CHAPTER I
INTRODUCTION

1.1 Drug development through Medicinal chemistry

Medicinal chemistry is the science that focuses on the invention and designing of new chemical agents and their development into useful medicines. It also deals with the construction of structure activity relationships and the relationships between chemical structure and pharmacologic activity of compounds developed. Establishing a new pharmaceutical agent is a highly complex process that requires the talents of people from a variety of disciplines such as chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics etc.

Earliest drug discoveries were mainly relied on the plant sources. Herbal remedies play an important role all the way through human narration. In pharmaceuticals, many natural products are used in their original chemical structures. Subsequently therapeutic properties of these natural agents are successfully improved by their structural modifications.

The advancements in biological sciences, it is probable to know more about body functions at the cellular and molecular level. Now most of the researches in the drug manufacturing industry or institute begin with the recognition of an appropriate target and designing of a proper agent to interact with that target. In this approach, understanding the structure and function of the goal and the mechanism by which it interacts with the potential drug is an important one.

1.2 Drug development

Innovation and progress of a therapeutic agent are a lengthy, enthusiastic and interdisciplinary effort, mostly described as a consecutive process that starts from the detection of targets.

Different stages of drug development are summarized and shown in Figure 1.1.
Figure 1.1: Summary of new moiety development
Drug development composed of 2 phases.

1.2.1 Pre-drug discovery phase

   This phase consists of 3 important stages.

1.2.1.1 Understanding the disease

   Initially, scientists focused on the knowledge gathering about the disease and its causes. Information about a modification in the genes caused by the disease, alterations emerged in the encoding proteins and their interactions with each other and the changes that developed in the particular affected cells and tissues are the essential one in the understanding of disease and the improvement of proper drug to face it.

1.2.1.2 Identification of the target

   Generally target is a single molecule associated with a particular disease, which may be a protein or gene. The drug molecule interacts with target (receptor) and either enhances or inhibits the receptor activity. Basically, there are 2 chief groups of receptor proteins. One is proteins that float surrounding the cytoplasm of the cell and the other is proteins that are incorporated into the cell membrane. In the second case, drugs need not enter the cell; they can bind to an extracellular binding site of the protein and control intracellular reactions from the outside.

1.2.1.3 Validation of the target

   Association within the target and a particular disease is analyzed through various experiments conducted on both living cells and in animal models.

1.2.2 Drug discovery phase

   This phase initiates the evaluation of drug target that leads to identify a chemical moiety with pharmacological activity, known as ‘hit’. After confirming its pharmacological activity and chemical possibility, the hit moiety that becomes a lead structure, which is improved through lead optimization until it fulfils the criteria for a drug candidate. This phase consists of 3 stages.

1.2.2.1 Lead identification and screening

   Traditionally, leads are mainly obtained from natural resources. It also obtained from an established drug or competitors’ patents or publications. But recently, for many diseases, screening of herbal products for lead shows a poor track record comparing
with other approaches. Nowadays, most of the ‘leads’ are derived from the High Throughput Screening (HTS) of collection of compounds. In these methods, the selection was performed on the basis of chemical structure, physicochemical properties, and classes of compounds with a historically high rate of success or use the structural information of the target.

1.2.2.2 Optimization of lead

The successful lead compounds after the initial screening are further subjected to optimization or alteration for making them more effective and safer one.

1.2.2.3 New chemical entities

These are the final compounds obtained from the method of drug discovery. These compounds have capable activity against selective pharmacological target associated with a particular disease.

1.3 Current trends in drug design

Nowadays, structural modification in the new drug moiety gains popularity in the design of drug and the development of new therapeutic agents. The immense growth of genomics, proteomics and bioinformatics sectors and the improvement of technologies such as HTS, virtual screening and *in silico* screening make an innovative change in the method of drug discovery.

In recent years, application of computers and computational methods spread into all levels of molecule development and they play a most important task in the structure based drug design. Arrival of software technologies into the field of drug design make improvement of new therapeutic agent is a more specific and quick one. Computers are useful to replicate a chemical moiety and in the designing of chemical structures. Data generation, conversion of multifaceted biological data into workable knowledge is simply possible with advanced computer technologies. Now drug designing is a rapid process and less cost one due to the availability of these computational tools.

1.4 Inflammation

Inflammation is a complex biological reaction in vascular tissues against the harmful stimulus, for example, pathogens, harmed cells, or irritants. It is a defensive mechanism accepted by the organisms to eliminate the injurious stimuli and to start the therapeutic process. Thus, inflammation is considered as a vital survival methodology
embraced by the body to avoid real harm. Inflammation can be categorized as either chronic or acute. Acute inflammation is the beginning effect of the body to tissue harm. A course of biochemical events proliferates and extend into an inflammatory reaction, which include an extensive variety of chemical mediators and different cells inside of the injured tissue to modify local vascular and immune responses. The resident inflammatory cells, for example, macrophages, mast cells and dendrite cells at the spot of harm undergo activation following the onset of tissue harm and discharge a host of inflammatory mediators, including tumor necrosis factor α (TNFα), interleukin 1β (IL-1β) and an assortment of chemokines.

The cytokines TNFα and IL-1β form a vital part of the inflammatory response of the body against infection by entering into adjacent blood vessels expanding the outflow of bond factors ICAM-1 and VCAM-1 on endothelial cells of vascular endothelium. These bond molecules permit the connection of leukocytes (white blood cells) to the endothelium and empower their ensuing transmigration into peripheral tissue.

The chemokines assume a noteworthy part by going about as chemo attractant and controlling the inflammatory cells to spots of inflammation (chemotaxis). The flowing leukocytes identify a small concentration of chemokines and follow a signal of rising concentration (concentration gradient) towards the resource of chemokines, i.e. site of harm.

The leukocytes, so enrolled from the lumen of the blood vessel to the spot of harm inspire an immune response and make a coordinated effort to resolve injury or harm. It adds to the feedback control of inflammation by different mechanisms to influence the extent and period of the inflammatory response.

Tumor necrosis factor-alpha (TNFα) is a focal regulator of inflammation and as accordingly TNFα antagonists might be efficient in treating inflammatory disorders in which TNFα acting an important pathogenetic part. Acute inflammation is a general inflammatory disorder with frequencies that have expanded altogether in the course of the last few decades (Bhatia, et al., 2000). Prolonged inflammation, prompts a dynamic movement in the kind of cells present at the site of inflammation and is described by concurrent destruction and healing of the tissue from the inflammatory procedure. The prolonged inflammation prompts the onset of chronic inflammatory disorders (Steven Highfill, et al., 2011).
In acute inflammation, the cellular phospholipases are actuated to separate membrane phospholipids into arachidonic acid, which thus is metabolized to inflammatory prostaglandins and leukotrienes correspondingly by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX) as portrayed in Figure 1.2. The developments of these inflammatory intermediaries exaggerate the inflammation by causing expanded permeability, vasodilation and bronchoconstriction and platelet aggregation. The development of prostaglandins and leukotrienes from arachidonic acid can be concealed by hindering cyclooxygenase and lipoxygenase correspondingly.

The investigation for inhibitors of these enzymes in this way forms the premise for improvement of new anti-inflammatory agents. In the enzyme family unit of cyclooxygenases, hindrance of cyclooxygenase-2 (COX-2) is more enviable. Be that as it may, current studies revealed that specific restraint of COX-2 reduces inflammation, however, causes side effects, especially those prompting to cardiovascular complications (Ong, et al., 2007). Despite what might be expected, the hindrance of the 5-lipoxygenase (5-LOX) enzyme of the option pathway of inflammation not only decreases inflammation as well as enhances cardiac health by reducing risk of atherosclerosis (De Caterina and Zampolli, 2004). The option pathway interceded by

**Figure 1.2: Inflammation pathways mechanism**

[Diagram of inflammation pathways mechanism]
5-LOX has along these lines turned into a critical focus for the advancement of new anti-inflammatory drugs.

1.4.1 Inflammatory medication compounds

Subsequently the compounds that can balance the action or articulation of inflammatory enzymes, cytokines and chemokines can possibly be helpful as anti-inflammatory medications. The anti-inflammatory medications as of now being used might be of synthetic or natural or biological origin. Some of the main anti-inflammatory drug compounds 1.01 to 1.33 of synthetic origin are concise in Figure 1.3.
Piroxicam (1.20)

Meloxicam (1.21)

Tenoxicam (1.22)

Lornoxicam (1.23)

Isoxicam (1.24)

Mefenamic acid

Meclofenamic acid (1.26)

Flufenamic acid
Tuberculosis (TB) is a typical and lethal irresistible disease brought on by *Mycobacterium tuberculosis* (Mtb) and congeners. More than 33% of the world's populace now have the TB bacterium in their bodies and new diseases are happening at a rate of one every second (WHO, 2011). In 2004, 14.6 million individuals had dynamic TB, there were 8.9 million new cases and 1.7 million passing's, for the most part in developing nations (WHO, 2011). An increasing number of reactivation TB cases are seen in immunosuppressive ailment, pharmacological immunosuppression or
HIV/AIDS patients. Drug-safe strains of TB have developed and are spreading (MMWR, 2006). TB is traditionally treated by a 6-month to one year regimen of 4 orally-regulated medications under the direct perception (DOTS) as prescribed by WHO, and executed in expansive parts of India under the Revised National Tuberculosis Control Program (RNTCP).

1.5.1 The Pathogen

Mtb is a non-motile and rod-shaped facultative intracellular parasite, a committer aerobe which can survive and increase inside macrophages and other mammalian cells. The measure of the mycobacterium is 2-4 J, one meter long and 0.2-0.5 J and one meter in width. Mycobacteria are not ordered into Gram-positive or Gram-negative since they don't have the chemical attributes of either, in spite of the fact that the microscopic organisms do contain peptidoglycan (murein) in their cell wall. In phylogenetic studies, 16S rRNA sequencing demonstrated that Mtb has a place with a gathering of 'moderate producers', otherwise called 'M tuberculosis complex' (Rogall, et al., 1990). The era time of mycobacterium is regularly ~24 h in solid medium. This variety incorporates 6 individuals: M. tuberculosis the causative operators of human tuberculosis cases; M. africanum, contaminating people in sub-Saharan Africa; M microti, the causative specialists of TB in some amphibian rodents; M bovis, which taints a wide assortment of mammalian species, including people; BCG, a constricted variation of M bovis; and M canetti, a smooth variation that is once in a while experienced however motivations human infection. The critical elements shared by all individuals from the Mycobacterium variety incorporate a cell divider made out of a complex external cell wall, containing a lot of cell wall lipid. It comprises of a few one of a kind parts, for example, lipoarabinomannan, lipomannan, pthiocerol dimycocerostate, mycocerostate, mycolic corrosive, trehalose dimycolate and sulpholipids (Brennan, et al., 1990; Besra, et al., 1994). These segments are proposed to be in charge of mycobacterial hydrophobicity, the capacity to shape clusters or ropes, capacity to survive intracellularly and it is the cell wall that gives acid- fastness, empowering it to hold fundamental dyes in the vicinity of acid alcohol. Many clinical strains of pathogenic microbes have been accounted in the literature. 2 lab strains are most usually used to create comprehension of TB. The destructive H37Rv strain ATCC 25618 and its nearby relative, the weakened H37Ra strain ATCC 25177. Harmful strains share the capacity to
attack macrophages and get by inside of them in development captured phagosomes, which don't meld with lysosomes.

1.5.2 Macrophage-mycobacterium interactions

Alveolar macrophages are the essential cell sort included in the introductory uptake of Mtb. Alongside these, dendritic cells and monocyte-inferred macrophages additionally join in the phagocytosis process (Henderson, et al., 1997; Thurnher, et al., 1997).

1.5.2.1 Pathogen Entry Into Host Cell

The phagocytosis of Mtb includes diverse receptors on the macrophage, which either tie to nonopsonized microscopic organisms or perceive opsonins on the surface of the pathogen. (Schlesinger, 1993; Gaynor, et al., 1995; Hoheisel, et al., 1995; Schlesinger, 1996; Zamzami, et al., 1996; Aderem, et al., 1999).

1.5.2.2 Phagosomal Development restrained by mycobacteria

Internalization of particles by the macrophage at first structures a phagosome, trailed by a progression of consecutive combination occasions with different vesicles from the endocytic pathway finishing in a phagolysosome. Mtb makes due inside host macrophages by repressing phagosome-lysosome combination (Vergne, et al., 2004), which is fundamental for killing the pathogen (Armstrong, et al., 1971; Vergne, et al., 2004). Deficient acidification of the phagosome is because of the restraint of insertion of proton pumps in the vacuolar film (Sturgill-Koszycki, et al., 1994) and non appearance of full grown lysosomal hydrolases. The deficient luminal fermentation permits intracellular survival and development of mycobacteria inside tainted macrophages (Russell, 2001).

1.5.2.3 Other host components restrained by mycobacteria

Antigen handling

An imperative part of the mycobacterial phagosome is its wasteful antigen preparing limit (Pancholi, et al., 1993; Ramachandra, et al., 2001). It has been indicated utilizing purified phagosomes that the greater part of peptide-MHC-II edifices are shaped inside phagosomes, by stacking MHC-II atoms without earlier export of bacterial antigens from phagosomes to conventional antigen handling compartments. In these measures, heat-eliminated mycobacteria were prepared more promptly than live Mtb (Ramachandra, et al., 2001).
1.5.2.4 Reactive oxygen intermediates (ROI) and Reactive nitrogen intermediates (RNI)

Bactericidal system inside phagolysomes of actuated macrophage incorporates the generation of ROI and RNI. Proteins discharged by Mtb for example, superoxide dismutase (SOD) and the catalase-peroxidase framework are adversarial to ROI (Dahl, et al., 1996). Mycobacterial segments, for example, sulphatides, LAM and phenolic-glycolipid I (PGL-I) are intense oxygen radical foragers (Chan, et al., 1989; Chan, et al., 1991). Mycobacteria are impervious to executing in vitro by ROI, for example, superoxide and hydrogen peroxide (Chan, et al., 1992). Hydrogen peroxide (H$_2$O$_2$), one of the ROI produced by macrophages by means of the oxidative burst, was the initially recognized effector particle that intervened mycobactericial impacts of mononuclear phagocytes (Walker, et al., 1981). Be that as it may, the capacity of ROI to slaughter Mtb has been exhibited just in mice (Flesch, et al., 1990) and stays to be affirmed in people. Thinks about demonstrate that Mtb disease actuates the aggregation of macrophages in the lung furthermore H$_2$O$_2$ generation (Selvaraj, et al., 1988). Comparative neighborhood resistant reaction in tuberculous ascitic liquid has likewise been illustrated (Swamy, et al., 1988). Be that as it may, the expanded generation of hydrogen peroxide by alveolar macrophages is not particular for TB (Selvaraj, et al., 1988). Also, alveolar macrophages created less H$_2$O$_2$, than the comparing blood monocytes. RNI is created by initiated mouse macrophages are significant components in antimicrobial movement. Phagocytes, upon actuation by IFN-y and TNF-a, produce nitric oxide (NO) and related RNI by means of iNOS2 utilizing L-arginine as the substrate. Disease of human alveolar macrophages with M bovis BCG in vitro brought about expanded iNOS mRNA and hindrance of iNOS is trailed by expanded bacterial development (Nozaki, et al., 1997). In TB patients, alveolar macrophages show expanded creation of iNOS (Nicholson, et al., 1996). In any case, whether iNOS quality expression prompts in vivo NO generation stays indeterminate, as in people post translational adjustment of iNOS might be important for utilitarian movement (Salh, et al., 1998). In this way, the careful commitment of RNI in human TB stays to be explained. Supported intracellular development of Mtb might rely on upon its capacity to maintain a strategic distance from obliteration by lysosomal compounds, ROI and RNI. The centrality of these dangerous nitrogen oxides in host guard against M
tuberculosis has been very much reported, both in vitro and in vivo, especially in the murine framework (Chan, et al., 2001). In iNOS quality thump out mice Mtb duplicates much quicker than in wild sort creatures, suggesting a critical part for NO in mycobacterial host resistance (Mac Micking, et al., 1997).

1.5.2.5 Cytokines Involved in the Immune reaction

Colonization of phagocytic cells by Mtb prompts cell actuation and cytokine generation, which affects further initiation and cytokine creation in a mind boggling procedure of auto regulation and cross-regulation. This cytokine system assumes an urgent part in the provocative reaction and dynamic illness. Some key cytokines in the process incorporate y Interferon IFN-y is delivered basically by both CD4+ and CD8+ T cells, and also by NK cells not withstanding macrophages themselves. IFN-y is vital for macrophage actuation in TB, and is thought to be crucial for compelling host reaction against contamination. The defensive part of IFN-y in TB is entrenched, basically in the setting of antigen- particular T cell insusceptibility (Garred, et al., 1997). IFN-y is delivered by T cells from solid PPD+ subjects and also those with dynamic TB. Albeit a few studies recommend that IFN-y levels are discouraged in patients with dynamic TB (Nicholson, et al., 1996; Cynamon, et al., 1999), this cytokine may not be perfect as a marker to connect assurance against disease. A report that Mtb has created instruments to constrain the enactment of macrophages by IFN-y (Oddo, et al., 1998) recommends that the measure of IFN-y delivered by T cells might be less prescient of result than the capacity of the cells to react to this cytokine. IFN-y is the real activator of macrophages in mouse, however, not human, macrophages to repress the development of Mtb in vitro (Cooper, et al., 1993).

1.5.2.6 Tumor Necrosis Factor (TNF-a)

TNF-a is viewed as "urgent" to the control of the contamination. M tuberculosis supresses TNF-a discharge in a strain-particular manner. Avirulent strains are not ready to repress TNF discharge, but rather harmful strains restrain TNF emission by contaminated macrophages (Riendeau, et al., 2003). In mice inadequate in TNF-an or TNF receptor, Mtb contamination brought about fast demise of the mice, with generously higher bacterial weights contrasted with control mice (Bean, et al., 1999). TNF-an in cooperative energy with IFN-y affects NOS2 expression (Liew, et al., 1990). TNF-a is vital for walling off contamination and forestalling dispersal. Persuading
information on the significance of this cytokine in granuloma arrangement in TB and other mycobacterial maladies has been accounted for (Flesch, et al., 1990; Flynn, et al., 1995). TNF-α influences cell movement and restriction inside of tissues in Mtb disease. Amid endless contamination, NOS2 expression in the lungs was lessened after TNF-α balance (Mohan, et al., 2001). TNF-α impacts articulation of bond particles and in addition chemokines and/or their receptors and influences the development of useful granuloma in tainted tissues. TNF-α has likewise been embroiled in immunopathologic reaction and is frequently a main consideration in host-intervened devastation of lung tissue (Moreira, et al., 1997).

1.5.2.7 Interleukin-1β

A second proinflammatory cytokine included in the host reaction to *M. tuberculosis* is IL-1β. Like TNF-α, IL-1β is mostly created by monocytes, macrophages, and dendritic cells. In TB patients, IL-1β is articulated in overabundance at the site of infection (Law, et al., 1996). IL-1β is main in affecting the course of experimental TB in mice: IL-1α and - 1~ 2fold KO mice (Sugawara, et al., 2000) and IL-1R sort 1-inadequate mice (which don't react to IL-1~) show an expanded mycobacterial trouble furthermore imperfect granuloma development after contamination with Mtb (Leemans, et al., 2001).

1.5.2.8 Interleukin-2

IL-2 has a crucial role in generating an immune response against specific antigen by inducing an expansion of the pool of lymphocytes. Therefore IL-2 secretion by the protective CD4Th1 cells is an important parameter to be measured protection against specific antigen.

1.5.2.9 Interleukin-4

IL-4 is a classic Th2 cytokine. The part of IL-4 in TB is still dubious. In TB patients a discouraged Th1 reaction, however, not an upgraded Th2 reaction was seen in segregated PBMC (Robinson, et al., 1994; Nicholson, et al., 1996; Ottenhoff, et al., 1998; Cynamon, et al., 1999). Hoisted IFN-y expression was identified in granuloma inside of lymph hubs of patients with *tuberculosis lymphadenitis*, yet little IL-4 mRNA was distinguished (Nicholson, et al., 1996).
These outcomes demonstrated that in people a strong Th2 reaction is not connected with TB. In mice, studies (Cooper, et al., 1993) recommend that the non appearance of a Th1 reaction to Mtb does not as a matter of course advance a Th2 reaction and an IFN-γ deficiency, as opposed to the vicinity of IL-4 or other Th2 cytokines, avoids spread of disease. The occurrence or non appearance of IL-4 did not connect with enhanced clinical result or contrasts in granuloma stages or pathology. The pernicious impacts of iL-4 in intracellular infections (including TB) have been credited.
to this present cytokine's suppression of IFN-y production (Powrie, et al., 1993). Some of the main tuberculosis drug compounds 1.34 to 1.33 of synthetic and semisynthetic origins are concise in Figure 1.4.

1.6. CANCER

Cancer is a collection of more than 100 diseases categorized by uncontrolled cellular growth which results in the changes of genetic information in cells. Cells and tissues are complex systems with critical stages and checkpoints to ensure normal growth, development, and function. Normally the division, differentiation, and death of cells are carefully regulated. All cancers begin as a sole cell that has gone astray, control of its usual growth and replication processes (Marmot, et al., 2007). Human adults are formed with around $10^{13}$ cells, which are renewed and replaced constantly. About 5 to 10 % of tumor results unswervingly from inheriting genes related to tumor, but the widely held that involved in the alterations or injure accumulated over time to the genetic material within cells. The causes of damage are both endogenous (internal) and exogenous (environmental). Food, nourishment, and physical activity are important environmental factors in the formation of cancer. The apoptosis and tumor formation in human have been placed in Figure 1.5.

![Figure 1.5: Apoptosis and Tumor](image-url)
Tumors that stay in a single spot and reveal limited expansion are normally considered to be benign (Kerr, et al., 1994)

1.6.1 Cancer in India

From the beginning of 2002, 16 oncology clinical trials were granted approval to date. Of these, 2 were phase I studies for chemotherapeutic agents manufactured by Indian Pharmaceutical companies. The studies were for non-small cell lung tumor, neck and head tumor and breast cancer. The year 2003 saw more clinical protocols being submitted for permission to conduct multinational, global studies with India as part of a global drug development plan. Studies that are ongoing include cancers of head & neck, Chronic Myelogenous Leukemia, breast, ovarian, colorectal and lung (Radhika, et al., 2003).

Etoposide (1.39)

5-fluorouracil (1.41)

Oxaliplatin (1.43)

Doxorubicin (1.40)

Etoposide (1.42)

Methotrexate (1.44)
India has become a destination of choice for multinational studies in the field of oncology due to the large patient numbers, improving regulatory processes that are being implemented, investigators who are research and academically inclined and the huge number of patients. The anticancer medications as of now being used might be of synthetic or natural or biological origin. Some of the main anticancer drug compounds 1.39 to 1.48 of synthetic origin are concise in Figure 1.6.
Literature Review