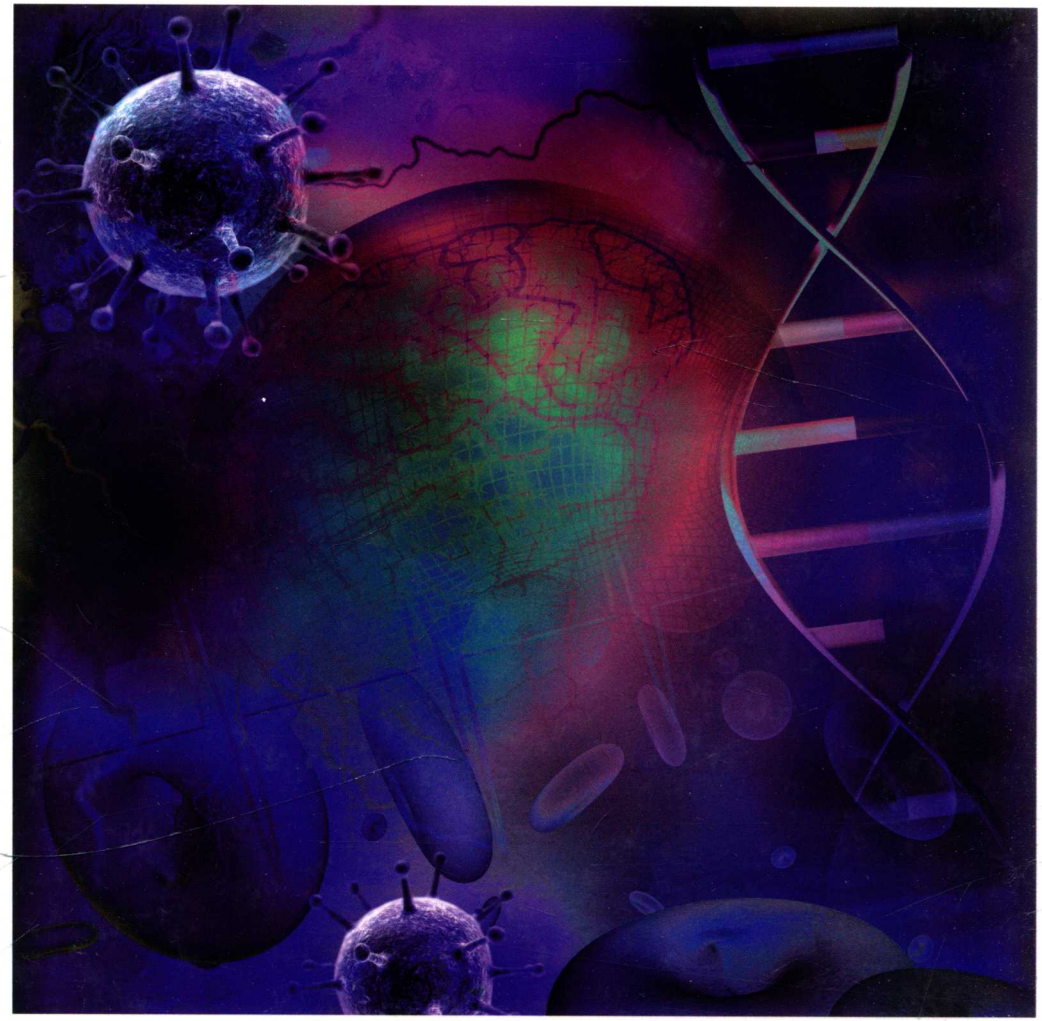
RSC Drug Discovery

Edited by Ben M. Dunn

Proteinases as Drug Targets



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Proteinases as Drug Targets

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Proteinases as Drug Targets

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Preface

Proteolytic enzymes play important roles in many metabolic processes in the human body. In recent years, the roles of proteinases in many cellular processes have been revealed, thus placing this type of enzyme into the forefront in efforts to control normal and abnormal physiological function. In the chapters that follow, the terms proteolytic enzyme, protease, and peptidase will be used interchangeably to mean the same thing: a protein with the ability to catalyze the extremely rapid cleavage of peptide bonds of other proteins with sometimes remarkable specificity for the peptide bond cleaved and sometimes with more general cleavage of multiple peptide bonds.

In the last few decades inhibitors of proteolytic enzymes have been discovered that are valuable in treating some human conditions. Among the first of these was the development of inhibitors of angiotensin converting enzyme, or ACE, including Captopril®. By blocking the conversion of angiotensin I to angiotensin II, ACE inhibitors provide a mechanism to lower blood pressure, as angiotensin II has a strong pressor activity. In the same pathway, scientists have long sought inhibitors of the first enzyme, renin, with many starts and stops. Recently, orally available inhibitors have been developed that show promise in controlling the start of the angiotensin cascade.

At the end of the 1980's and into the decade of the 1990's, a major focus of research was on developing compounds that would block the replication and spread of the Human Immundeficiency Virus (HIV). It was recognized that the virus contained a segment of nucleotides coding for a protein that had sequence identity to one half of an aspartic proteinase, such as pepsin or cathepsin D. The discovery of HIV proteinase led to the eventual development of Saquinavir, Indinavir, and Ritonavir which provided a second set of drugs to combine with inhibitors of the viral reverse transcriptase. After considerable effort and clinical trials, the combination therapy for treatment of HIV

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infection was optimized for human use. This has been followed by seven other compounds that inhibit the viral protease with increasing potency and with improved efficacy versus the drug-resistant forms of HIV that develop upon prolonged use of the older inhibitors. It should be appreciated that the earlier work on development of renin inhibitors noted in the above paragraph was vital in the rapid development of the HIV protease inhibitors.

This book collects chapters from scientists who are continuing the effort to identify new proteolytic enzymes that may serve as targets for new drug discovery. Eleven chapters provide details on recent studies of important enzymes.

The enzymes known as dipeptidyl peptidases are involved in many different processes such as "...liver disease, obesity, type II diabetes, arthritis, inflammatory bowel disease and cancer", quoting from the introduction to the chapter from Cathy Abbott. In addition, effects on innate immunity and the processing of a variety of peptide hormones can be mediated by members of this family. The chapter from Mark Gorrell and colleagues continues this discussion by focusing on dipeptidyl peptidase IV (DPPIV), which is a new target for drug discovery in diabetes. DPPIV has generated a lot of excitement in the research community that studies diabetes and drugs are beginning to reach the marketplace.

Vivian Hook has contributed a chapter that discusses the role of the cysteine proteinase B in Alzheimer's Disease. While considerable effort has been underway on other enzymes that are involved in cleavage of the beta amyloid precursor protein to produce the fragments that form plaques in patient's brains, Dr. Hook points out data that indicates that cathepsin B may play an important role there as well.

The chapter contributed by Pampalakis and Sotiropoulou discusses the kallakreins, including plasma kallakrein and tissue kallakreins. The former is involved in the release of bradykinin from High Molecular Weight Kininogen and the latter, also known as tissue kallakrien-related peptidases (KLKs), is a group of serine proteases that involved in release Lys-bradykinin from Low Molecular Weight Kininogen. In addition, the KLKs are involved in tissue-specific cascades to control physiological functions. As such they are excellent targets for pharmacological intervention.

Christoph Becker-Pauly discusses meprins α and β and the multitude of pathophysiological roles they play in human health and disease. These enzymes are representatives of the metallo-peptidase family and expand the coverage of this book. There is a large family of related enzymes in this group and they play different roles in different tissues. Another metallo-peptidase is glutamate carboxypeptidase II, discussed by Jan Konvalinka and colleagues. This enzyme is overexpressed in metastatic prostate cancer and could present a new target for drug discovery.

Two chapters discuss aspects of proteins known as inactive serine proteases, especially those from a significant human parasite, the skin mite *Sarcoptes scabiei*. This mite can cause devastating disease in severe cases and little is known about effect therapies. Katja Fischer focuses on the role of the title

proteins in the evasion of the complement system, while the chapter from Ashley Buckle discusses the structural reasons for the lack of activity in the inactive serine proteases.

There are a large number of parasitic organisms that live by feeding on the blood of their hosts. Alex Loukas considers the proteases from another major human pathogen, the hookworm. The chapter compiles information on a wide variety of proteolytic enzymes from the hookworm and discusses both vaccine strategies as well as drug development for control of this pest.

The book concludes with two chapters that focus on enzymes from the malaria parasite, *Plasmodium falciparum*. These continue the theme developed by Alex Loukas in his discussion of the hookworm and feeding upon blood. First, Sheena McGowan discusses the neutral aminopeptidases of the malaria parasite and the great potential for development of anti-malarial agents through work that has been accomplished to determine the three dimensional structure. Finally, the last chapter by this author discusses the members of the aspartic proteinase family present in the malaria parasite and, where possible, details of the structure and properties of the enzymes are presented. A discussion of the development of inhibitors of these enzymes is presented.

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CHAPTER 1

Dipeptidyl Peptidases: Substrates and Therapeutic Targeting in Human Health and Disease

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1.1 Introduction

Dipeptidyl peptidase 4 (DP4), fibroblast activation protein (FAP), DP8, and DP9 are the enzymatic members of the serine protease S9b DP4-like gene family. One of the most important features of the DPs is their ability to preferentially cleave the N-terminal post-prolyl bond of regulatory peptides and small protein substrates. DP4 proteolysis results in the inactivation, activation, or alteration of its substrates function via changes in receptor selectivity; thus DP4 plays an important role in regulating biological function. Together, DP4 and FAP have been implicated in a number of diseases including liver disease, obesity, type II diabetes, arthritis, inflammatory bowel disease and cancer. Recently, evidence has emerged to implicate both DP8 and DP9 in innate immunity, and DP8/9 *in vitro* cleavage of well-known DP4 substrates, including neuropeptide Y (NPY), glucagon-like peptide (GLP)-1, and a number of chemokines, has been demonstrated. Despite this, the true pathophysiological roles of DP8/9 and their involvement within human biology and disease are still to be elucidated. Identification of the *in vivo* substrate repertoire of each DP will be an important step toward elucidating the biochemical pathways in which each protease is involved. This will allow us to unravel further the roles that the

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