International Journal of Pharmacy and Pharmaceutical Science

Review article
ISSN Print: 2664-7222
ISSN Online: 2664-7230
Impact Factor: RJIF 8
IJPPS 2023; 5(1): 40-44
www.pharmacyjournal.org
Received: 03-04-2023
Accepted: 07-05-2023

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Design, synthesis, characterization and pharmacological evaluation of mTOR inhibitors for anticancer activity

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DOI: https://doi.org/10.33545/26647222.2023.v5.i1a.32

Abstract
Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. 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 1016 (10 million cells being replaced every second!). Risk of cancers are increased by infectious agents including viruses [Hepatitis B virus (HBV1), Human Papillomavirus (HPV), Human Immunodeficiency Virus(HIV)-increase risk of Nasopharyngeal, Cervical carcinomas and Kaposi's Sarcoma] and bacteria such as Helicobacter pylori (Stomach cancers). Candidate molecules were docked for anticancer activity against the modeled protein target mTOR using drug design software (Maestro 9.1). Twenty five scaffolds were screened with high docking score against mTOR inhibitor. These compounds also passed Lipinski's rule. The scaffold containing quinoline nucleus was selected on the basis of synthetic feasibility.

Keywords: Synthesis, anticancer activity, DNA mutations, mTOR inhibitor

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. 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 1016 (10 million cells being replaced every second!). Risk of cancers are increased by infectious agents including viruses [Hepatitis B virus (HBV1), Human Papillomavirus (HPV), Human Immunodeficiency Virus(HIV)-increase risk of Nasopharyngeal, Cervical carcinomas and Kaposi's Sarcoma] and bacteria such as Helicobacter pylori (Stomach cancers). 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 six hallmarks of cancers are:

Immortality: continuous cell division and limitless replication

- Produce 'Go' signals (growth factors from oncogenes)
- Override 'Stop' signals (anti-growth signals from tumour suppressor genes)
- Resistance to cell death (apoptosis)
- Angiogenesis: Induction of new blood vessel growth
- Metastasis: Spread to other sites

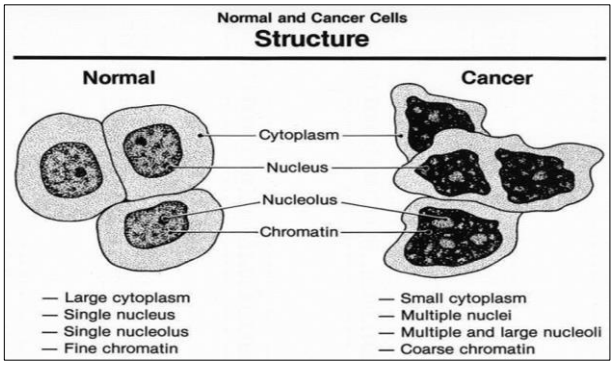

Fig 1: Structure of Normal and cancer cells

Types of cancer

Cancers may be categorized based on the functions/locations of the cells from which they originate. The following terms are commonly used to categorize by their tissue (cell type) of origin.

- **Carcinoma:** A tumor derived from epithelial cells, those cells that line the⎩ surface of our skin and organs. This is the most common cancer type and represents about 80-90% of all cancer cases reported.
- **Sarcoma:** A tumor derived from muscle, bone, cartilage or connective tissues.
- **Leukemia:** a cancer derived from white blood cells or their precursors. The⎩ cells that form both white and red blood cells are located in the bone marrow.
- **Lymphoma:** A cancer of bone marrow derived cells that affect the lymphatic⎩ system.
- **Myelomas:** a cancer involving the white blood cells responsible for the production of antibodies (B lymphocytes or B-cells)

Each type of cancer is unique with its own causes, symptoms, and method of treatment. The most common cancers are:

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

Globally, cancer of the colon and rectum5, 6, 7 is the third leading cause of cancer in males and fourth leading cause of cancer in females. The frequency of colorectal cancer varies around the world. It is common in the western world and is rare in Asia and Africa. In countries where the people have adopted western diets, the incidence of colorectal cancer is increasing. Factors that increase a person's risk of colorectal cancer include high fat intake, a family history of colorectal cancer and polyps, the presence of polyps in the large intestine, and chronic ulcerative colitis.

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)
- Interferon receptors (IFN)

The mammalian target of rapamycin (mTOR) 8, 9, 10, 11 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.

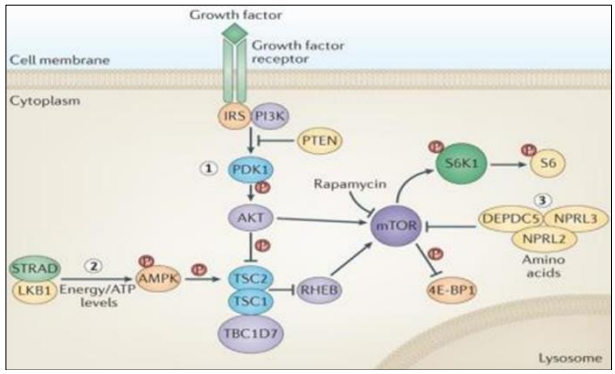

Fig 2: mTOR signaling pathway

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 phosphatidylinositol 3-kinase (PI3K)/AKt signaling pathway 12,13,14, one of the most frequently dysregulated pathways in cancer. This pathway has been shown to cooperate in prostate 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.

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:

- Template recognition and initial alignment
- Alignment correction
- Backbone generation
- Loop modeling
- Side-chain modeling
- Model optimization
- Model validation

Drug discovery [16, 17, 18]

Medicinal chemistry blends synthetic chemistry, molecular modeling, computational biology, structural genomics and pharmacology to discover and design new drugs, and investigate their interaction at the molecular, cellular and whole-animal level.

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 structurebased drug design (SBDD) essential tools for drug discovery.

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

- Low costs, no compounds have to be purchased externally or synthesized by a chemist.
- 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 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.

Lipinski's rule of five19 is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule is important for drug development where a pharmacologically active lead structure is optimized-step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule.

- A molecular weight less than 500
- No more than 5 hydrogen bond donor groups
- No more than 10 hydrogen bond acceptor group
- A calculated log P value less than +5 (log P is a measure of a drug's hydrophobicity)

Molecular docking [20, 21]

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 have 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

Conclusion

In current study a novel series of coumarin sulfonamides and amides derivatives were found to be the most potent derivative, which displayed favorable antiproliferative activities respectively. Moreover, scrutinising results of the cell cycle analysis unravelled that compound arrested the cell cycle mainly in the G0/G1 phase. Compounds is not only with significant anticancer activity, but also possessed promising pharmacokinetic properties. This work presents information that is helpful for the design and synthesis of new coumarin derivatives as potential antitumor drug candidates.

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