CHAPTER-1
INTRODUCTION TO PROCESS DEVELOPMENT OF APIs AND A BRIEF REVIEW ON INDOLE AND FUSED INDOLE DERIVATIVES
1.1 **Introduction to Active Pharmaceutical Ingredients (APIs)**

A pharmaceutical drug is a substance which is used in the treatment, cure and prevent the diseases. These compounds are generally chemical substances (organic compounds) including small organic molecules, biopolymers, peptides and nucleosides. However, inorganic compounds like *cis*-platin series of platinum-containing complexes have been found as *anti*-cancer agents.¹

Medicinal chemistry or pharmaceutical chemistry is a vast subject, where pharmaceutical drugs are developed by combining chemistry, molecular biology, statistics and pharmacology. By utilizing medicinal chemistry, novel compounds (hits) will be identified by screening the compound library for specific target (figure 1.1).² Other sources of hits can be isolation from the natural sources such as fungi, plants and animals. Next step in the development is further chemical modification of candidate library to enhance the biological and physicochemical properties.

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**Figure 1.1:** Development journey of new drug from discovery to launch
Active component in the drug (drug product) is called active pharmaceutical ingredient (drug substance). This active ingredient will be combined with other suitable excipients like carbohydrates, stearate, antioxidants, etc. which will be administered through a particular route of administration.

Development of new drugs is one of the major challenges for the pharmaceutical industries because the key activities involved in the development of new drugs are extremely expensive and prolonged process. Since branded drugs are expensive, generic drugs became popular in US market. Generic drugs are available relatively low price due to the low production cost and competition between the generic companies.

A generic drug is a drug which is produced and distributed without patent protection. The generic drug may still have a patent on the formulation but not on the active ingredient. As per the USFDA regulations generic drug should contain same drug substance with satisfactory bioequivalent range compare to branded drug with respect to pharmaco properties.

When the innovator makes/markets the drug, it will be under patent protection. Generic company can be legally produced the drugs where
i) para-I: No patent information exists on the related product.
ii) para-II: Patent has expired on the related product. iii) para-III: Date on which relevant patent will expire is noted in application. iv) para-IV: the
generic company certifies the branded company's patents are either invalid, unenforceable or will not be infringed.

Paragraph I or II certification permits the drug product to be approved instantly. Paragraph III certification specifies that the ANDA can be approved on the date of the patent expiry. If the Orange Book (OB) lists unexpired patents, the generic company seeking ANDA approval before to the patent expiration, must challenge the listing of the patent under the paragraph IV certification, that the patent is invalid or will not be infringed by the manufacture use, or sale of the drug product. File a statement that the application for use is not claimed in the listed patent.

This highlights the care and attention that should be taken when filing patent applications. A wrong word or missed word in a claim might create debatable lawful opportunity for competitors which may possibly have a significant impact on the exclusive rights of a company.

Hence, it is vital when developing new processes to existing substances that an extensive patent search is conducted. This search should establish that which processes or intermediates have been used and patented by whom and in which countries, whether the patents have expired, and the validity of the claims. Thorough knowledge in the patent laws, claim construction and the synthetic chemistry is the key aspects of the success. The further opportunities are designing around a patent or varying to alternative technology eventually enhances the success to enter in the regulatory markets avoiding the IP fence.
Synthetic procedures used for the synthesis of libraries during the drug development are rarely applicable for commercial scale production. To produce the API on ton scale, the synthetic process must be thoroughly studied to obtain the optimum process.

### 1.2 Process Development

Development of active pharmaceutical is related to process chemistry and it is main application of organic synthesis, which refers to the design and development of synthetic routes and manufacturing at commercial scale.\(^3\) Process research and development is an integral part of the generic pharmaceutical industry that plays a major role in providing the safe, efficient, robust, cost effective and environmentally friendly synthesis of medicines to treat the human disease.\(^4\) The demand for cost effective drugs is a growing trend across the globe. Process development flow diagram of active pharmaceutical ingredients (API) in the generic pharmaceutical company is shown in figure-1.2.

A significant feature of robustness is the controlling of impurities\(^5\), getting the required polymorph, yield, purity and particle size consistently within the specification limits.

Process chemistry established its professional identity with the advent of modern pharmaceuticals that required multiple-step synthesis and stringent requirements for quality of the active pharmaceutical ingredients.\(^6\) Process chemists should have process knowledge to enable commercial production. This includes suitability of the synthetic routes
with respect to scalability, economics, optimization and identification of critical process parameters, development and validation of analytical methods, characterization of impurities, and establishment of quality control strategies etc. Close collaboration with chemical engineers and analytical chemists is essential to ensure the accuracy of these tasks.

**Figure 1.2:** Typical process development flow diagram of API synthesis

Process chemistry is interplay of several underlined factors such as freedom to operate, polymorphism, efficient analytical methods to
determine quality as well as green chemistry. In current scenario, the main objective of process R&D of a generic API player is to establish a non-infringing process that meets the above-defined issues under tremendous patent restrictions. Overcoming the bunch of process patents imposes a genuine challenge to the chemists working in generic process R&D field both at chemistry as well as formulation. The cost of API depends on the following parameters.

1. Raw materials (reactants, catalysts, solvents and reagents).
2. Robustness of the process (concise process and high yields).
3. Cycle time.
4. Recovery.

Following are the key factors involved in the process development, these points need to be followed to produce the APIs in safe and eco-friendly manner.

1. Design the synthesis with safe chemicals.
2. Minimize the usage of solvents.
3. Ensure the material balance (All the raw materials used in the synthesis should be converted to the end product).
4. Minimize the usage of energy; preferably design the experiments at room temperatures.
5. All the raw materials, reagents, solvents or feedstock must be renewable instead of depleting.
(6) Avoid/minimize the usage of protecting groups. Unnecessary usage of protecting groups can generate a lot of waste.

(7) Plan for catalytic reactions instead of stoichiometric reactions.

Identification, synthesis and characterization of impurities also play detrimental role in the process development of API. All the impurities levels must comply with the guidelines of ICH.

Impurities are the unwanted chemicals present in the active pharmaceutical ingredients (APIs). The small amounts of impurities present in the API may influence the effectiveness and safety of the drug products. Impurity profile study is necessary as per various regulatory requirements. Now, the United States Pharmacopoeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP) and British Pharmacopoeia (BP) are slowly incorporating limits to acceptable levels of impurities present in the APIs or formulations.

According to the ICH guidelines impurities in the API are classified into three categories (a) Organic impurities (Process and Drug-related), (b) Inorganic impurities and (c) Residual solvents.

(a) Organic impurities: Organic impurities can be formed during the optimization/manufacturing process and/or storage of the API. These impurities are starting materials (SM) and their impurities, intermediates and their impurities, degradation impurities, by-products, enantiomeric impurities (If API is chiral molecule) and polymorphic impurities.
As per the ICH guidelines, identification of impurities below the 0.1 % level needs not to be considered unless the impurities are potent or toxic. In all cases, impurities should be qualified. If data is not available to qualify the proposed specification level of an impurity, studies to obtain such data may be necessary (when the usual qualification threshold limits given below are exceeded). According to ICH, the maximum daily dose qualification threshold is considered as follows: \( \leq 2 \text{ g/day } 0.1 \% \text{ or } 1 \text{ mg per day intake (whichever is lower)}; \geq 2 \text{ g/day } 0.05 \% \)

(b) **Inorganic impurities:** Inorganic impurities can be generated from the reagents, ligands, catalysts, heavy metals or other residual metals, inorganic salts, other materials, e.g. filter aids, charcoal.

(c) **Residual solvents:** Residual solvents are low boiling liquids (organic volatile) which are present in the drug substance. Generally, these impurities obtain from the solvents used in the reactions. Based on the toxicity, residual solvents were categorized into three classes *i.e.* i) class-I, ii) class-II and iii) class-III solvents.

   i) **Class-I:** These solvents to be avoided due to strongly suspected human carcinogens and environmental hazards [Ex: benzene (2 ppm) and carbon tetrachloride (4 ppm)].

   ii) **Class-II:** These solvents to be limited. Non-genotoxic, animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity. [Ex: methanol (3000 ppm), acetonitrile (410 ppm) and tetrahydrofuran (720 ppm)].
iii) **Class-III**: Solvents with low toxic potential to humans; no health-based exposure limit is needed (Ex: acetone, ethyl acetate and acetic acid). Note: Class-3 residual solvents have PDE of 50 mg or more per day.

Identified impurity is an impurity for which structure has been characterized. Specified impurity is an impurity that is separately listed and limited within the specification limit. Unidentified impurity has no structural characterization and that is defined exclusively by qualitative analytical properties (e.g. chromatographic retention time).

Polymorphism is a new area of research and has become the major deciding factor in formulation, because it plays critical role in solubility, flowability and rate of dissolution etc. In the same way, achieving the required particle size and bulk density of API is a great challenge as it plays a key role in deciding the solubility of API.

Many pharmaceutical solids can exist in different physical forms. Polymorphism is often characterized as the ability of a drug substance to exist as two or more crystal phases that have different arrangements and/or conformations of the molecules in the crystal lattice.

Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice.

Solvates are crystalline solid adducts containing either stoichiometric or non-stoichiometric amounts of a solvent incorporated within the
crystal structure. If the incorporated solvent is water, the solvates are also commonly known as hydrates.

Polymorphs and/or solvates of a pharmaceutical solid can have different chemical and physical properties such as melting point, chemical reactivity, apparent solubility, dissolution rate, optical and electrical properties, vapor pressure and density. These properties can have a direct impact on the process-ability of drug substances and the quality/performance of drug products such as stability, dissolution and bioavailability.

A metastable pharmaceutical solid form can change crystalline structure or solvate/desolvate in response to changes in environmental conditions, processing, or over a time period.

Identification of polymorphs can be done by performing so called polymorph screening and choosing one of the polymorphs, often the one having the lowest free energy for further development. Such screenings are mainly performed by crystallization from various solvents and by using different crystallization techniques. The obtained crystals are then analyzed by one or a combination of techniques is crucial. Following are the different analytical techniques to analyze the polymorphs.

(a) IR
(b) X-ray powder diffraction
(c) Thermal analysis procedures (like DSC, TGA and DTA).
(d) Raman spectroscopy


(e) Optical microscopy

(f) Solid state $^{13}$CNMR

1.3 A brief review on indole and fused indole derivatives

1.3.1 Introduction to indoles

Heterocyclic compounds are cyclic compounds in which one or more ring carbons are replaced by heteroatoms. Indole 1 (figure 1.3) is a bicyclic compound consisting of a benzene ring fused to a pyrrole ring. Indole is the key component in fragrances, dyestuffs and the synthon to many pharmaceuticals.

![Structure of indole 1](image)

**Figure 1.3:** Structure of indole 1

However, the isolation of indole alkaloids as the active principles from medicinal plants (*i.e.* antibiotics, *anti*-inflammatory, *anti*-hypertensive and antitumor agents), the indole nucleus has taken on considerable pharmacological importance (figure 1.4). These features contribute to the importance of indole derivatives and have motivated the efforts made towards the development of new and efficient synthetic routes.
Figure 1.4: Biological activities of indole

Indole is an important heterocyclic system, because it is built into proteins in the form of amino acid tryptophan. It is the basis of drugs like indomethacin, alkaloids and biologically active compounds from plants including strychnine and LSD. Indole is a key sub structure in several natural compounds such as complex alkaloids, marine natural products and fungal metabolites as well as in pharmaceutical compounds such as naratriptan 2, zolmitriptan 3, rizatriptan 4, eletriptan 5, almotriptan 6, sumatriptan 7, frovatriptan 8, zafirlukast 9 and indomethacin 10 (figure 1.5). 10

The building of a substituted indole frame has been a topic of great interest for many years due to their miscellaneous biological activities. Several methods are available in the literature for the synthesis of indole moiety 11 and construction of indole ring are Fisher-indole synthesis, Buchwald modification [modification in Fischer-indole], Bartoli indole
synthesis, Dobbs modification [modification in Bartoli-indole], Reissert-indole synthesis, Leimgruber-Batcho indole synthesis, Japp-Klingemann indole synthesis and etc. One of the popular methods for the construction of indole ring is Fisher indole synthesis.¹²

![Figure 1.5: Pharmaceutical indole derivatives](image)

Fisher indole method is one of the ancient methods falls under sigma tropic rearrangement category. In which, synthesis of indole 12 was achieved from aryl hydrazones 11 under acidic conditions (scheme 1.1). This method was extensively used during several years to access wide variety of indole derivatives.
Scheme 1.1: Fisher indole reaction

Indole inhibitors of cPLA2a with promising pharmacokinetic parameters that were active in both an isolated enzyme assay and in cell-based assays were discovered. Modeling these compounds into the cPLA2a structure validated the assumptions made at the start of the SAR effort.

For this purpose substrate mimic derivatives 13 and 14 were designed by modifying the substitution on indole nitrogen, indole C-3 position and indole carbocycle (figure 1.6).\textsuperscript{13} Synthesis of these derivatives achieved in a simple method and tested against phospholipase.

Figure 1.6: Substituted indole derivatives

Nettekonven \textit{et al.},\textsuperscript{14} reported the synthesis of C-acyl 18 and N-acyl 20 indole derivative libraries (18 & 19). The first series of derivatives 2-acyl-3-amino-indoles 18 were synthesized from amino benzonitrile 15.
Acylation of amine 15 followed by cyclization provided the analogs 18 via intermediate 17 in a one-pot reaction sequence (scheme 1.2).

Scheme 1.2: Synthesis of substituted indole derivatives

Whereas the other series of derivatives 20 prepared from isolated intermediates 19 (R₃=aromatic, heteroaromatic, or cycloalkyl) with acid chlorides. These derivatives were obtained in multi-milligram quantities in acceptable yields.

Browns group¹⁵ explored cysteinyl leukotriene Dd (LTD₄) antagonistic activity of novel zafirlukast derivatives 21 and 22 by modifying the substitution on indole nitrogen of zafirlukast (figure 1.7).

Figure 1.7: N-Substituted zafirlukast derivatives
A variety of functionalities incorporated on indole nitrogen and studied the pharmacokinetic profile. Promising results were not observed from these derivatives.

Matassa et al.,16 reported the synthesis of 1,3,5-substituted indoles and indazoles and studied as receptor antagonism of the peptidoleukotrienes (figure 1.8).

**Figure 1.8:** Indole and indazole derivatives

Best results were obtained when a methyl group at the \( N-1 \) position and other group at \( C-5 \) position [(cyclopentloxy) carbonyl] amino or 2-cyclopentylacetamido or \( N^1 \)-cyclopentylureido group] and an aryl sulfonyl amide group at the \( C-3 \) position of the ring.

### 1.3.2 Introduction to fused indoles

Fused indoles are another class of heterocyclic compounds. Fused indoles usually contain an additional fused ring, and in most case a six membered ring such as in carbazole 25 and \( \beta \)-carboline 26 (figure 1.9).
Graebe and Glazer isolated carbazole from coal tar in 1872. Chakraborty et al.,\textsuperscript{17} described the isolation and antibiotic properties of murrayanine from \textit{Murraya koenigii} spreng. Several synthetic methods are available in the literature for the synthesis of carbazole. There has been a great demand in this area due to the intriguing structural features and biological activities exhibited by many carbazole alkaloids. For example ditercalinium \textbf{27} is a pyrido[c]-fused carbazole, used clinically for the treatment of cancers. The other carbazole derivatives \textbf{28} and \textbf{29} have been shown to bind to estrogen receptors and inhibit the growth of mammary tumours in rats and cell growth inhibition in tumour cell lines (figure 1.10).

Substituted carbazole derivatives have been explored for their potential application as antiviral agents and in the treatment of tumors. Due to various biological activities displayed by carbazole and its numerous derivatives, these are continued to capture the attention of synthetic organic chemists.
Figure 1.10: Biologically active carbazole derivatives

Zhou et al.,\textsuperscript{18} synthesized a new series of \( N \)-substituted carbazole derivatives (30, 31 and 32) and evaluated biological activities. Some of these derivatives showed comparable or even better \emph{anti}-bacterial and \emph{anti}-fungal activities than existing drugs (norfloxacin, fluconazole and chloramphenicol) (figure 1.11).

30 Carbazomycin A, R = CH\textsubscript{3} \quad 32 Murrayafoline A
31 Carbazomycin B, R = H

Figure 1.11: Substituted carbazole derivatives

Jan Bergman and co-workers\textsuperscript{19} synthesized symmetric and non-symmetric indolo [2,3-\textit{c}] carbazoles 34 from 3,9-\textit{bis}-indolyls 33. Two methods were followed for the synthesis of these derivatives. Both the
routes consist one step and started from readily available precursors (scheme 1.3).

\[ \text{Scheme 1.3: Indolo [2,3-c] carbazole derivatives} \]

In the method A, bis-indolyls were heated in diphenyl ether to give desired product and the same reaction was carried out in acetic acid by method B to yield indolo carbazoles.

In the other communication, synthesis of 11-alkylbenzo[a]carbazoles and their 5,6-dihydro derivatives with hydroxy substitution on the phenyl and their binding affinities were studied for the estrogen receptor.\(^{20}\)

\[ \text{Figure 1.12: Carbazole derivatives} \]

Out of these derivatives one hydroxy group at C-3 and a second one at position C-8 or C-9 proved to be the better for the receptor binding. Low binding affinities observed with dihydro derivatives, but still high
with planar structure of these molecules. Other derivatives inhibited the growth of dimethyl benzanthracene-induced hormone-dependent mammary tumors.

Carbazole is a conjugated compound with a wide band gap originated from the bridging nitrogen atom and biphenyl unit. These derivatives exhibit interesting optoelectronic properties such as photoconductivity, photo refractivity, electroluminescence, and used as whole transport, blue emission materials for photo electronic devices (figure 1.13). Electroluminescent (EL) devices based on organic thin layers have attracted much attention because of the potential application to large-area flat-panel displays and light-emitting diodes (LEDs).²¹

Figure 1.13: Carbazole derivatives

β-Carboline and tetrahydro β-carboline derivatives are most widely used drugs in the treatment of many diseases. Tetrahydro β-carboline
derivative tadalafil 39 (figure 1.14) is a PDE-5 inhibitor, currently marketed in pill form for treating erectile dysfunction (ED) under the name Cialis.

![Structure of tadalafil](image)

**Figure 1.14:** Structure of tadalafil

Abecarnil 40 (figure 1.15) is an anxiolytic drug from the β-carboline family. It is a partial agonist acting selectively at the benzodiazepine site of the GABA_A receptor.22

![Structure of abecarnil](image)

**Figure 1.15:** Structure of abecarnil

To develop potent therapeutic derivatives, Bi et al.,23 prepared more potent analogs by combining the anti-inflammatory moiety (1,3-dioxane derivative) to the key pharmacophore moiety of melatonin (figure 1.16).
Figure 1.16: Melatonin and 1, 3-dioxane hybrid tetrahydro β-carboline derivatives

β-Carboline derivatives 41 and its isomers showed potent anti-inflammatory anti-oxidant effects and exert a protective effect against skeletal muscle injury.

Domning and co-workers²⁴ developed a convergent 2-step process for the synthesis of tetrahydro β-carboline derivatives 48 by using an Ugi and a Pictet-Spengler reaction. Several novel derivatives were produced by different starting materials with unprecedented complexity (scheme 1.4).

Scheme 1.4: Synthesis of tetrahydro β-carboline derivatives
When the tryptophan derived isocyanide was used, two diastereomers were observed with slightly more trans-stereoisomer. This synthesis allows producing many derivatives which can be used to construct the indole alkaloids type natural product derivatives.

1.4 CONCLUSION

In this chapter, we have taken up the detailed discussions on biological importance of indole and fused indole derivatives. Also, discussions on importance of process development, impurity profile and polymorphic studies.

With this above context, the present thesis entitled “Synthesis and Characterization of some Active Pharmaceutical Indole Derivatives and their Analogs” is mainly focused on the establishment of scalable improved/alternative processes, their impurity profile of few API`s containing indole, fused indole derivatives and their new analogs.

The total study carried out as part of this research programme has been divided into five chapters. Chapter-1 explains introduction to process development of APIs and a brief review on indole and fused indole derivatives.

In chapter-2, focused for development of zafirlukast and it is an oral leukotriene receptor antagonist (LTRA) used for the treatment of asthma. Owing to this important feature various research groups have developed to synthesize the zafirlukast and amorphous polymorph, those methods are not cost effective at industrial scale-up. In this context our efforts
were directed towards developing cost effective, scalable, improved processes for the synthesis of zafirlukast and prepared the stable amorphous and acetonitrile new solvate polymorphs.

Structure of zafirlukast

In chapter-3, focused to identify, synthesis, characterization of impurities and root cause of their formation other than metabolites.

In chapter-4, focused to synthesis of new zafirlukast analogs, utilizing well known chemical reactions.

Chapter-5 illustrates an alternate novel synthesis of tadalafil and it is PDE5 inhibition would be of therapeutic utility in treating male erectile dysfunction (MED). Also described the complete impurity profile of tadalafil, including identification, synthesis, characterization and root cause of their formation in two commercial viable schemes.
1.5 REFERENCES


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