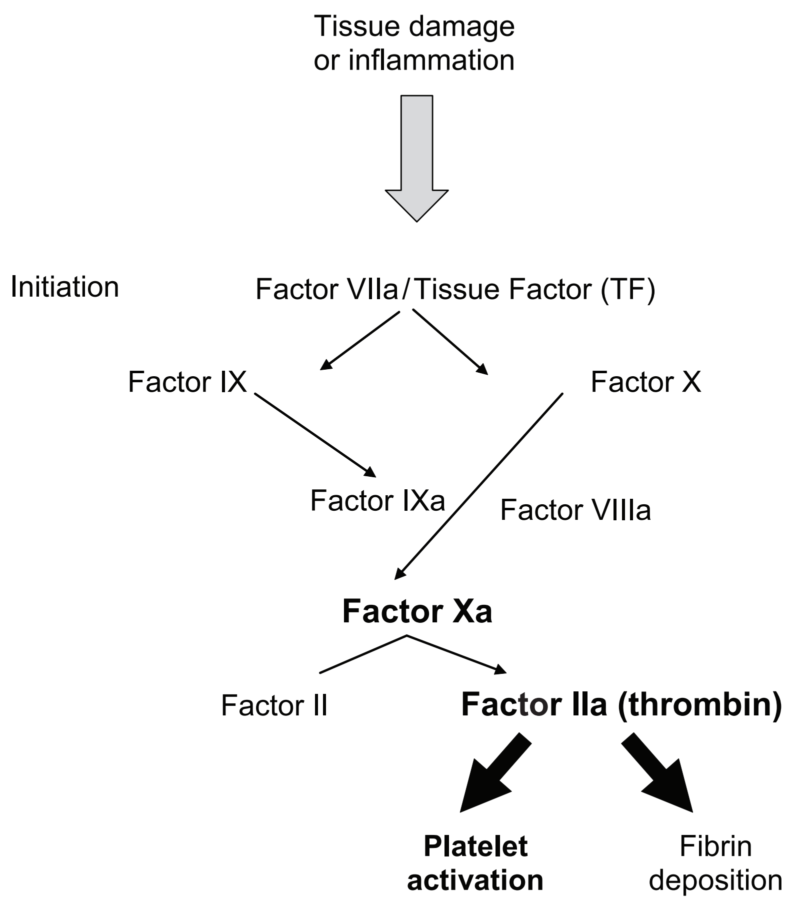
Figure 1.2. Schematic of the Coagulation Cascade



Source: Norman Consulting

1.4. Multiplicity of Potential Drug Targets

The above sections indicate that there are significant numbers of proteases, most or all of which have some potential as drug targets. Because of the disparate nature of the physiological roles of proteases and the diverse nature of their substrates, it has proved less straightforward to identify the number of human proteases that have the greatest potential as drug targets in contrast to either G protein-coupled receptors (GPCRs) or protein kinases. In 2001, it was estimated that the human genome would contain some 500 proteases.[2] This total has been

Table 2.15. Threonine Proteases (cont.)

Code	Protease	Gene
T02.003	glycosylasparaginase-3	AGA3
T02.004	taspase	TASP1
T03.006	gamma-glutamyltransferase 1	GGT1
T03.017	gamma-glutamyltransferase-like 3	GGTL3
T03.022	gamma-glutamyltransferase 6	GGT6
T03.015	gamma-glutamyltransferase 2	GGT2
T03.016	gamma-glutamyltransferase m-3	GGTL4
T03.002	gamma-glutamyltransferase 5	GGTLA4

Source: http://degradome.uniovi.es/

The four members of the T02 subfamily are also expressed intracellularly. The glycosylasparaginases appear to be essential for normal function, as the expression of mutant proteases has been shown to be linked to the lysosomal storage disease aspartylglucosaminuria.[26] Characterization of taspase has not yet been performed. The six gamma-glutamyltransferases constitute the T03 subfamily and play a major role in regulating oxidative stress as well as being markers of impaired liver function. These proteases are not believed to represent rational drug targets.

2.6. Cysteine Proteases

In cysteine proteases, a nucleophilic cysteine residue is a key element of the catalytic triad involved in the proteolysis of their substrates. The 178 cysteine proteases plus the three pseudogenes have been classified into 22 distinct subfamilies. These comprise four major groups: the caspases (C14), the cathepsins (C01), the calpains (C16), and the USP proteases (C19), and many smaller families.

Caspases

The caspases are a family of cysteinyl-aspartic proteases, in which an aspartate residue is also found in the catalytic site. They are intracellular enzymes that are post-translationally regulated, being rapidly activated by certain signaling pathways. Their function appears to be to regulate apoptosis, necrosis, and also inflammation.

3.5. Screening Issues

In establishing a screening program for protease inhibitors, a number of factors need to be taken into consideration. While it may be desirable to screen for activity using the endogenous substrate, both the cost of supplying sufficient substrate and optimizing the sensitivity of the assay protocol may lead to the development of modified protocols in which a non-natural (synthetic) substrate is employed. In such instances, it is generally regarded as desirable to incorporate a label, usually a fluorescent label, in the modified substrate. Fluorescence-based assays generally offer higher sensitivities than other assay formats.

Another consideration that influences the choice of screening assays is whether to screen using the full-length protease or simply the catalytic domain of the protease. The use of this was especially common practice within those companies working on matrix metalloprotease (MMP) inhibitors, with both cost considerations and the greater ease of producing the protein prompting the use of the latter option in many cases. However, in such instances it remains desirable to validate results for preferred compounds using the natural target protein.

Fluorescence-based assays generally offer higher sensitivities than other assay formats.

The use of multiple truncated proteins also facilitates compound profiling against both the intended target and closely related targets, thus guiding the design process at an earlier stage. Designing selectivity screening programs is less straightforward than for kinase inhibitors because of the much greater structural diversity of proteases and their substrates than of kinases. A careful process of selecting targets to counter-screen against is thus desirable.

3.6. Summary

This chapter has only sought to provide a brief overview of technical issues that impact upon the design and selection of protease inhibitors as potential development candidates. Key issues that should be addressed are: understanding the role of the target, knowledge of its structure, and the development of effective screening strategies.

as of greater potential relevance to the treatment of allergic disease, but a body of evidence from animal studies has indicated the potential of such inhibitors in treating cardiovascular disease, probably via effects on angiotensin II production.

TTP-889

TransTech Pharma's TTP-889 is the only factor IX inhibitor in development that specifically targets the intrinsic coagulation pathway. TransTech (High Point, NC) reports that TTP-889 is entering a second Phase II trial in patients with vascular assist devices. The initial Phase II trial was in 212 patients post–hip fracture surgery, but failed to demonstrate significant efficacy although TTP-889 was well tolerated.[94] The investigators concluded that TTP-889 lacked potential as an antithrombotic agent.

Metabolic Diseases

DPP IV Inhibitors

A large number of DPP IV inhibitors are in clinical development, as shown in Table 5.4. As discussed in Chapter 3, two agents in this class have already been approved for use, both alone and in combination with metformin. To date, 2008 has seen the filing of three further NDAs for alogliptin, alogliptin plus pioglitazone, and saxagliptin, while linagliptin, dutogliptin, and a saxagliptin combination formulation are currently in Phase III. A further ten agents are reported to be in Phase II studies.

Table 5.4. DPP IV Inhibitors in Development

Drug	Developer	Indication	Status
alogliptin benzoate	Takeda	type II diabetes	filed
saxagliptin (Onglyza)	Bristol-Myers Squibb/ AstraZeneca	type II diabetes	filed
alogliptin + pioglitazone	Takeda	type II diabetes	filed
linagliptin (Ondero)	Boehringer Ingelheim	type II diabetes	Phase III
saxagliptin + metformin	Bristol-Myers Squibb/ AstraZeneca	type II diabetes	Phase III
dutogliptin (PHX-1149)	Phenomix	type II diabetes	Phase III
AMG-222	Amgen/Servier	type II diabetes	Phase II
melogliptin	Glenmark	type II diabetes	Phase II

Continued

6.2. Selected Biotechnology Companies

This section contains brief profiles of some of the smaller companies that are significantly involved in the development of certain protease inhibitors and the technologies or indications that they have tended to focus on. It is notable that a number of these companies are virology specialists, a field in which the utility of protease inhibitors has been clearly established.

Ambrilia Biopharma

Ambrilia is a very small, Montreal-based company focusing on the development of drugs for the treatment of cancer and viral infections. In September 2008, it announced its intention to reduce its staff by one-third from the then 43 employees to concentrate its financial resources on its active programs. Its September 2008 presentation focused on the company's expertise in virology, which it is currently applying to a number of targets for the treatment of HIV, hepatitis C, and influenza. However, the only protease target that it has pursued is HIV protease, successfully licensing PPL-100 to Merck in September 2006 for global development. This deal provided Ambrilia with $17 million initially and potentially a total of $230 million, while providing Merck with all Ambrilia's intellectual property relating to this target. The patent filings relating to this effort represent a considerable portion of Ambrilia's patent filings to date, while other filings provide no evidence of Ambrilia's current interests in any other protease target.

Amura Therapeutics

Amura (Cambridge, UK) specializes in the application of structure-based design methods. It describes its AMcore scaffold as a proprietary scaffold that provides a turnkey solution for the design of inhibitors of the CAC1 family of cysteine proteases, which include cathepsins K and S and the falcipains. It has applied this technology to the generation of a series of patent applications, with seven filings relating to cathepsin K inhibitors all filed in July 2006. Amura's therapeutic focus is thus on disease that such CAC1 inhibitors are relevant to, such as osteoporosis, malaria, and autoimmune diseases. Amura currently has no deals with major companies, but its strategy is to out-license all development compounds at a relatively early stage, which it will probably do at the conclusion of Phase I studies.

Chapter 8

EXPERT INTERVIEWS

8.1. Mark Whittaker, PhD

Mark Whittaker, PhD, is Senior Vice President Drug Discovery, Evotec OAI, Abingdon, UK, and Scientific Director of Evotec's Services Divisions. Prior to joining Evotec he was Director of Chemistry at British Biotech, where he worked extensively on the identification and development of MMP inhibitors including batimistat and marimistat.

CHI Insight Pharma Reports (CHI): *In your opinion, how do proteases compare with other classes of drug targets?*

Mark Whittaker: Proteases offer very attractive targets, some of which have been highly validated. It is relatively straightforward to obtain nanomolar-potency inhibitors early in the medicinal chemistry efforts. However, it is also highly frustrating because of the problems in achieving selectivity against relevant enzymes, problems in achieving desirable pharmacokinetic properties due to the over-reliance on large extended molecules of relatively high molecular weight.

CHI: *What proportion of human proteases do you think might be viable drug targets?*

Dr. Whittaker: It is hard to assess, but I would anticipate a relatively small fraction. Some classes of proteases are more druggable than others.

CHI: *Which technologies have made the biggest impact upon the design of protease inhibitors?*