



1.1 INTRODUCTION

The well being of mankind is very much dependent on the food habits, environmental and ecological factors. It is well known that any imbalance between them can result in adverse affects for which human race has to bear the brunt. History has shown that such imbalances had occurred and human race has learnt to survive the on-slaught of disease, destruction and pest from “Mother Nature”. Despite the knowledge gained about survival, human beings continue to face challenges with respect to new diseases, pests, strains of viruses and bacteria that continue to mutate, or become resistant to drugs that were once active against the particular strain. In addition, there are number of diseases (Swine flu, AIDS, etc) for which there is no correct medication available to affect a permanent cure.

Ancient medicine was centric around plant extracts and products that were readily available from nature. As new diseases came to light at periodic intervals of the earth time machine, the relevance of these medicines subsided either due to lack in curing the diseases or instant relief provided by modern synthetic medicines. In this direction, the first leap was attained by the introduction of synthetic organic molecules as medicaments. The success of these compounds rose due to the fact that they were curing a number of diseases. This potential was recognized by several pharmaceutical companies and huge investments were attracted in the health care business. There are several intricacies involved in the

lead compound identification to product launch in the market. Moreover, as the end users are human beings, the scrutiny of these compounds faces several hurdles like toxicological tests, clinical trials and a whole bunch of regulatory requirements. This is a time consuming process where patience and money are required. A comparison of the number of hit molecules identified that translates into the final drug candidate, which is launched into the market, indicates the success rate is very low. There are several cases where even after the launch of the drug in the market, the product had been withdrawn due to side effects or safety. As time progressed, a better understanding of the diseases was obtained by people trained in the art. For simplification and better understanding, the biologically active molecules were categorized according to their respective therapeutic relevance. The current scenario indicates the therapeutic areas that have received great attention from researcher's world over are namely

- a) Anti-inflammatory
- b) Antihypertensive
- c) Antidiabetic
- d) Antidepressant
- e) Anticancer

Presently the human medicine segment alone constitutes huge market value. Estimates suggest that this will continue to grow as more and more people come onto the planet and new diseases emerge. Of

particular interest, our attention was drawn towards medicines containing azole moiety namely isoxazole, pyrazole and carbazole due to the fact that the revenues from this class of medicines are attractive and their synthesis poses a huge challenge to the organic chemists. Molecules containing these functional groups possess various therapeutic activities.

1.1.1 Isoxazole and Pyrazole Derivatives

A moiety with an oxygen and nitrogen atoms at positions 1 and 2, respectively is termed as isoxazole **1** (figure 1.1).

Figure 1.1: Structure of isoxazole

Natural products, such as ibotenic acid **2** (figure 1.2) contains isoxazole moiety.¹ Number of drugs, including the COX-2 inhibitor valdecoxib possesses isoxazole as the core pharmacophore.

Figure 1.2: Ibotenic acid

Isoxazole derivatives have been used in the treatment of multiple diseases irrespective of therapeutic areas such as anti-inflammatory,

antidepressant, anticonvulsant and antibacterial. Some of the isoxazole derivatives and their pharmacological activities are listed in the table 1.1.

Table 1.1: Pharmacological activity of isoxazole derivatives

S.No.	Compound Name	Structure	Activity	Compd. No.
1	Valdecoxib ²		Anti-inflammatory	3
2	Perisoxal ³		Anti-inflammatory, Analgesic	4
3	Isocarboxazid ⁴		Antidepressant	5
4	Zonisamide ⁵		Anticonvulsant	6
5	Sulfisoxazole ⁶		Antibacterial	7

Pyrazole **8** is a five membered heterocyclic aromatic compound containing two nitrogen atoms at adjacent positions, 1 and 2 (figure 1.3). Renowned chemist Ludwig Knorr had coined “pyrazole” for such compounds.

Figure 1.3: Structure of pyrazole

From the seeds of watermelons, the first natural pyrazole derivative, 1-pyrazolyl-alanine **9** (figure 1.4) was isolated in 1959.⁷



Figure 1.4: 1-Pyrazolyl-alanine

Derivatives of pyrazoles are well known in medication as anti-inflammatory, analgesic, antipyretic, antiarrhythmic, anticonvulsant, tranquilizing, muscle relaxing, psychoanaleptic, antidiabetic and antibacterial activities. A few pyrazole derivatives are given in the table 1.2.

Table 1.2: Pharmacological activity of pyrazole derivatives

S.No.	Compound Name	Structure	Activity	Compd. No.
1	Celecoxib ⁸		Anti-inflammatory	10
2	Lonazolac ⁹		Anti-inflammatory	11
3	Muzolimine ¹⁰		Antihypertensive, Diuretic	12
4	Aminopyrine ¹¹		Antipyretic, Analgesic	13
5	Sulfaphenazole ¹²		Antibacterial	14
6	Betazole ¹³		Gastric secretion stimulant	15
7	Norphenazone ¹⁴		In treatment of stroke	16

Major application of isoxazole and pyrazole derivatives has been found to be in the anti-inflammation category. Anti-inflammatory drugs are divided into three classes.

- a) Steroids.
- b) Non-steroidal anti-inflammatory drugs (NSAIDs).
- c) Immune selective anti-inflammatory derivatives (ImSAIDs).

Isoxazole and pyrazole derivatives fall into the class of non-steroidal anti-inflammatory drugs.

Non-steroidal anti-inflammatory drugs mainly function by inhibiting the cyclooxygenase (COX) enzyme, which facilitates in producing prostaglandin from arachidonic acid. So far three types of cyclooxygenase enzymes are identified, namely COX-1, COX-2 and COX-3.¹⁵ COX-1 is mainly responsible for producing prostaglandin, which is responsible for protection of gastrointestinal tract (GIT). COX-2 is responsible for production of prostaglandin, which is the main cause of inflammation, pain and fever. COX-3 is the most recently identified cyclooxygenase enzyme, whose role in prostaglandin mediated physiological response is not fully understood. Based on their selectivity towards the COX inhibition, NSAIDs are classified into two categories

- i) Non-selective NSAIDs
- ii) Selective NSAIDs

Non-selective NSAIDs inhibit both the COX-1 and COX-2 enzymes. As they inhibit COX-1 enzyme, prostaglandin responsible for protection of

gastrointestinal tract will not be produced and hence lead to the damage of gastrointestinal tract.

Selective NSAIDs will inhibit only COX-2, which produces prostaglandin that causes inflammation.¹⁵ Hence the production of prostaglandin responsible for GIT protection will not be affected significantly and will reduce the risk of peptic ulcers and intestinal bleeding.

Functionalized isoxazole, valdecoxib and pyrazole, celecoxib are widely used as selective COX-2 inhibitors.

1.1.2 Carbazole Derivatives

Carbazole **17** is a tricyclic aromatic compound possessing a pyrrole ring fused on either sides at 2, 3 and 4, 5 positions with benzene rings (figure 1.5). Carbazole was first isolated by Graebe and Glaser¹⁶ from the anthracene fraction of coal tar in 1872. Bhattacharya *et al.*¹⁷ isolated carbazole from *Glycosmis pentaphylla* plant.

Figure 1.5: Structure of carbazole

Carbazole is basic in nature with fluorescent properties. Due to extended *pi*-electrons, this is used in luminescence chemistry as a photosensitizing agent. Carbazole and its derivatives are widely used as an intermediate in synthesis of pharmaceuticals, agrochemicals, dyes

and other organic compounds. Carbazole structure is a motif in pharmaceuticals such as carvedilol and carazolol (table 1.3) used to treat high blood pressure and to prevent cardiac arrhythmias and angina.

The carbazole ring is the main component in the most of the alkaloids, few of them are shown in the figure 1.6. Wu *et al.*¹⁸ isolated 3-methylcarbazole (**18**) from the root bark of *M. euchrestifolia*. The isolation of 3-formylcarbazole (**19**) from the root bark of *M. euchrestifolia* was reported by Furukawa *et al.*¹⁹ From the roots of *Clausena lansium*, Mc Chesney and El-Feraly²⁰ have isolated 3-formylcarbazole (**19**) and methyl carbazole-3-carboxylate (**20**). Chakrabarty and co-workers²¹ have published a process for the isolation of 9-carbethoxy-3-methylcarbazole (**21**) and 9-formyl-3-methylcarbazole (**22**) from the roots of *M. koenigii*.



Figure 1.6: Structures of various carbazole based alkaloids

A variety of drugs containing carbazole ring system have been used for different therapeutic activities. The known examples of carbazoles

containing compounds along with their pharmacological activity have been listed in table 1.3.

Table 1.3: Pharmacological activity of carbazole derivatives

S.No.	Compound Name	Structure	Activity	Compd. No.
1	Elliptinium ²²		Antitumor	23
2	Carvedilol ²³		Antihyper-tensive	24
3	Carazolol ^{24,25}		Antihyper-tensive, antianginal	25
4	Carprofen ²⁶		Anti-inflammatory	26
5	Rimcazole ²⁷⁻²⁹		Antipyretic, neuroleptic	27

1.1.3 Importance of Impurity Profile Study in Drug Substances

The end users of drug substance are human beings, hence the producers of these compounds need to be adhered the stringent rules and regulations. In this direction, to bring about uniformity around the globe, an organization called “International Conference on Harmonisation

of Technical Requirements for Registration of Pharmaceuticals for Human Use” (ICH) was formed. This organization lays down certain guidelines that should be met in order for the compound to be considered as a drug substance.

Related substances are the unwanted chemicals (impurities) that remain with the drug substance, or formed during the storage of drug substance. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the drug products.

According to ICH guidelines,³⁰ impurities can be classified into following three categories.

- i) Organic impurities (process and drug related)
- ii) Inorganic impurities.
- iii) Residual solvents.

Organic impurities can arise during the manufacturing process and/or storage of drug substance. These may be identified or unidentified, volatile or nonvolatile, may include starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts.

Inorganic impurities are reagents, ligands, catalysts, heavy metals or other residual metals, inorganic salts and materials such as filter aids and charcoal. These can result from the manufacturing process and normally known and identified.

Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacturing process of drug substances or drug products. Since there is no therapeutic use, all residual solvents should be removed to the possible extent to meet the specification. Solvents are classified into four categories, viz class-1, class-2, class-3 and class-4.³¹ Solvents [benzene (2 ppm), carbon tetrachloride (4 ppm), 1,2-dichloroethane (5 ppm), 1,1-dichloroethane (8 ppm), etc.] known to cause unacceptable toxicities (class-1) should be avoided in the manufacturing process of drug substances. Solvents [acetonitrile (410 ppm), tetrahydrofuran (720 ppm), pyridine (200 ppm), dichloromethane (600 ppm), toluene (890 ppm), chloroform (60 ppm), etc.] associated with less severe toxicity (class-2) should be limited. Less toxic solvents [ethanol, ethyl acetate, acetic acid, acetone, heptane, isopropyl alcohol, etc.) are classified as class-3, which can be used in the manufacturing process of the drug substances and are acceptable up to 5000 ppm. Solvents whose toxicological data are unavailable come under class-4 (petroleum ether, isopropyl ether and isooctane etc.)

According to ICH guidelines, limit of known impurities are below 0.15 % and unknown impurities are below 0.10 %, in case of daily dosage is less than 2 g. If the maximum daily dosage is more than 2 g, known and unknown impurities should be controlled by 0.05 %. In view of this, impurity profile study is mandatory for identification and control of

impurities to fulfill the requirements of regulatory authorities and to produce the high quality drug substances.

Impurity profiling is a study, which involves understanding the formation, identification, synthesis and characterization of impurities present in a drug substance or a drug product.

1.2 PURPOSE AND OUTLINE OF THE THESIS

Among the selective COX-2 inhibitors, our attention was drawn towards valdecoxib (isoxazole containing compound) and celecoxib (pyrazole possessing compound) due to the fact that their synthesis on commercial scale poses a great challenge. Therefore a need for efficient, cost effective, robust and environmental benign process was required. As these drugs are intended for human use, the safety and efficacy are important. Presence of impurities in the drug substance will impact the quality, thereby affects the safety and efficacy of drug product. In this regard, it is required to study the root cause for the formation of impurities to minimize/avoid its contamination in the drug substance. In addition to this, synthesis and characterization is also required in order to confirm the proposed structures and develop the purification methods.

In view of above, the development of valdecoxib and celecoxib was under taken to have an alternative and efficient synthesis, to study the formation of impurities in detail, synthesis and characterization of possible impurities.

In addition, carbazole based β -amino alcohols, carvedilol and carazolol possess antihypertensive and antianginal activity. It was envisioned that variously substituted carbazole based β -amino alcohols would also exhibit similar activity. Further, we intended to utilize the generated β -amino alcohols as versatile synthons for the synthesis of carbazole based five and six membered heterocyclic ring systems namely oxazolidine, oxazolidinone, morpholine, morpholinone and morpholinedione derivatives.