CHAPTER-I

INTRODUCTION: CASPASES, THEIR IMPORTANCE AND DIFFERENT PHARMACOPHORES REQUIRED TO INHIBIT CASPASE-3
INTRODUCTION

Apoptosis is a form of cell death that is involved in the regulation of a wide range of physiological systems including normal cell turnover, control of the immune system, embryonic development and hormone-dependent tissue atrophy. Inappropriate apoptosis has been implicated in diseases ranging from Alzheimer's disease, autoimmune disorders to AIDS and cancer. Regulation of apoptosis is vital to the development and long-term survival of metazoan animals. Apoptosis is required to maintain the balance between cell proliferation and cell death and therefore, disruptions in the apoptotic program have been associated with pathologies such as cancer, where there is too little cell death, and degenerative diseases, where there is too much cell death. Increased levels of apoptosis and caspase activity have been frequently observed at sites of cellular damage in both acute (e.g. sepsis, stroke, spinal cord injury, myocardial infarction, alcoholic hepatitis) and chronic (e.g. Alzheimer’s, Parkinson’s and Huntington’s disease) diseases.

However, Inappropriate and excessive apoptosis, underlines the etiology of some of the most intractable of human diseases. The apoptotic pathway is predominantly executed by a series of cysteine proteases designated as “Caspases” (Cysteiny1 aspartate - specific proteinases). Caspases are intracellular proteases that play significant roles in both cytokine maturation and programmed cell death (apoptosis). Caspases are responsible for the proteolytic
degradation of more than 100 different protein substrates, including proteins that have been involved in DNA repair, nuclear membrane integrity, and cell structural integrity.

1.1 Discovery of caspases, functions

Robert Horvitz initially established the importance of caspases in apoptosis\(^1\) and found that the *ced*-3 gene is required for the cell death during the development of the nematode C. elegans. Horvitz and his colleague Junying Yuan found that the protein encoded by the *ced*-3 gene is a cysteine protease with similar properties to the (mammalian) interleukin-1-beta converting enzyme (ICE) (now known as caspase-1) which at the time was known as caspase\(^2\). Subsequently, other mammalian caspases have been identified in organisms such as fruit fly (Drosophila melanogaster). In many instances, a particular caspase has been identified simultaneously by more than one laboratory, and given a different name. For example, caspase-3 was known as CPP32, apopain and Yama. Hence, researchers have decided upon the nomenclature\(^3\)\(^-\)\(^5\) of the caspase in 1996. Therefore, caspases have been numbered in the order in which they were discovered. ICE\(^6\) has been therefore renamed as caspase-1. ICE is the first mammalian caspase to be characterized because of its similarity to the nematode death gene *ced*-3, but it appears that the principal role of this enzyme is to mediate inflammation rather than cell death.
Recent studies have demonstrated that caspase proteases have also been regulators of non-death functions, most notably have been involved in the maturation of a wide variety of cells such as red blood cells and skeletal muscle myoblasta. About 14 different members of the caspase-family have been identified in mammals. Phylogenetic analyses of the caspases have revealed that the caspases belong to three different subfamilies: 1) The ICE subfamily includes caspases-1, -4, and -5; (2) The CED-3/CPP32 subfamily includes caspases-3, -6, -7, -8, -9 and -10; and (3) The ICH-1/Nedd2 family caspase-2.

Two main pathways have been involved in the activation of caspases. The first pathway is initiated by the death factors, leading to the activation of caspase-8 by its recruitment to the death receptor. Activated caspase-8 can activate downstream effector caspases, such as caspases-3, -6 and -7. The second pathway is initiated by the release of cytochrome-c from mitochondria. This leads to the activation of caspase by formation of a complex with Apaf-1/cytochrome activated caspase-9, which then cleaves and activates downstream effector caspases-3, -6 and -7. Once they have been activated by either pathway, effector caspases disassemble the cell by cleavage of the vast majority key cellular proteins.

Inhibition of caspases with the aim of reducing cell death, either broad spectrum or caspase specific, would be therapeutically beneficial. Among the caspase inhibitors that have been known until now, the most noted irreversible inhibitors are (Figure-1).
All the above inhibitors exhibit their activity based on the common mechanism that they irreversibly inactivate the enzyme to suppress the cell apoptosis (irreversible, broad-spectrum inhibitor). It has been reported that irreversible inhibitor has much more effective inhibitory activity when compared to reversible inhibitors\textsuperscript{8}. Both IDN-1965 (I) of IDUN Co. and MX-1013 (III) of Maxim Co. have been reported to show activity in cell apoptosis model for hepatic injury\textsuperscript{9,10}. These compounds are in preclinical stage of development and both the compounds have same side chain fluoromethyl ketone. The irreversible inhibitor IDN-6556 (Figure-2), the structure of which has been reported, is now discontinued from further development after phase-II clinical test as a therapeutic agent for liver diseases\textsuperscript{11}.
Figure-2

[Chemical structure image]

Compound V and VI (Figure-3) are other caspase peptidyl inhibitors having 2, 6-difluorophenoxy side chain\textsuperscript{12} which have shown good potency against caspase-3.

Figure-3

[Chemical structure images for V and VI]

Beside the above peptidyl inhibitors it has also been reported in literature that compounds possessing 2, 6-difluorophenoxy side chain are potent caspase inhibitors VII and VIII\textsuperscript{13} (Figure-4) and found that the size, lipophilicity and binding affinity of the warhead play an important role in the overall inhibitory activity of these compounds, in terms of caspase potency and selectivity. The number and substitution pattern of fluorines on the phenol have an impact on the SAR.
Compound IX (Figure-5) is another potent caspase inhibitor which possess 4-fluorophenoxy side chain\textsuperscript{13a}

In literature it has been reported that to inhibit caspase-3 either one of the following pharmacophores (war head) are required as shown in above examples. Hence, have used some of these pharmacophores in our research study to develop potent caspase-3 inhibitors.

1) Fluromethylketones (FMK)\textsuperscript{14}

2) Phenoxy methylketones (TFPMK, DFPMK and FPMK)\textsuperscript{15}
3) A warhead (like CHO or N₃) attached to the aspartyl side chain<sup>16</sup>

![Azide and Aldehyde](image)

Few examples with warhead like CHO and N₃ are as follows (Figure-6).

**Figure-6**

[X](image) [XI](image)

Recently, a number of isatin-based inhibitors of caspase-3 and caspase-7 have been reported in literature<sup>17-20</sup> indicating the importance of below side chains XII and XIII (Figure-7).

**Figure-7**

[XII](image) [XIII](image) [XIV](image) [XV](image)
5-Dialkylaminosulfonylisatins\textsuperscript{17} have been identified as potent, nonpeptide inhibitors of caspase-3 and -7. The most active compounds XIV and XV (Figure-7) inhibit caspase-3 and -7 in the 2-30nM range. These compounds exhibit 1000-fold selectivity for caspase-3 and -7 versus a panel of five other caspases (1, 2, 4, 6, and 8) and also are 20-fold more selective versus caspase-9. Sequence alignments of the active site residues of the caspases strongly suggest that the basis of this selectivity is due to binding in the S\textsubscript{2} subsite comprised of residues Tyr204, Trp206 and Phe256 which have been unique to caspase 3 and 7.

1.2 OBJECTIVE OF THE PRESENT WORK

In our endeavor to identify potent caspase-3 inhibitors, derive Structure Activity Relationships and synthesize more potent analogues, we investigated indole-N-acetic acid and oxalamic acid derivatives of fluoromethyl ketone, substituted phenoxy aspartyl ketones. The following chapters in this thesis summarize our findings. Indole-N-acetic acid derivatives of FMK are more potent and its biological evaluation and synthetic studies performed are described in chapter-2. Details of our study on oxalamic acid derivatives using war head and non-war head groups and rationale behind indole as main scaffold for activity are given in chapters 3-5 respectively.