International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

Design, Synthesis, Characterization andPharmacological Evaluation Of mTOR INHIBITORS FOR Cytotoxic Activity

Mr.Solunke Shivaji Hanumant, *DBATU University, Aditya college of Pharmacy Beed*
Dr.L.D.Hingane, *Aditya college of Pharmacy Beed*

Date of Submission: 04-07-2023 Date of Acceptance: 16-07-2023

ABSTRACT:
Cancers arise approximately in one among every three individuals. DNA mutations arise normally at a frequency of 1 in every 20 million per gene per cell division. The average number of cells formed in any individual during an average lifetime is 10 (10 million cells being replaced every second!). Risk of cancers are increased by infectious agents including viruses [Hepatitis B virus (HBV1), Human Papilloma virus (HPV), Human Immunodeficiency Virus(HIV)-increase risk of Nasopharyngeal, Cervical carcinomas and Kaposi's Sarcoma] and bacteria such as Helicobacter pylori (Stomach cancers).

KEYWORDS: DESIGN, SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION

I. INTRODUCTION:

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Initiation and progression of cancer is also due to exposure to cancer-causing agents (carcinogens, mutagens). These are present in the food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers originate from epithelia (carcinomas). In less than 10% of cases, a genetic predisposition increases the risk of cancer developing a lot earlier. (e.g. Certain childhood leukemia's, retinal cancers etc.) Although cancer can occur in persons of every age, it is common among the aging population. 60% of new cancer cases and two thirds of cancer deaths occur in persons >65 years. The incidence of common cancers (e. g. breast, colorectal, prostate, lung) increases with age. The exponential rise in many cancers with age fits with an increased susceptibility to the late stages of carcinogenesis by environmental exposures. Lifetime exposure to estrogen may lead to breast or uterine cancer; exposure to testosterone leads to prostate cancer. The decline in cellular immunity may also lead to certain types of cancer that are highly immunogenic (e.g. lymphomas, melanomas). Accumulation of DNA mutations have to be amplified to constitute a cancer, therefore the longer the life span, the higher the risk of developing cancer. The available anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. A single "cure" for cancer has proved elusive since there is not a single type of cancer but as many as 100 different types of cancer. In addition, there are very few demonstrable biochemical differences between cancerous cells and normal cells. For this reason the effectiveness of many anticancer drugs is limited by their toxicity to normal rapidly growing cells in the intestinal and bone marrow areas. A final problem is that cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. For this reason cancer chemotherapy may consist of using several drugs in combination for varying lengths of time. The majority of drugs used for the treatment of cancer today are cytotoxic (cell-killing) drugs that work by interfering in some way with the operation of the cell's DNA. Cytotoxic drugs have the potential to be very harmful to the body unless they are very specific to cancer cells - something difficult to achieve because the modifications that change a healthy cell into a cancerous one are very subtle. A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects. The reason for this is simple: cancer cells are not foreign to the body but are simply subtly mutated forms of normal human cells, and it is very different to synthesize drugs that can tell the difference.

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

II. Types of cancer:

- Breast cancer
- Colorectal cancer
- Lung cancer
- Prostate cancer
- Skin cancer
- Bladder cancer
- Renal cell carcinoma

III. The various receptor targets for cancer are as follows:

- Mammalian target of rapamycin receptor (mTOR)
- Epidermal growth factor receptor (EGFR)
- Platelet-derived growth factor receptor (PDGF)
- Adenosine receptor
- Estrogen receptor
- G-protein-coupled receptors
- Chemokine receptors
- Toll-like receptors
- Cyclin-dependent kinase receptors (CDK)
- Cannabinoid receptors
- Fibroblast growth factor receptors (FGF)
- Insulin-like growth factor receptors (IGF)
- Hepatocyte growth factor receptors (HGF)

IV. mTOR Signaling pathway

The mammalian target of rapamycin (mTOR) is an intacellular kinase that controls the production of several proteins through its phosphorylation of translational machinery. mTOR-activated proteins promote several hallmarks of cancer such as cell growth and proliferation, angiogenesis, and bioenergetics. Since mTOR acts as a neoplastic switch that is frequently turned on by many mutations found in cancer, inhibition of mTOR may offer a promising new strategy for cancer therapy. The mammalian target of rapamycin (mTOR), also known as FKBP 2-rapamycin associated protein (FRAP), a phosphatidylinositol 3-kinase (PI3K) related serine/threonine kinase. The pathway in which it plays a prominent part regulates the growth, proliferation, motility and survival of cells and also angiogenesis. This central regulation of cell growth and proliferation is activated by growth factor/mitogenic stimulation activation of the cancer progression and the transition to androgen-independent disease. Rapamycin,a known mTOR inhibitor is a bacterial product that was originally of interest for its antifungal properties. It was subsequently found to have immunosupressive and antiproliferative properties. While it was being tested as an immunosuppressive agent to prevent organ rejection in transplant patients, the drug rapamycin was also discovered to have anti tumor properties. Rapamycin shows promise against few types of cancers particularly mantle cell lymphoma, endometrial cancer, and renal cell carcinoma. The known mTOR inhibitor rapamycin and its analogues (RAD001, CCI-779, and AP23573) bind to the FKBP12/rapamycin complex binding domain (FRB) and suppress the signaling to the downstream targets p70S6K and 4E-BP1. The potent but non-specific inhibitors of PI3K, LY294002 and wortmannin, have also been shown to inhibit the kinase function of mTOR but it inhibits by targeting the catalytic domain of protein. Recently it has been shown that mTOR exists in two complexes. mTORC1, a rapamycin sensitive complex signaling to p70S6K and 4E-BP1 and mTORC2, a rapamycin insensitive complex that signals to AKt. Inhibition of mTORC1 alone can block a desirable negative feedback mechanism, thereby causing an increase of PI3K/AKt signaling and reducing the effectiveness of the inhibitors. This negative feedback mechanism can be restored byinhibiting mTORC2. Therefore it is proposed that direct targeting of the kinase domain of mTOR would inhibit the signaling through both mTORC1 and mTORC2 and that such a compound would exhibit a different pharmacology compared with rapamycin. Since there is no available crystal structure of mTOR, mTOR homology model was built based on the X- ray crystal structure of the closely related protein PI3Kγ.

Homology modeling:

The ultimate goal of protein modeling is to predict a structure from its sequence with an accuracy that is comparable to the best results achieved experimentally. Protein modeling is the only way to obtain structural information if experimental techniques fail. Many proteins are simply too large for NMR analysis and cannot be crystallized for X-ray diffraction.

Homology modeling is a multistep process that can be summarized in seven steps:

1. Template recognition and initial alignment
2. Alignment correction
3. Backbone generation
4. Loop modeling
5. Side-chain modeling

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

6. Model optimization
7. Model validation

The percentage sequence identity between template and target. If it is greater than 90%, the accuracy of the model can be compared to crystallographically determined structures, except for a few individual side chains. From 50% to 90% identity, the root mean square (rms) error in the modeled coordinates can be as large as 1.5 A°, with considerably larger local errors. If the sequence identity drops to 25%, the alignment turns out to be the main bottleneck for homology modeling, often leading to very large errors.

Drug discovery:

It combines empirical knowledge from the structure-function relationships of known drugs with rational designs optimizing of known drugs with rational designs optimizing the physiochemical properties of drug molecules.

The process of drug discovery involves the identification of candidate molecules, synthesis, characterization, screening for therapeutic efficacy and toxicity studies. The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target.

Drug discovery and development can broadly follow two different paradigms- Physiology-based drug discovery and Target-based discovery. The main difference between these two paradigms lies in the time point at which the drug target is actually identified.

Physiology-based drug discovery follows physiological readouts, for example, the amelioration of a disease phenotype in an animal model or cell-based assay. A purely physiology-based approach would initially forgo target identification/validation and instead jump right into screening. Identification of drug target and the mechanism of action would follow in later stages of the process by deduction based on the specific pharmacological properties of lead compounds.

By contrast, the road of target-based drug discovery begins with identifying the function of a possible therapeutic target and its role in disease.

One way to find promising drug candidates is to investigate how the target protein interacts with randomly chosen compounds. This is done by using compound libraries which can contain more than a million synthetic and natural compounds. These libraries are then tested against the target protein. This is most often done in so called high-throughput screening facilities. The most promising compounds obtained from the screening process are called hits- these are the compounds showing binding activity. Some of these hits are then promoted to lead compounds-candidate structures which are further refined and modified in order to achieve more favourable interactions and less side-effect.

Advances in computing power and in structure determination by X-ray crystallography and NMR have made computer-aided drug design (CADD) and structure- based drug design (SBDD) essential tools for drug discovery.

The main advantages of computational methods over wet-lab experiments are asfollows:

- Low costs, no compounds have to be purchased externally or synthesized by achemist.
- It is possible to investigate compounds that have not been synthesized yet.
- Conducting high-throughput screening (HTS) experiments is expensive and virtual screening (VS) can be used to reduce the initial number of compounds before using high-Throughput Screening (HTS) methods.
- Huge chemical search space. The number of possible virtual molecules available for VS is much higher than the number of compounds presently available for HTS.

CADD of lead compounds:

A detailed knowledge of a target binding site significantly aids in the design of novel lead compounds intended to bind with that target. In cases, where enzymes or receptors can be crystallized, it is possible to determine the structure of the protein and its binding site by X-ray crystallography. Molecular modeling software can then be used to study the binding site, and to design molecules which will fit and bind to the site-de novo drug design.

In some cases, the enzymes or receptor cannot be crystallized and so X-ray crystallography cannot be carried out. However, if the structure of an analogous protein has been determined, this can be used as the basis for generating a computer model of the protein (Homology Modeling). Homology Modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

template sequence. The sequence alignment and template structure are then used to produce a structural model of the target. The quality of the model is dependent on the quality of the sequence alignment and template structure.

Molecular docking:

Molecular docking programs try to predict how a drug candidate binds to a protein target without performing a laboratory experiment. Molecular docking software consists of two core components.

- A search algorithm (sometimes called an optimization algorithm). The search algorithm is responsible for finding the best conformations of the ligand and protein system. A conformation is the position and orientation of the ligand relative to the protein. In flexible docking, a conformation also contains

information about the internal flexible structure of the ligand and in some cases about the internal flexible structure of the protein. Since the number of possible conformations is extremely large, it is not possible to test all of them, therefore sophisticated search techniques have to be applied. Examples of some commonly used methods are Genetic Algorithms and Monte Carlo Simulations.

- An evaluation function (sometimes called a score function). This is a function providing a measure of how strongly a given ligand will interact with a particular protein. Energy force fields are often used as evaluation functions. These force fields calculate the energy contribution from different terms such as the known electrostatic forces between the atoms in the ligand and in the protein forces arising from deformation of the ligand, pure electron-shell repulsion between atoms and effect from the solvent in which the interaction takes place.

Pharmacophore mapping:

Pharmacophore mapping is a geometrical approach. A pharmacophore can be thought as a 3D model of characteristic features of the binding site of the investigated protein. It can also be thought of as a template, a partial description of a molecule where certain blanks need to be filled. Like QSAR models, pharmacophores can be built without knowing the structure of the target. This can be done by extracting features from compounds which are known experimentally to interact with the target in question. Afterwards, the derived pharmacophore model can be used to search compound databases (libraries) thus screening for potential drug candidates that may be of interest.

Identifying 3D pharmacophore is relatively easy for rigid cyclic structures. With more flexible structures, it is not so straightforward because the molecule can adopt a large number of shapes or conformations which place the important binding groups in different positions relative to each other. Normally only one of these conformations is recognized and bound by the binding site. This conformation is known as the active conformation.

In order to identify the 3D pharmacophore, it is necessary to know the active conformation. There are various ways in which this might be done. Rigid analogues of the flexible compound could be synthesized and tested to see whether activity is retained. Alternatively, it may be possible to crystallize the target with the compound bound to the binding site. X-ray crystallography could then be used to identify the structure of the complex as well as the active conformation of the bound ligand.

Lead optimization:

Lead optimization is the complex, non-linear process of refining the chemical structure of a confirmed hit to improve its drug characteristics with the goal of producing a preclinical drug candidate. This stage frequently represents the bottleneck of a drug discovery program.

Once the important binding groups and pharmacophore of the lead compound have been identified it is possible to synthesize analogues that contain the same pharmacophore. Very few lead compounds are ideal. Most are likely to have low activity, poor selectivity, and significant side effects. They may also be difficult to synthesize, so there is an advantage in finding analogues with improved properties.

The following strategies are used to optimize the interactions of a drug with its target in order to gain higher activity and selectivity.

- Variation of substituents
- Extension of the structure
- Chain extension/contraction
- Ring expansion/contraction
- Ring variations
- Ring fusions
- Isosteres and Bioisosteres
- Simplification of the structure
- Rigidification of the structure
- Conformational blockers

Quinoline:

Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially.

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic. One striking structural features inherent to heterocycles, which continue to be exploited to great advantage by the drug industry lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations.

For more than a century, heterocycles have constituted one of the largest areas of research in organic chemistry. Between them nitrogen and sulfur containing heterocyclic compounds have maintained the interest of researches through decades of historical development of organic synthesis.

Quinoline is a heterocyclic aromatic nitrogen compound characterized by a double ring structure that contains a benzene ring fused to pyridine at two adjacent carbon atoms.
Its principal use is as a precursor to 8-hydroxyquinoline, which is a versatile chelating agent and precursor to pesticides. Its 2- and 4-methyl derivatives are precursors to cyanine dyes.

Oxidation of quinoline affords quinolinic acid (pyridine-2, 3-dicarboxylic acid), a precursor to the herbicide sold under the name "Assert". Like other nitrogen heterocylic compounds, such as pyridine derivatives, quinoline is often reported as an environmental contaminant associated with facilities processing oil shale or coal. Owing to high water solubility quinoline has significant potential for mobility in the environment, which may promote water contamination. Fortunately, quinoline is readily degradable by certain microorganisms, such as *Rhodococcus* species Strain Q1, which was isolated from soil and paper mill sludge.

V. MATERIALS AND METHODS

Homology modeling:

Homology model of mTOR kinase domain was built using Accelrys discovery studio. Modeler algorithm was used to generate the 3D structure of mTOR based on the crystal structure of PI3K gamma (PDB ID: 3S2A). The structure has a resolution of 2.5 Å and exists in complex with a quinoline inhibitor. The c-terminal region of human mTOR protein sequence was taken from uniprot database (P42345). The sequence alignment was carried out using the ClustalW program to identify homologous regions between the two proteins. The catalytic domain of mTOR and PI3K gamma shows maximum of 45% similarity. After the identification of structurally conserved and variable regions, restraints, distances and dihedral angles were extracted from the template structure and applied to mTOR. Stereochemical restraints, viz., bond length and bond angle preferences, were obtained from the molecular mechanics force field CHARMM. The quinoline inhibitor from the crystal structure was extracted and transferred to mTOR homology model for further guidance in docking studies. mTOR homology model was further refined using 600 ps MD simulations in explicit water. Minimization was carried out using the consistent valence force field (CVFF) with a van der Waals cutoff of 9.5 ˚A and a distance-dependent dielectric constant of 1×r. One thousand steps of steepest descents were performed followed by 1000 steps of conjugate gradients until the root mean square (RMS) gradient reached a value of less than 0.001kcal/mol/Å. The homology models were each solvated with a 10 ˚A water layer and optimized using MD simulations for 2 ns at a 300 K temperature. The quality of the model was assessed by PROCHECK. The model was evaluated by a Ramachandran plot and found that 97% of the residues are in favorable region.

Drug design:

A drug is a small molecule ligand that binds to a specific protein which either increases its activity (an agonist) or decrease/block its activity (an antagonist). One way to find promising drug candidates is to investigate how the target protein interacts with randomly chosen compounds. Drug designing basically of two types namely ligand based approach or receptor based approach.

- Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.
- Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

Pharmacophore studies using catalyst:

Pharmacophore means "a molecular framework that carries (phoros) the essential features responsible for a drugs (pharmacon) biological activity".

For Rational design of small molecules as drug candidates using 3D pharmacophore and shape-based models, and to suggest potentially active compounds suitable for synthesis and biological testing. The typical features are hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HY), hydrophobic aliphatic, hydrophobic aromatic, positive ionizable, negative ionizable and ring aromatic(RA).

- HipHop

Generates a set of common feature pharmacophore models from set of compounds known to be active (No activity data) at a specific target.

- HypoGen

Develop SAR hypothesis models from a set of molecules for which activity values (IC50 or Ki) on a given biological target are known.

- HypoRefine

Permits consideration of exclusion volumes in pharmacophore-based 3D QSAR optimization. The result is to better model predictivity where biological activity is determined by considerations of molecular shape.

- Exclusion volume

An excluded volume can be added to a hypothesis (or to a template molecule) to specify one or more spherical spaces that must not contain any atoms or bonds. An exclusion volume can represent a region in space that might impinge sterically on a receptor. An exclusion volume can be interpreted as a geometrical constraint, and this is how it is treated in catalyst

Data set:

Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features in a set of active analogues. The compounds in this study were collected from a series of mTOR inhibitors published in recent years, considering both structural diversity and wide coverage of the activities. A set of 297 human mTOR inhibitors with an activity range (IC_{50}) 0.0016-11000 nM was selected. This initial group was then divided into the training set and test set. The training set of 24 molecules was designed to be structurally diverse with a wide activity range. Molecules with K_i, ED_{50}, EC_{50} and other activity type values were ignored and not considered for modeling studies. The training set molecules play a critical role in the pharmacophore generation process and the quality of the resultant pharmacophore models relies solely on the training set molecules. The test set of remaining 273 molecules is designed to evaluate predictive ability of the resultant pharmacophore. Highly active, moderately active, and inactive compounds were added to the training set to obtain critical information on pharmacophoric requirements for mTOR inhibition. The molecules selected as the training set are given in fig.6 and a few molecules from test set are given in fig.7. This training set was then used to generate quantitative pharmacophore models. While generating a quantitative model, a minimum of 0 to a maximum of 4 features involving hydrophobic acceptor (HBA), hydrophobic donor (HBD), hydrophobic aliphatic and ring aromatic (RA) were selected and used to build a series of hypotheses using a default uncertainity value of 3. The quality of HypoGen models is best described in catalyst user guide in terms of fixed cost, null cost and total cost and other statistical parameters. According to which, a large difference between the fixed cost and null cost, and a value of 40-60 bits for the unit of cost would imply a 75-90% probability for experimental and predicted activity correlation. In general, pharmacophore models should be statistically significant, predict the activity of molecules accurately, and retrieve active compounds from a database. The derived pharmacophore models were validated using a set of parameters including cost analysis, test set prediction, enrichment factors, and goodness of fit. HipHop and HypoGen modules within catalyst were then used to generate qualitative pharmacophore and quantitative pharmacophore models respectively

Database searching:

Virtual screening of chemical databases can serve the purpose of finding novel, potential leads suitable for further development. Database searching methodology provides the advantage that the retrieved compounds can be obtained easily for biological testing when compared to any de novo design methods. A molecule must fit on all the

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

features of the pharmacophore model that is used as 3D query in database searching to be retained as hit. Two database searching options such as Fast/Flexible and Best/Flexible search are available in DS. Better results can be achieved using Best/Flexible search option during database screening.

Molecular Docking:

Molecular docking is a computationally intensive SBVS technique that generates and scores putative protein-ligand complexes according to their calculated binding affinities. Given the crystal structure of the target, molecular docking automatically samples ligand conformations and protein ligand interactions with a specified region of the protein surface. It has been successfully used for identifying active compounds by filtering out those that do not fit into the binding sites. In absence of the structural information of the target, a homology model can be constructed and used for the molecular docking. In this study, molecular docking was performed with a homology model of mTOR by the program Glide

IN VITRO ANTI CANCER ACTIVITY:

The human colorectal carcinoma cell line (HCT116) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Dulbeccos Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. one hundred micro liters per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred micro liters per well of each concentration was added to plates to obtain final concentrations of 100, 10, 1.0 and 0.1 µM. The final volume in each well was 200 µl and the plates were incubated at 37^0C, 5% CO2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT Assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using GraphPad Prism software.

BIBLIOGRAPHY

[1]. Momna Hejmadi. Introduction to cancer biology. Ventus Publishing ApS; 2010.p.6-13.
[2]. Cancer [Online].Available from: http://www.elmhurst.edu/~chm/vchembook/655cancer.html [Accessed on February 21, 2012].
[3]. B. Ganesh, Sanjay D. Talole, Rajesh Dikshit, A case–control study on diet and colorectal cancer from Mumbai, India. Cancer Epidemiology, 2009; 33:189–93.
[4]. Rajaraman Swaminathan, Ramanujam Selvakumaran, Pulikattil Okkuru Esmy, P. Sampath, Jacques Ferlay, Vinoda Jissa, Viswanathan Shanta, Cancer pattern and survival in a rural district in South India. Cancer Epidemiology, 2009; 33:325–31.
[5]. J. Cardoso, J. Boer, H. Morreau, R. Fodde, Expression and genomic profiling of colorectal cancer. Biochimica et Biophysica Acta, 2007; 103–37.
[6]. H.E. Blum, Colorectal Cancer: Future Population Screening for Early Colorectal

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 602-609 www.ijprajournal.com ISSN: 2456-4494

Cancer. Eur. J. cancer, 1995; 31A:1369-72.
[7]. Suzanne Hector, Jochen H.M. Prehn, Apoptosis signaling proteins as prognostic biomarkers in colorectal cancer: A review. Biochimica et Biophysica Acta, 2009;117–29.
[8]. Laura Asnaghi, Paola Bruno, Marcella Priulla, Angelo Nicolin, mTOR: a protein kinase switching between life and death. Pharmacological Research, 2004;50:545– 49.
[9]. David A. Guertin and David M. Sabatini, Defining the Role of mTOR in Cancer.
[10]. Cancer Cell, 2007; 12:9-22.
[11]. JB Easton and PJ Houghton, mTOR and cancer therapy. Oncogene, 2006; 25:6436–46.
[12]. Carlos Garcia-Echeverria, Allosteric and ATP-competitive kinase inhibitors of mTOR for cancer treatment. Bioorg. & Med. Chem. Lett., 2010; 20:4308-12.
[13]. mTOR pathway [Online].Available from: www.abcam.com
[Accessed on February 21,2012].
[14]. Margit Rosner, Michaela Hanneder, Nicol Siegel, Alessandro Valli,Christiane Fuchs, Markus Hengstschlager, The mTOR pathway and its role in human genetic diseases. Mut. Res., 2008; 659:284-92.
[15]. Dos D Sarbassov, Siraj M Ali and David M Sabatini, Growing roles for the mTOR pathway. Current Opinion in Cell Biology, 2005; 17:596–603.
[16]. Jie Chen, Yimin Fang, A novel pathway regulating the mammalian target of rapamycin (mTOR) signaling. Biochemical Pharmacology, 2002; 64:1071-77.
[17]. Koji Ogata and Hideaki Umeyama, An automatic homology modeling method consisting of database searches and simulated annealing. J. Mol. Graphics Mod., 2000; 18:258–72.
[18]. Anthony Ivetac and J. Andrew McCammon. A Molecular Dynamics Ensemble- Based Approach for the Mapping of Druggable Binding Sites. In: Riccardo Baron, editor. Computational drug discovery and design, methods in molecular biology. USA: Humana Press; 2012.p.3-9.
[19]. Daniel Seeliger, Bert L. de Groot, Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J. Comp. Aided Mol. Des., 2010; 24:417-22.
[20]. Povl Krogsgaard-Larsen, Tommy Liljefors and Ulf Madsen. Textbook of Drug Design and Discovery.3rd ed. London and Newyork: Taylor & Francis; 2005.
[21]. Lipinski C.A, Lombardo.F, Dominy B.W and Feeney P.J. Experimental and Computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev., 1997; 23(1-3):3-25.
[22]. Kitchen DB, Decornez H, Furr JR, Bajorath J, Docking and scoring in virtual screening for drug discovery: methods and applications. Nature reviews, Drug discovery, 2004; 3(11):935-49.
[23]. Lengauer T, Rarey M, Computational methods for biomolecular docking. Curr. Opin. Struct. Biol., 1996; 6(3):402-6.
[24]. Solomon V R, Lee H, Quinoline as a priviledged scaffold in cancer drug discovery. Curr. Med. Chem., 2011; 18(10):1488-508.
[25]. Karunakar Tanneeru, Lalitha Guruprasad, Ligand-based 3-D pharmacophore generation and molecular docking of mTOR kinase inhibitors. J.Mol.Model., 2011;
[26]. Drug design [Online]. Available from: www.celgene.com/research/drug_discovery_and _development.aspx [Accessed on February 14, 2012].
[27]. Drug design [Online]. Available from: www.scfbio_iitd.res.in/tutorial/drugdiscovery.htm [Accessed on February 22, 2012].
[28]. Catalyst, version 4.11; Accelrys Inc: San Diego, CA 92121, USA, 2010. Cerius, version 4.11; Accelrys Inc: San Diego, CA 92121, USA, 2010.