drawn that as the derivative compound 21 has shown positive results as a potent anti-cancer inhibitor the possibility of the future studies might add as a steppingstone towards the discovery of proper treatment of cancer (Rao et al., 2015).

The attempts in order to discover a proper inhibitor for the receptor c-met has led studies for quite some time now. A study where the investigation of the [E] -N'- benzylidene hydrazides for the inhibition of c-met receptor used virtual drug screening approach. When the inhibition pathways for c-met and VEGFR-2 were studied in vitro it was found that one of the derivative compounds that is compound 10b showed very promising inhibition

reactions for the tyrosine kinase receptor c-met (Liang et al., 2020) with the inhibitory concentration as 0.37 nM. Another derivative compound that showed mulit targeting inhibition activities for the receptor c-met was the compound 11b. The inhibitory concentration for the receptor c-met was observed as 3.41 nM whereas the inhibitory concentration for the receptor VEGFR-2 was 25.34 Nm. This conclusion shows that not only the derivative compounds 10b and 11b can lead to the inhibition of the receptors c-met and VEGFR-2 but they can eventually lead to a promising targeted therapy for the cancer (Liang et al., 2020). One of the surveys that explored the multiple targeted protein kinase inhibitors hybrid for the treatment of cancer found that one of the approaches to treat cancer was the molecular hybridization (Soltan et al., 2021).

The expression and mechanisms of VEGFR-2 and c-met have been studied in numerous studies. A similar study that investigates and explores the expression of the receptors VEGFR-2, c-met and PDGFR-beta in the hepatocellular carcinoma. The approaches that were used to explore expression of the receptors included an immunohistochemical staining examination. All the ninety-three patients' prognostic measures were studied after the immunohistochemical staining. It was observed that expressions of VEGFR-2 were not only elevated in the patients with hepatocellular carcinoma but also it corelated with the hepatitis B infection and liver cirrhosis. Similarly, it was observed that PDGFR-beta can be seen as a marker for less progression whereas the elevated expression of C-Met receptor exhibits the signs of some impacts of an inhibitor among the patients with hepatocellular carcinoma (Chu et al., 2013).

Various kinase inhibitors were included in the study such as EGFR, VEGFR-2, c-Met, PDK and CDK were explored with the chemotherapy agents which would eventually form the hybrids including tublin polymerization. The couple of benefits that led to the investigations in this study were less resistance of drugs not only that but also the less interactions among drugs and elevated efficiency (Soltan et al., 2021). Various studies have explored the metastatic nature of the cancer cells among different types of cancer. One of the studies investigating the metastatic characteristics of the tumours in the renal cell carcinoma included some of the potent therapies sorafenib tosylate, sunitinib malate and termsirolimus (Hutson and Figlin, 2009). As the investigations of discovering not only a potential therapy but also finding of potential inhibitors is still underway some of the strategies can assist in the therapy discovery for future. The strategies that can assist in the discovery of a valid treatment for cancer include synthesizing the agents that not only show the chances of full remissions but also are tested in the patient (Hutson and Figlin, 2009).

Chapter 5 - Conclusion

The aim of this study was to find a novel inhibitor (FDA approved drug) that targets VEGFR-2 and C-met receptor in cancer simultaneously. In this study out of 2016 potential FDA approved drugs 11 FDA approved drugs were shortlisted to test and target VEGFR-2 and C-met, the two tyrosine kinase receptors. The in-silico techniques; molecular docking and virtual drug screening were used to achieve the aim. It was concluded that among the 11 FDA approved drugs one drug was observed to be a dual inhibitor for both the receptors (VEGFR-2 and C-met). The dual inhibitor was Triamterene for both the receptors c-met and VEGFR-2. As for the single inhibitor in case of the receptor c-met out of 11 shortlisted FDA drugs Entacapone and for the receptor VEGFR-2 was Telmisartan.

For further research the three FDA approved drugs (Two single inhibitor and one dual inhibitor) Entacapone, Telmisartan and triamterene can be tested in the vitro with the receptors c-met and VEGFR2 to prove that triamterene is the dual inhibitor of c-met and VEGFR-2 and that it can aid in curing cancer in the future.

Chapter 6 - References

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Chapter 7 - Appendix

1. **The FASTA sequence of VEGFR2 (P35968):**

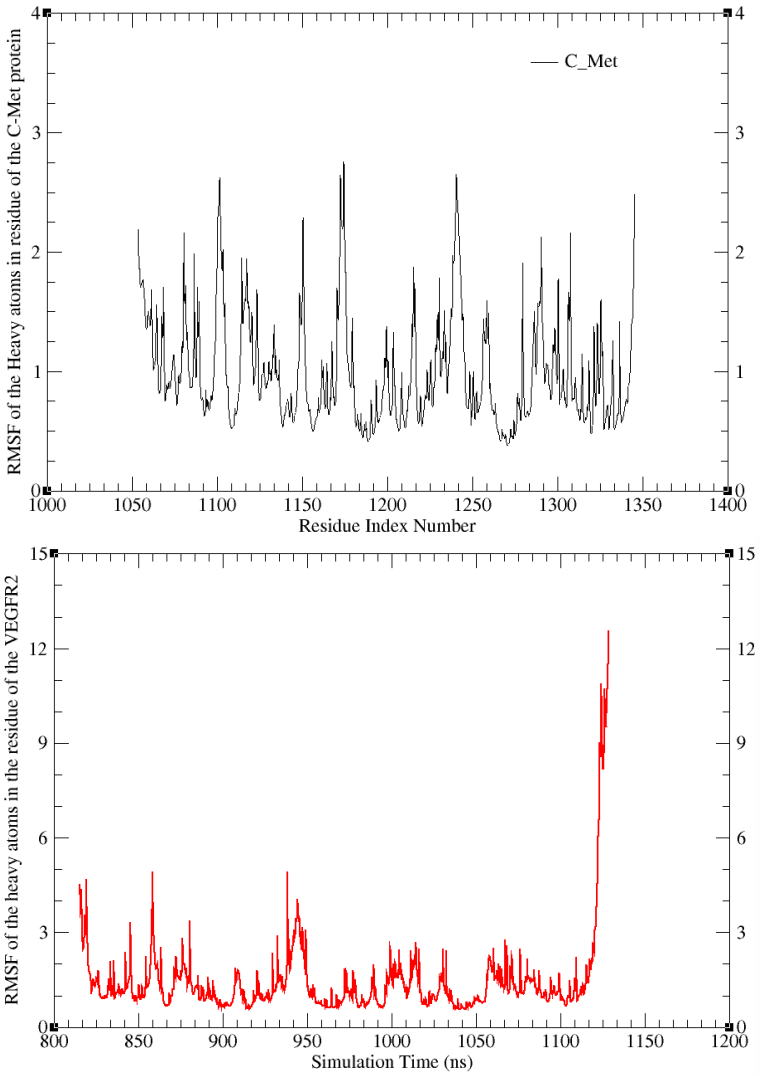
>sp|P35968|VGFR2_HUMAN Vascular endothelial growth factor receptor2OS=HomosapiensOX=9606GN=KDRPE=1SV=2MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAMFFWLLLVIILRTVKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVPYKTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFLTLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQTDSGMVLASEELKTLEDRTKLSPSFGGMVPSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSEEAELLKLIEIGVQTGSTAQILQPDSGTTLSSPPV

2. **The FASTA sequence of C-Met (P08581):**

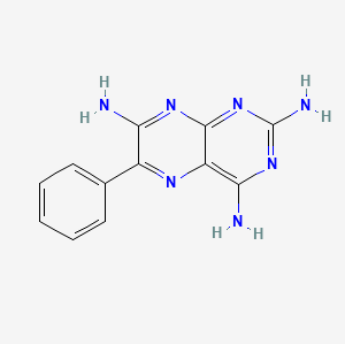
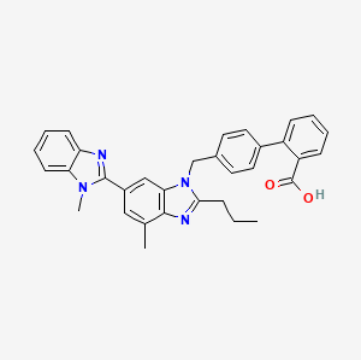
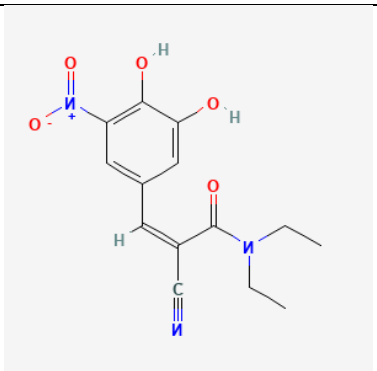
>sp|P08581|MET_HUMAN Hepatocyte growth factor receptor OS=Homo sapiens OX=9606GN=METPE=1SV=4MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPIQNVILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSGGVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFSPQIEEPSQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLKETKDGFMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHTRIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQIGASLNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQHFYGPNHEHCFNRTLLRNSSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLTSISTFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHTLNQNGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDKCVRSEECLSGTWTQQICLPAIYKVFPNSAPLEGGTRLTICGWDFGFRRNNKFDLKKTRVLLGNESCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPVITSISPKYGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEFAVKLKIDLANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLNSVSVPRMVINVHEAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIYVHNPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSEAVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQPDQNFTGLIAGVVSISTALLLLLGFFLWLKKRKQIKDLGSELVRYDARVHTPHLDRLVSARSVSPTTEMVSNESVDYRATFPEDQFPNSSQNGSCRQVQYPLTDMSPILTSGDSDISSPLLQNTVHIDLSALNPELVQAVQHVVIGPSSLIVHFNEVIGRGHFGCVYHGTLLDNDGKKIHCAVKSLNRITDIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEGSPLVVLPY

MKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLASKKFVHRDLAARNCMLDEKFTVKVADFGL
ARDMYDKEYYSVHNKTGAKLPVKWMALESLQTQKFTTKSDVWSFGVLLWELMTRGAPPYPDVNTF
DITVYLLQGRRLLQPEYCPDPLYEVMLKCWHPKAEMRPSFSELVSRISAIFSTFIGEHYVHVNATYVNV
KCVAPYPSLLSSEDNADDEVDTRPASFWETS

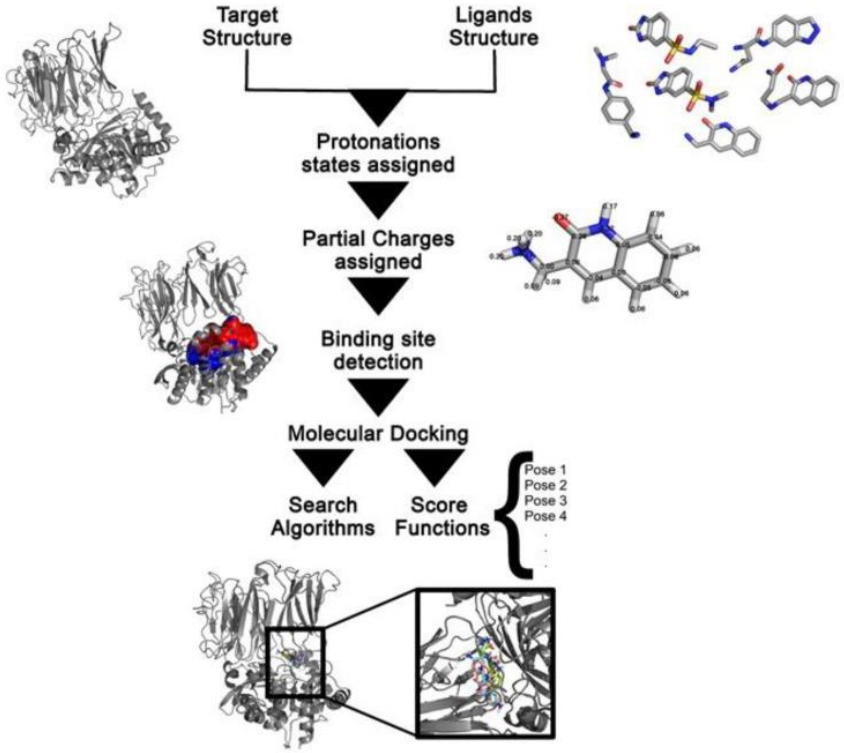
3. **The Root Mean Square Fluctuation for heavy atoms in (C-Met and VEGFR2) systems is sampling interval of 15 ns**



4. Dual inhibitors & Single Inhibitors Drugs 2D chemical structures

FDA Approved Drugs	2D Chemical Structure
Triamterene **(Dual Inhibitor for C-Met & VEGFR-2)** (PubChem, n.d.)	
Telmisartan **(Single Inhibitor for VEGFR-2)** (PubChem, n.d.)	
Entacapone **(Single Inhibitor for C-Met)** (PubChem, n.d.)	

5. Workflow of molecular docking approach



(Torres et al., 2019)