CHAPTER-1

INTRODUCTION

1.1 General introduction

Medicines innovated in current scenario are the results of highly devotional efforts done by human being and civilization of and on. When the age of synthetic and medicinal drugs started, it paved thousand of possibilities for the growth and discovery of variant synthetic molecules with slight biological activities and therapeutic efficacy. But developing a new molecule every time was neither easy nor a wise step too, so concept of derivatization came. It is always better to synthesize a derivative of known molecule with known properties rather than to synthesize a totally unknown new molecule. In fact it is a wise step, in minimizing the toxicity as well as improving potency of the parent molecule. It is a rational approach towards the drug design and development, based upon the various physical and physiochemical parameters. That’s why most of the drugs in practice are derivatives of known molecules, whether it is β-lactam antibiotics (Penicillin G, Penicillin V, and Ampicillin), Irreversible proton pump inhibitors (Omeprazole, Lansoprazole, Rabeprazole), or CNS drugs such as BDZ derivatives i.e Diazepam and Clonazepam etc. Hardman et al (2001)

The earlier sources of drugs were from minerals, animals and plants, but due to the lack of potential, fewer efficacies, definitive cure, delayed in cure of diseases and some time more toxicity, the discovery of new drugs begins that are more potential and less toxic is nature. The synthesis of derivatives has been become an important part and is focused on modifying the action of drugs, mainly to reduce the side effects and to potentiate the drug action. Today’s more than 60% drugs used in practice are the derivatives of known molecules, and day-by-day the scope of synthetic and medicinal chemistry is broadening and long-lasting. Isatin (1H-indole-2,3-dione) is a synthetic universal substrates and is used for the synthesis of a huge variations of heterocyclic compounds. Indole and quinoline moieties are the examples containing isatin, and used as basic component for the synthesis of numerous drugs. Isatins have also been recognized in various tissue of mammalian and their biological functions are being analyzed as a initiator in biochemical processes in human beings which has been the subject of several discussions.

The isatin has turned in to the synthetic versatility and compatibility to the comprehensive use and in organic synthesis this compound is needed. The approaches of isatin in organic synthesis have stemmed from the interest of its pharmacological and biological components. Joaquin et al (2001)

The compound was considered synthetic for almost 140 years until it was found to be present in plants from the genus isatis Guo and Chen (1986). Bergman et al (1985) proposed presence of isatin in Calanthe discolor LINDL, and Couroupita guianensis Aubl, and has also been recognized in the secretion of the parotid gland of bufó frog Wei et al (1982) whereas in human it is the metabolic derivative of adrenaline. Ischia et al (1988)

Halket et al (1991); Kapadia (1976); Kapadia et al (1980); Ansaasamoah et al (1990) investigated the presence of substituted isatin in various plants e.g. the melasotin alkaloid (methoxy phenylpentyl isatins) which are derived from the Caribbean tumorigenic plant Melochia tomentosa. Isatin substituents have also been found in fungi: Streptomyces albus kapadia and Shukla (1993); Grafe and Radics (1986) symbiotic bacteria Phelan et al (2012)
and marine mollusks Schunck (1879); Letellier (1890); Baker and Sutherland (1968); Benkendorff et al (2001) where they are postulated to play a defensive role against pathogenic organism.

The picture shows adult Australian muricid mollusks (*Dicatharis Orbita*) amongst freshly laid egg capsules a rich source of cytotoxic isatin derivatives tyrindoleninone (6-bromo-2-methylthio-\(3H\)-indol-3-one). Westley et al (2006)

In human and other mammals isatin is found as an endogenous molecule although the synthesis and metabolism of isatin have not yet been fully studied. It has been assumed worthwhile that it has been synthesized *in-vivo* from phenylalanine and tryptophan rich foods such as meat, dairy and whole grains. In this pathway tryptophan is converted into indole by intestinal bacteria or flora and then transported into liver where it is absorbed and oxidized Hamane et al (1999); Gillam et al (2000). Isatin also plays a role in many physiological pathways. Pandeya et al (2005); Medvedev et al (2005); Medvedev et al (2007)

Isatin contains a relatively large number of functionalisable groups and this together with its long term use in the synthetic dye industry has led to its wide spread exploitation as a substrate/intermediate for organic synthesis. A number of review articles including a comprehensive survey by Silva et al (2001) describing the synthesis and chemistry of isatin have been published Sumpter (1944); Popp et al (1975); Mesropyan and Avetisyan (2009). Reviews that discuss the utility of isatin as a precursor for the synthesis of other heterocyclic compounds are also available. Ivashehenko and Dziomko (1977); Shvekhgeimer (1996); Joshi and Joshi (1999)

Recently it has been shown that cytochrome P450 2A6 efficiently catalyses the reaction and known for its CNS activity too Arias et al (2003) as well investigated the binding order of isatin in Wistar rat brain and studied by \(\beta\) imaging technique and the order was found as cerebellum, cortex, hypothalamus > hippocampus > brain stem > thalamus = striatum.

1.2 History of Indole

Isatin (1H-indole-2,3-dione) an oxidized derivative of indole was first discovered by Erdmann and Laurent in 1840 as a product arising from the oxidation of indigo using nitric acid and chromic acid \textbf{Erdmann (1840); Laurent (1840)}. Bayer first prepared indole in 1866 by zinc distillation of ox-indole. The IUPAC name of indole is 1-H-benzo-pyrrole. Indole is a planar molecule with a conjugated system of 10 \(\pi\) electrons. Indole possesses resonance with a resonance energy of 47-49 K cal/mole and acts as a very unenergetic base with pKa value 3.6

In structure a, b, and d the benzenoid 6-\(\pi\) system is preserved. The electrophilic attack occurs at 3\(^{rd}\) position due to the presence of greater density of electrons and the distance from the nitrogen atom present in indole ring. Molecular orbital method (MOA) and calculation of \(\pi\) electron density revealed the presence of high electron density at 3\(^{rd}\) position. \textbf{Solomons et al (1996); Mehta and Mehta (2010)}

Indoles can be used in formation of various metal complexes and in various organic reactions like acylation, protonation, nitration, sulfonation, halogenations, etc \textbf{Bansal (2005)}. It produces electrophilic substitution reactions at 3\(^{rd}\) position less preferably at position 2\(^{nd}\) due to the greater stabilization of the intermediate carbonium ion formed in 3-substitution than in 2\(^{nd}\) substitution, it also offers nucleophilic substitutions reactions also at 2\(^{nd}\) position. Probably,
Indoles are the most widely distributed heterocyclic compound in nature. Essential amino acid and Tryptophan is the major constituent of most of the proteins. While in animals serotonin (5-HT) and melatonins are also indole derivatives. These are also widely used for the treatment of disorders example sumatriptan, for the treatment of migraine and nausea, alosteron for treatment of irritable bowel and allergic syndrome. In plant kingdom substances derived from tryptophan are also very useful example indole-3-yl acetic acid act as a plant growth hormone. Vincristine, a dimeric indole alkaloid is all the same highly important in treatment of cancer, Brassinin, isolated from turnips is a phytoalexin which is being used for the treatment of microbial infection. Rheumatoid arthritis is also treated by indomethacin containing indole nucleus. These concerned reports explains the reason why indole and its derivatives are even now a very interesting and universal molecule from when it has been synthesized in the year of 1866. Joule and Mills (2004)

1.2.1 Structure and Chemistry
It forms red needles m.p 200-201°C and readily undergoes clean aromatic substitution reaction at C-5, N-alkylation via anions, and ketonic reaction at the C-3 carbonyl groups. If the 5<sup>th</sup> position is already occupied then electrophile takes the 7<sup>th</sup> position. The carbonyl group at position 2 is adjacent to the hetero atom and is stabilized by resonance. Thus it behaves as typical amide in its properties. It can be recrystallized from the hot water or ethanol. Isatin fused with various substituents on the aromatic ring which are usually obtained from the succeeding anilines, and can be synthesized by electrophilic aromatic substitution reactions also. The conversion of isatin in to other heterocyclic molecules has been discussed with many synthetic methodologies.

The generalization of chemistry of indole can be proposed by one of the following method:
   a) The reduction of the heterocyclic ring weather it is partial or complete leads conversion in to indoles and its derivatives.
   b) Isatin ring can be oxidized in to isatoic anhydride with subsequent conversion to other heterocyclic systems.
   c) Position C-3 is in favour of Nucleophilic addition reaction, which is further involved in a cyclization process to form a ring and this reaction is resulted by a spiro-annelation at position C-3 position in the presence or absence of N1-C2 bond cleavage.
d) The opening of the heterocyclic ring is favoured by nucleophilic substitution which is to take place at C-2 position. This procedure may be further proceeded by an either intramolecular or intermolecular bonding or by an exo-trig cyclization.

![Diagram]

fig: Nucleophilic substitution in isatin and its derivatives can take place at C-2 or C-3 position.

e) The chemo selectivity of the chemical reactions depend on the nature of the substituents and nucleophile fused to the isatin moiety. Sometimes it may also relate to the bonded nitrogen atom and nature of the solvent or temperature employed. Cyclization products are formed by the substances obtained initially in the presence of a second nucleophilic group. That is why reactions have been taken out by the nature of the nucleophile. Joaquim et al (2001)

### 1.2.2 Spectral Analysis of Isatin

The structural information about the isatin derivative is given by crystallographic data and proposed that the structure of isatin is almost planar and has a bond length of 1.55 Å between the two-carbonyl carbons. This large value is due to the existing repulsion between lone pair of electrons present on two oxygen atoms.

The FT-IR spectrum of isatin showed stretching vibrations for the functional groups present in it and indicate a broad band at 3190 cm⁻¹ due to the N-H stretching, which is because of the presence of by many sub-bands. On deuteration all bonds moved to lower frequency. Two strong bands at 1740 cm⁻¹ and 1620 cm⁻¹ are due to the carbonyl stretching vibrations. This affects presence of various bands in the region 1400-1100 cm⁻¹ which corresponds N-H functional group in-plane bending. Glasso et al (1968); Naumov and Jovanovski (2001)

The ¹H NMR spectra of isatin showed the peaks for the aromatic protons at 6.86 (d), 7.00 (t), 7.47 (d) and 7.53 (t) (DMSO-d₆), that are correspond to H-7, H-5, H-4 and H-6 respectively, and 1H (-NH) peak at 11.12 δ ppm.

The electron-impact mass spectrum of isatin and its related derivatives generally undergoes following fragmentation pattern. Verma et al (2005); Preet and Barbuch (1984)
The M⁺ peak (molecular ion peak) in acetylated derivatives appears at relatively low intensity. The fragmentation process shows loss of ketene (ion d') and of CO (ion e'). Isatin does not show fluorescence or phosphorescence under any condition. Haucke et al (1987)
1.3 Benzoyl hydrazide and Quinazoline

Benzoyl hydrazide and 3-amino-2-phenyl quinazoline is a moiety having a free amino group which is much susceptible to attach with the isatin moiety at 3\textsuperscript{rd} position. It can be prepared either by conventional method or microwave method, with the melting point 108 °C (lit- 113-114 °C). Desai and Desai (2005)

1.4 Antimicrobial Activity

The term chemotherapy was coined by Ehrlich at the beginning of the century to describe the use of synthetic chemicals to destroy infective agents. Chemotherapeutic agents are chemicals that are intended to toxic for the pathogenic organism but for the host. These substances may be prepared in the chemical laboratory or obtained from microorganisms, plants and animals. Rang et al (2005)

Initially the term chemotherapeutic agent was restricted to synthetic compounds, but now since many antibiotics and their analogues have been synthesized. This criterion has become irrelevant; both synthetically and microbiologically produced drugs need to be including together. However it would be more meaningful to use the term antimicrobial agent (AMA) to designate naturally obtained drugs as well as synthetic that attenuate microorganisms.

Antibiotics are chemical compounds produced by living microorganisms (bacteria, fungi, actinomycetes), semi synthetic and synthetic origin. Which are active at high dilution capable of inhibiting or killing bacteria, & other micro organisms. Synthetic anti-infective drug differ from naturally derived antibiotics only their origin, in that they are synthetic. Basically the molecules of these entire agents react with microbial cell molecules in a way interferes with the normal metabolic processes of the microorganisms.

The modern era of antimicrobial chemotherapy was started in 1936 with the induction of sulfanilamide with the clinical practice. In 1941 discovered new drug penicillin used for clinical purpose. Streptomycin, chloramphenicol and chlortetracycline are the main drugs in the category of antibiotics that were identified at the end of or early after World War II Block et al (2005); Williams and Lemke (2002). Since then various categories of antimicrobial agents have been discovered and literally hundreds to thousands of drugs are available for use in today human.

Antimicrobial agent may act either by destroying the organism (bactericidal) or by inhibiting it’s growth (bacteriostatic). The cell wall of bacteria is a thick rigid envelope, mainly compound of sugar and peptides called mucopeptide or murein. The mucopeptide is much thicker in Gram positive cell.
Underneath the rigid cell wall is a cytoplasmic membrane, which encloses the cytoplasm. This membrane has lipoprotein structural element which account for selective permeability to water, ions and nutrients. Antimicrobials attacking this membrane may alter permeability of the cell or may disorganize the lipoproteins leading to cell death. Satoskar and Bhandarkar (2005); Tripathi (2014)

1.5 Anti-tubercular Activity

The most prevailed communicable infectious disease on earth is Tuberculosis which is uncontrollable in developing countries. Among all the infectious diseases, TB is the only infectious disease and causes the highest numbers of death in human being leading to 3 million casualties every year in short we can say 5 casualties in every minute. In the whole world TB is the only main reason of death caused by infectious disease in comparison to malaria and AIDS. Every year near about 8 to 10 million people are affected with this disease producing agent. In India about 500000 persons die annually due to TB. That is why world health organization (WHO) declared in 1993 TB to be the world wide emergency Dutt and Stead (1999). Overtime antibacterial infection generates severe defects in cell mediated immunity and brings to heavy reduction of CD4 T-lymphocytes (T-cells) which causes infection like TB. According to world health organization (WHO), TB is the leading infectious disease among humans and world’s one third population is being infected with mycobacterium tuberculosis, less developed countries are mostly exist with TB while in other parts of the world does it very often Mantu et al (2010).

Spreading of HIV in the world may worsen the situation of TB. The virus which is responsible for spreading TB weakens the situation of TB immunity and permitt latent TB for the reactivation and grow and makes the patient only impressed emotionally towards regenerate infection with either drug resistant strains or drug susceptible. The harmful combination of HIV infection and drug resistant TB are the major two growing problems that present serious challenges for the control of TB effectively.

Mostly human illnesses are caused by microbial infections like bacteria, virus and fungi. Where certain tubercular, fungal, viral and bacterial infections are quite common because their tendency to develop resistance against the classical drugs and forming new strains under any conditions. Tuberculosis (TB) and HIV are the main diseases belongs to it. It seems that the mostly used drug for the treatment of patients suffereing from HIV should crush the replication of HIV so act as that of anti HIV drug and also should be used for the treatment of opportunistic infection like TB. Kuete et al (2011)

Especially in the tropical region of south east asia and sub-saharan Africa, Tuberculosis (TB) has made severe back path since 1980’s. The resurgence of TB is directly connected to the tremendous spreading of the HIV epidemic mainly in Africa, which makes the effect that, two - thirds of HIV patients are suffered with TB. During the last two decades Russia and Eastern Europe have been found new TB existing countries. The major problem in TB therapy is that Mycobacterium tuberculosis became more and more resistant to the classical anti tubercular drugs Mantu et al (2010). According to a recent report about the TB and data collected related to it by WHO, it was found that the numbers of new cases aggregated of TB in 2002 in the whole world had been raised up 9 million approximately. If the present and same trend continues, about 60 million people will die suffering from TB by 2020. In spite of this, in addition, multi drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) is also marked its presence in order to increase incidence in many regions.
1.5.1 Multiple Drug Resistant Tuberculosis (MDR-TB)

Patients suffering MDR-TB are more resistant to anti-tubercular drugs especially isoniazid and rifampicin. This is a human generated problem and more fatal than tuberculosis itself. Such cases are being difficult to treat the disease and remain infectious for longer period of time. MDR-TB can be treated with the combination of remaining 1st line and 2nd line drugs Lippincott et al (2010). The treatment duration takes up to two years. In general, treatment of MDR-TB requires more complex intervention, longer hospitalization and extensive laboratory monitoring.

1.5.2 Extensively Drug Resistant Tuberculosis (XDR-TB)

Extensively drug-resistant TB (XDR-TB) is also found to resist first line anti-tubercular drugs isoniazid and rifampin whereas among second-line drugs, fluoroquinolone and one among the three injectable drugs Katzung (2015). XDR-TB is virtually and unfortunate untreatable form of tuberculosis. Because XDR TB is resistant to first-line as well as second line drugs. The reason of high mortality rate is that who are left with the treatment is less effective.

1.5.3 Direct Observed Therapy (DOT)

Direct observed therapy (DOT) is a therapy that provides the anti-tubercular medicines to the patient direct suffering from tuberculosis, all the same also observe as the patient takes the medicines. This treatment is generally recommended for the entire person suffering from TB by the core management.

Patient-centered programs are identified in order to enhance the therapy completion. These programs are utilized to wide area of approaches that are decided on the situation and requirements of the patient who is suffering from TB. DOT is the most recommended strategy at the initial stage and as one of approaches. This strategy favors to put particular attention to the patient.

In low affected areas DOT has not been a matter of controlled trials but the United States firmly proposed DOT after observation and findings and meta analysis. So it is the best remedy suggested for the treatment of TB. Three publications on the study on DOT have come in forth so far, but they have been published in highly affected areas, two of them could not show any benefit while third one has appropriate advantage for DOT. The apparent data which came from the mentioned studies are that it cannot be appropriate to limit DOT to passive observation of medication. An interference with an aggression must be there if an affected person missed a dose. The result can be enhanced and improve only when DOT is used in this manner.

DOT can be given to the patient any time any where i.e. it can be given either daily or alternatively under the supervision of appropriate personnel in office, clinic or in the field. It is advisable regarding use of DOT that, it may be prescribed to all patients who are admitted in hospitals and nursing homes. It is necessary that all the persons affected with TB should be treated with such precautions that administration of drug with an interval and must be given all doses under DOT due to possible dangerous aftermath of missed dose. Dipirio and Talbert (2010)

One of the most plus point of administering DOT, is that it makes us capable to identify the disobedience, reaction of wrong medication and making TB clinically worst. Its close connection is with the group of patients affected with TB, by proving them DOT for the looking after their health purpose. There is no guarantee from DOT regarding the intake of other medicine. Patients perhaps may not take medicines or may knowingly eject the pills taken. The result is that all the
patients along with who are being treated with DOT must be watched constantly whether there is any sign of failure of medicine, so all programs which are patient-centered comprehensively; DOT is only one of them which require encouragement. Those patients who are found serious to transmit this disease to others or likely having the problem with entanglement should be given DOT on priority basis if the other means are constraint. Suppose DOT is not in use or being administered, then a certain amount of dose combination prepared with INH and RIF or INH, RIF and PZA can be lessen the risk of the patient who has been taking only one medicine and can help to check the enhancement of drug resistance. Formulations of combination are easy for administration and can be lessen the types of drug mistakes. When DOT is not being used, fixed dose combination preparations containing INH and RIF or INH, RIF, and PZA reduce the risk of the patient taking only one drug and may help to prevent the development of drug resistance. Combination formulations are easier for administration and also may reduce kind of medication errors.

1.5.4 Vaccine

Tuberculosis can be prevented by vaccination with BCG vaccine.

BCG Vaccine

Bacillus Calmette-Gurein (BCG) is a prophylactic vaccine against TB. It is considered as a live bacterial vaccine, obtained from an attenuated bovine strain of tubercle bacilli. It induces benign and artificial primary infection which is able to stimulate an acquired resistance to the possible infection with virulent tubercle bacilli and thus reduce the morbidity and mortality from primary TB among those at risk. There are two types of BCG vaccine- liquid and freeze dried vaccine. Dipirio and Talbert (2010)

The freeze dried vaccine is more stable and hence widely used. Consequences these vaccines are highly active but most probably they will not cause any result in sterilizing immunity and therefore, will not able to any solution regarding unexplained TB problem in the world. This means vaccines do not usually check the disease and cure of unexplained TB or regeneration of lung related disease in adult life but help to find the target of eliminating TB in the world by 2050.