Inhibitors of proteases as anticancer drugs*
Minireview

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Proteolytic processes are necessary for normal physiological functions in the body. Failure in the biological control mechanisms of proteolytic activities may cause various diseases, for example, it may enable tumor invasion and metastasis. In the metastatic process, proteolytic enzymes play an important role in mediating passage of the malignant cell through the cell membrane. Tumor cell migration and invasion into the surrounding extracellular matrix is facilitated by a variety of cell surface-associated proteolytic enzymes: matrix metalloproteinases (MMPs), cysteine proteases including cathepsins B and L, aspartic protease cathepsin D, and serine proteases including plasmin and urokinase plasminogen activator (uPA). Many of the natural and synthetic inhibitors of the proteases prevent the dissemination of cancer cells and have also inhibitory effect on tumor growth. Thus inhibition of protease activity by low molecular weight inhibitors represents a promising strategy for anticancer and antimetastatic therapy. The review surveys low molecular inhibitors of MMPs, uPA and lysosomal proteases.

Key words: protease inhibitors, tumor metastasis, lysosomal proteases, anticancer drugs

Proteolytic enzymes (proteases) comprise a family of enzymes which hydrolyse protein or peptide substrates in the generalized process of intracellular protein degradation, a process essential for the normal functioning of all cells [47]. Proteolytic processes are necessary for normal physiological functions in the body, including normal blood vessel maintenance, new vessel formation (angiogenesis), clot formation and dissolution, bone remodelling and ovulation. The same enzyme system for the above roles is also used by the cancer cells for their growth and spread. These enzymes are produced by the tumor cells or cells surrounding them and can degrade the basement membrane and extracellular matrix (ECM), which consist of several components including collagens, glycoproteins, proteoglycans and glycosaminoglycans [83]. Degradation of the surrounding connective tissue is considered a necessary step to allow malignant cells to locally invade, to enter the lymphatic or blood circulation and to metastasize [18]. The process of metastasis is formed by several multi-step events. The steps involved in this process are: the escape of malignant cells from the primary tumor, entry of the cells into the vascular or lymphatic circulation (intravasation), survival and transport in the circulation, escape of the cells from the circulation (extravasation) and the growth of cells at the new site to form a secondary tumor [15]. The process of cell invasion and metastasis of solid malignant tumors is shown in Figure 1.

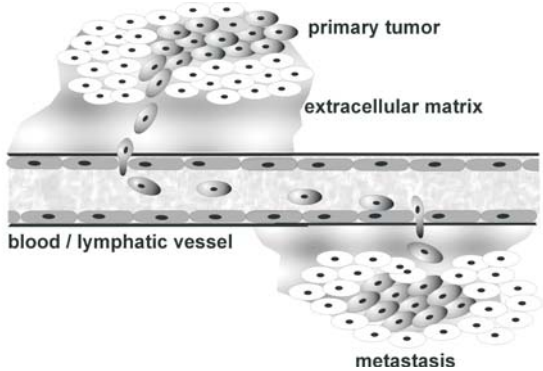

Figure 1. The process of cell invasion and metastasis of solid malignant tumors.

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During most of these steps, extracellular matrix and basement membrane have to be degraded. The breakdown of these barriers is catalysed by the proteolytic enzymes, which are released from the invading tumor [48]. The major proteases involved in this turnover are serine proteases (especially the urokinase-type plasminogen activator/plasmin system), matrix metalloproteases (a family of about 20 zinc-dependent endopeptidases including collagenases, gelatinases, stromelysins, and membrane-type metalloproteases), cysteine proteases [44] (cathepsin B, H and L) and aspartic proteases (cathepsin D). Many of the natural and synthetic inhibitors of the extracellular proteases prevent the dissemination of cancer cells and have also inhibitory effect on tumor growth. Thus protease inhibitors are emerging as promising potential therapeutic agents for treatment of cancer [22]. In the following paragraphs we will survey selected proteases involved in the tumor metastasis and review their inhibitors.

Matrix metalloproteases

Matrix metalloproteases (MMP) are a family of zinc-dependent endopeptidases that degrade various components of the ECM [15]. Based on primary structure, substrate specificity, and cell localization, these enzymes may be divided into at least four main subfamilies: collagenases (degrade fibrillar collagens), gelatinases (show high activity against gelatin and type IV collagen), stromelysins and membrane bound MMP (degrade variety of ECM components) [32]. The substrates targeted by MMPs are primarily insoluble proteins of the ECM, including interstitial and basement membrane collagens, glycoproteins such as fibronectin, laminin, vitronectin, tenascin and elastin as well as proteoglycans [17]. The MMP family is composed of at least 20 extracellular endopeptidases whose activities are regulated predominantly by expression as inactive precursors or zymogens [8, 88].

All the MMPs share common basic structure. Most MMPs contain five basic domains: a pre domain or signal sequence to direct secretion from the cell [15], a latency or prodomain, a zinc-binding catalytic domain, and a hinge region followed by a domain with sequence homology to hemopexin, a plasma heme-binding protein, and vitronectin, a cell adhesive protein [86]. The MMP family members differ in the presence or absence of additional domains that are involved in activities such as membrane binding, inhibitor binding and substrate specificity. The prodomain contains a PRCGVPDV consensus sequence in which the cysteine residue serves as a 4th Zn^{2+} ligand and maintains the catalytic pocket in an inactive state. A unique feature of these proteases is the presence of the HEXXHXXGXXH motif in their catalytic domain, in which the three histidine residues represent three zinc ligands and the glutamic acid residue forms the active site [17]. The C terminal region of the MMP molecule contains a hemopexin like domain that seems to determine the substrate specificity or the interaction with the cell surface receptor [65, 74, 85, 63].

MMPs are usually secreted as soluble, latent pro-enzymes, and are then activated by proteolytic cleavage of an amino-terminal domain [8, 69, 58] by several proteases [41]. This domain contains a cysteine residue which co-ordinates the active site zinc atom. Once activated, the matrix metalloproteinases are subject to control by endogenous inhibitors such as α2 macroglobulin and more specifically by tissue inhibitors of metalloproteinases (TIMPs) [24]. Four members of the TIMP family have been identified, of which TIMP-1, synthesized by most types of connective tissue cells as well as by macrophages, acts against all members of the collagenase, stromelysin and gelatinase classes [10]. TIMP-1 (28 kDa) and TIMP-2 (21 kDa) are found in the soluble form, while TIMP-3 (24 kDa) is regarded to be insoluble. TIMP-2 and TIMP-3 can inhibit all MMPs to varying degrees, while TIMP-1 is a poor inhibitor of MT1-MMP (MMP-14). A recently cloned TIMP family member, TIMP-4 (22 kDa), has been reported to inhibit MMP-1, -2, -3, -7 and -9 [20, 42]. The TIMPs were described to inhibit tumor progression. They can suppress human amnion (collagen types I, III, IV, and V) invasion and lung colonization by melanoma cells [76]. MMPs, alone or in concert with the plasminogen/plasmin system, are involved in the degradation of ECM components, a requirement for cell migration and tissue remodelling, which play an essential role in many (patho) physiological processes [41].

Design of MMP inhibitors

One approach to limit the activity of the MMPs in cancer has been the development of synthetic inhibitors of MMP. The first generation of metalloprotease inhibitors (MMPIs) was designed to mimic the part of the collagen peptide sequence that is initially cleaved by MMP-1 [15]. The key element needed to obtain potent enzyme inhibition has been the incorporation of a zinc-binding group (ZBG) into peptide analogues of the substrate sequence positioned either on the left-hand side (LHS) or the right-hand side (RHS), or in between both sides of the cleavage site [66]. It was found that RHS inhibitors featuring a hydroxamic acid ZBG represent the most promising drug candidates. The inhibitors fit tightly into the active site of the MMP in a stereo-specific manner. The ZBG, in this case hydroxamic acid (-CONHOH), is then positioned to chelate the active site zinc ion. Modification of the stereochemistry of the molecule results in the loss of inhibitory activity [13]. Several zinc binding groups have been tested including carboxylates, aminocarboxylates, sulphydryls, and derivates of phosphorus acids [7], but hydroxamates have proved to be the most useful and the majority of inhibitors currently in clinical testing contain this group. The first generation of MMP inhibitors to be tested in patients were potent compounds but were also characterized by poor oral bioavailability [13]. Representative examples of

this series of MMPIs featuring a succinyl scaffold are hydroxamates batimastat (BB-94) and marimastat (BB-2516) developed at the British Biotech [57]. Batimastat, one of the first synthetic MMPIs, has a potent activity against most MMPs with an IC_{50} in the low nanomolar range. It acts as a competitive, reversible inhibition by mimicking the substrate of MMPs [27]. Structural modification of Batimastat resulted in the synthesis of Marimastat. Marimastat is a broad-spectrum inhibitor for the MMP family with low nanomolar IC_{50} against all the MMPs except MMP-3 [15]. Marimastat was one of the first inhibitors to show improved oral bioavailability in both animals and man, differing from its predecessor Batimastat in the group adjacent to the hydroxamate and the group at the P2' position [13]. Most of the peptide-mimetic MMPIs have poor oral bioavailability so research focused on the development of a non-peptidic inhibitor based on the newly available X-ray crystallographic information about the MMP active site. AG3340 (Agouron), Ro 32-3555 (Roche), CGS- 27023A (Novartis), and BAY 12-9566 (Bayer), are examples for these non-peptidic inhibitors.

As yet it has not been possible to design an MMP inhibitor specific for just one MMP, but compounds can be made that favor the inhibition of MMPs with a 'deep pocket' at the active site (MMP-2, MMP-9, MMP-3) at the expense of MMPs with 'shallow pockets' (MMP-1 and MMP-7). Heterocyclic sulphonamide Prinomastat (AG3340) is a potent selective inhibitor for MMP-2 (gelatinase A), MMP-3, MMP-9 and MMP-13 [15] exhibiting inhibition constant (*Ki*) values in the picomolar range. Another representative of the non-peptidic inhibitors is Ro 32-3555. It is an inhibitor with relatively weak activity against gelatinase A and stromelysin-1 but display a good activity when given orally in animal models [39]. CGS-27023A is another orally administered, broad spectrum MMPI with antitumor activity in preclinical models. It inhibits MMP-1, 2, 3, 9 and 13 [15]. As with the sulphonamide CGS-27023A, the key to the discovery of this series was high throughput screening of combinatorial libraries of compounds, which identified a fenbufen derivative as a micromolar MMP-3 inhibitor [66]. Structural modification of fenbufen led to the discovery BAY-129566. This is a novel nonpeptidic biphenyl compound which inhibits MMP-2 and MMP-9 [15].

Urokinase plasminogen activator

Urokinase-mediated plasminogen activation is involved in many normal physiological processes, including tissue remodelling, embryogenesis, and wound healing and clot lysis [68]. In particular, the plasminogen activation (PA) system is considered to play a major part in enhancing tumor invasiveness [55]. Of these, the PA is the best characterized system, and many reports demonstrate its involvement in ECM degradation. The system is composed of one enzyme – the urokinase type plasminogen activator (uPA), one substrate (plasminogen), two receptors (uPA receptor (uPAR) and plasminogen receptor) and three inhibitors (plasminogen activator inhibitors PAI-1, PAI-2 and protease nexin 1) [83]. uPA is a serine protease with a Mr of approximately 50 kDa [2]. It is secreted by cells as a 411 amino acid single chain zymogen, pro-urokinase (pro-uPA), also known as single-chain uPA (scuPA) [54]. uPA is produced by tumor cells and normal cells (e.g. kidney tubule cells, phagocytic cells, pneumocytes, keratinocytes, fibroblasts, trophoblasts) as a proenzyme with little intrinsic enzymatic plasminogen activating activity [64]. The mature uPA protein is a disulfide bond-linked heterodimer containing an A chain (the N-terminal fragment) that binds to uPAR and the catalytic B-chain (the C-terminal fragment) [62, 64]. The uPA consists of three domains: N-terminal domain, kringle domain and C-terminal proteolytic domain. The active form of uPA is two-chain urokinase (uPA or tcuPA) [25]. The low enzymatic activity of scuPA secreted by cells increases ~300-fold after the proteolytic cleavage of the Lys158-Ile159 bond [40] that results in uPA conversion into the two-chain form consisting of two polypeptide chains joined via the Cys148-Cys279 disulfide bond [53]. This conversion can be catalyzed by plasmin [16], which is formed from the inactive zymogen plasminogen (Plg) by plasminogen activators [28].

Small molecule urokinase inhibitors

Clinical studies have demonstrated an association between high levels of expression of the components of plasminogen activator system in tumors and poor patient prognosis and outcome [49]. Moreover, high levels of urokinase activity are associated with many cancers, and furthermore, increased urokinase activity is an independent predictor of the diseased state [59]. The role of urokinase in the mechanism of tumor metastasis indicates that structural and functional characterization of the urokinase active site and the discovery of more potent small-molecule inhibitors should provide a significant step towards obtaining an anticancer clinical agent [52].

In 1987, the diuretic drug amiloride with acyl guanidine moiety, was found to efficiently inhibit uPA with a *Ki* value of 7 μM [80] and served as a prototype uPA inhibitor for X-ray crystallography [51, 89]. Taking into account the trypsin-like arginine specificity of uPA, (4-aminomethyl) phenyl-guanidine was selected as a potential P1 residue and an iterative derivatization of its amino group with various hydrophobic residues was carried out [67]. In addition, QSAR-based optimization of a spacer in terms of hydrogen bond 'acceptor/donor properties resulted in N-(1-adamantyl)-N-(4-guanidinobenzyl)-urea as highly selective nonpeptidic uPA inhibitor. This urea derivative WX-293 (Wilex) is the analog with the highest uPA affinity, K_i value of 2.4 μM [45]. Crystallographic analysis of this compound shows that the phenylguanidine moiety typically occupies the S1 site [61]. 2-pyridinylguanidines have recently been proposed to form uPA inhibitors by PFIZER [68]. Structural modification such

as the incorporation of a rigid aryl-containing side chain at position 3 of the pyridine led to the discovery of compounds with K_i of 0.49 and 0.17 μM respectively [4]. In addition, Pfizer developed 1-guanidino-4-chloroisoquinoline derivates with K_i values lower than 20 nM [3, 5].

Peptide containing mechanism-based uPA inhibitors were first reported by WAKSELMAN et al in 1993 [82]. These compounds are cyclic hexapeptides and were designed to impart selectivity for the trypsin family of proteases. These molecules contain either an arginine or lysine residue for specific interaction with Asp-189 in the S1 site [61]. Corvas developed tetrapeptide inhibitors with an arginine-mimicking aldehyde or an arginine ketoamide group at the P1 position, and D-serine at P3 [4]. One of the most potent compound showed high value of IC_{50}=3.1 nM [75]. Group from University of Jena developed a highly potent peptidomimetic compounds with a D-serine in the P-3 position and replaced the P1-arginine with a 4-amidinobenzylamide [71]. The highest uPA potency was found for the P2-Ala derivative with K_i=7.7 nM [37].

The discovery of substituted benzamidines and 2-naphtamidines identified by STÜRZEBECHER and colleagues, demonstrated a moderate uPA affinity with micromolar inhibition constants [70]. Pentapharm and Medical School of Erfurt developed inhibitors, containing an N-terminal 2, 4, 6-triisopropyl-phenylsulfonyl residue and substituted piperazine at C-terminus [72, 87]. The most potent inhibitor WX-UK-1 with *Ki*=0.41 μM, demonstrates remarkable potency in inhibiting tumor growth and metastasis [84]. On the basis of crystallographic data Abbott Laboratories have chosen 2-naphtamidine as the lead scaffold for structure-directed optimization [68]. Structural modification (substitution at the positions 6, 7 and 8) of 2-naphtamidine led to the discovery of highly potent compounds. As successful result has been observed 6,8 disubstituted naphthamidine inhibitor with K_i=6 nM potency to the uPA [60, 61]. 6,8-disubstituted naphtamidine-based compounds are the most potent and selective uPA inhibitors known.

A major improvement in the potency of uPA aryl-amidine inhibitors was described in 1993 by LITTLEFIELD and coworkers [11, 77]. They used benzo[b]thiophen-2-carboxamidine as the basis for their inhibitors. The unsubstituted benzo[b]thiophen-2-carboxamidine has nearly the same uPA potency (K_i=3.7 μM) as the naphtamidine with K_i of 5 μM [60]. Very potent selective active site directed uPA inhibitors are the substituted benzo[b]thiophen-2-carboxamidines, B-428 and B-623, with IC_{50} values of 320 nM and 70 nM respectively [77]. Amidinobenzimidazoles or amidinoindoles have been chosen by Axys Pharmaceuticals (now Celera) as the templates for the development of small molecule uPA inhibitors utilizing structure-based approach [31, 43]. Axis scientists proposed more selective uPA inhibitors based on the amidinoindole template. By incorporating a chlorine atom ortho to the amidine of a lead scaffold, highly potent compound with K_i=9 nM and significantly improved selectivity over tPA (1000-times), factor Xa (2000-times), thrombin (6000-times) and porcine kallikrein (90-times), has been observed. This halo group displaces an important water molecule in the S_1 subsite and eliminates a key hydrogen bond [68]. The these compounds bind to the S_1 specificity subsite of urokinase and also to the recently described S_{1beta} pocket [52]. The novel inhibitors are now serving as leads for drugs discovery efforts toward development of selective and orally available uPA inhibitors [81].

After screening several hundred amidines, guanidines, and amines, the 5-methylthiothiophene-2-carboxamidine (K_i=6 μM) was selected by 3-D Pharmaceuticals as the suitable P1 residue for structure-based design [73]. A molecular modelling comparison of template 5-methylthiothiophene-2-carboxamidine to inhibitor B-428 indicated that substitution in the position 4 of thiophene should lead to increased potency. This replacement led to discovery of new lead inhibitor with a K_i of 101 nM [23, 73]. Starting from a weakly active P1 scaffold, 3-D Pharmaceuticals constructed a novel series of potent and selective inhibitors of human uPA [73]. These compounds possess high selectivity for uPA and are able to inhibit tumor metastasis in a cell-based assay [68].

Flavonoids (phenolic compounds naturally occuring in the plants) represents other group of potential inhibitors of uPA. The compounds from the groups of flavones, flavanones, flavanoles, flavan-3-oles and isoflavones exhibited potent inhibitory effect towards uPA. The most potent inhibition effect exhibited quercetin and epigallocatechin gallate with IC_{50} values of 7 μM and 15 μM, respectively [46].

Lysosomal proteases

Lysosomal proteases, generally known as the cathepsins, play an important role in many physiological processes such as in bulk proteolysis, antigen presentation, processing of invariant chain of the major histocompatibility complex (MHC), bone resorption, chronic inflammation and others [9]. They have also been implicated in many pathological processes including tumor invasion, metastasis, Alzheimer disease, inflammation, rheumatoid arthritis and osteoarthritis, multiple sclerosis, muscular dystrophy, pancreatitis, liver disorders, lung disorders, lysosomal disorders, diabetes and myocardial disorders [21, 78, 79]. The physiological activities of cathepsins are regulated by endogenous intracellular stefins (A and B) and extracellular cystatins, such as cystatin C [38]. The cathepsins are subdivided on the basis of their active site residues (cysteine, serine and aspartate type). The papain-like lysosomal cysteine proteases are monomeric proteins with molecular weight between 22 and 28 kDa. The only exception is cathepsin C, which is a tetrameric molecule with a molecular weight of 200 kDa [78]. Cathepsins share an identical domain structure and a high sequence homology. All cathepsins are encoded as preproenzymes. The signal or pro-sequence consist of a 15–21 amino acid long peptide, which is required for the

entry into the endoplasmic reticulum and is cleaved off by the signal peptidase [12].

From cathepsins the cathepsin B is the best studied of the mammalian cysteine proteases [78]. In contrast to other cysteine proteases, cathepsin B is an exopeptidase with carboxydipeptidase activity as well as an endopeptidase activity [6]. Its powerful proteolytic activity towards extracellular matrix proteins and increased cathepsin B expression as well as secretion in tumors has been used to propose a role of cathepsin B in tumor invasion and metastasis [12, 36]. Cathepsin B was the first lysosomal protease to be associated with breast carcinoma [56]. Furthermore, cathepsin B has also been implicated in the invasive and malignant phenotype of tumors of the bladder, brain, lung, pancreas, prostate, stomach and thyroid [21]. Cathepsin B can activate certain MMPs and also receptor-bound uPA [33, 34], which then in turn, can convert plasminogen to plasmin [16] capable to degrade various components of ECM [35]. The second best studied mammalian cathepsin is an aspartic protease cathepsin D. Cathepsin D contains a pair of aspartic acid residues at the catalytic site and is active at acidic pH [22]. In contrast, cathepsin D seems not to have an endogenous inhibitor in mammalian cells, with the exception of its pro-peptide, removed after enzyme activation [38]. Cathepsin D degrades a large variety of endocytosed proteins and has been implicated in breast cancer invasion and metastasis, where relapse and metastatic disease correlate with high levels of cathepsin D expression [1]. The mechanisms by which cathepsin D increases the incidence of clinical metastasis are most likely different from other cathepsins, as the enzyme seems to act as an autocrine mitogen. Cathepsin D induces increased cell growth and decreased contact inhibition rather than the digestion of extracellular matrix and basement membrane components. Intracellularly, it might inactivate growth inhibitors and other pro-peptidases [38].

Low-molecular-weight inhibitors of cathepsins

Increasing understanding of the precise biological role of cysteine proteases and their inhibitors in the progression of cancer, founded on basic research and clinical investigations, has identified these proteins as promising therapeutic targets for the development of new anti-tumor drugs [79]. One of the approaches how to limit the activity of the cathepsins in cancer is the development of low molecular weight synthetic inhibitors. The rather short binding site seems to facilitate covalent interactions with low-molecular-weight inhibitors. Covalent interactions, however, impose hard constraints on the binding geometry [78]. At present, the active site thiol remains the key to selective synthetic inhibitors of cysteine proteases. Selective inhibitors of cysteine proteases (inhibitors with a strong preference for cysteine) typically form irreversible covalent bonds to the active site thiol. Cathepsins are inhibited by the various classes of inhibitors. The best known group of inhibitors of cathepsins are the epoxysuccinamide derivatives. E-64 is an irreversible selective inhibitor of cathepsin L [26], but inhibition activities have been reported on cathepsin H and cathepsin B too. It utilizes an epoxysuccinyl group to covalently interact with the reactive-site cysteine. Variations of E-64, in which the carboxyl group (that was shown to occupy the oxyanion hole of the protease) was esterified or otherwise changed, were shown to be highly specific for cathepsin B [50]. The best known synthesized derivative is CA030 with high specificity to cathepsin B. However, new derivatives of L-*trans*-epoxysuccinate and aldehyde, named CLICK were designed as specific inhibitors of cathepsin L and S [29]. CLICK-148 has been developed as a specific inhibitor of cathepsin L [30]. Pepstatin is a highly potent peptidomimetic inhibitor of cathepsin D. It is a typical representative of the group of statin-based analogs as perspective inhibitors of cathepsin D [19]. A large body of evidence has accumulated showing the association of lysosomal cathepsins, in most cases cathepsins B and D, with the progression of malignant disease [79]. One of the most perspective strategies for suppression of tumor growth seems to be the development of selective low molecular weight inhibitors of cathepsins. Inhibitors of cathepsins provide attractive target for the development of potential therapeutic agents to treat cancer.

Conclusion

Proteases play an important role in many physiological processes. Therefore, breakdown of the control of protease activities can lead to many pathological processes. Many cancer cells, especially those in fast-growing or aggressive neoplastic disease, express proteolytic enzymes, such as cathepsins B, D and L, matrix metalloproteinases, and plasminogen activators, either membrane-bound or secreted extracellularly. The MMPs (zinc-dependent endopeptidases) are one of key families of enzymes used by tumor cells to invade and metastasize. MMPs inhibitors remain an important and perspective class of drugs for cancer therapy. The development of synthetic MMP inhibitors is one of the possible treatments for controlling the metastatic potential of many tumors. This therapy alone cannot destroy the tumor, but if used in combination with other therapies, the MMPIs could stop the disease progression and slow down the spread of the tumor. The possibilities in future studies would lie at targeting the correct MMPs with other cytotoxic agents.

The importance of the plasminogen activator system in carcinogenesis, especially in metastasis formation and invasion, has stimulated the search for more potent a selective uPA inhibitors to block cell surface-associated proteolysis. Much effort has been made to generate novel S1 binding groups for uPA with the main goal of increasing oral bioavailability. The development of potent and selective small molecule inhibitors of uPA has been mainly focused on an aryl amidine or an aryl guanidine templates. Structure-based design has led to the discovery guanidino and

2-naphtamidino, benzo[b]thiophen-2-carboxamidino, amidinobenzimidazolo, thiophene 2-carboxamidino substituted aromates as potent and selective compounds. The X-ray analysis has allowed a detailed study of important inhibitor-protein interactions of existing uPA inhibitors and provided unique opportunities for engineering selectivity into the inhibitors. These novel inhibitors are now serving as leads in drug discovery efforts toward the development of selective, orally bioavailable uPA inhibitors with fully reversible binding mode, relative synthetic accessibility and non-peptidic nature.

Lysosomal proteases, generally known as the cathepsins, have also been implicated in tumor invasion and metastasis. One of the intensively studied fields is the development of cathepsin protease inhibitors as potential new antiinvasive drugs. The most promising inhibitor class comprises the epoxysuccinyl peptides, originally derived from E-64 a potent inhibitor of various cathepsins. No drug which directly targets cysteine proteases is currently on the market, therefore this area is still very attractive for pharmaceutical industry. The near future of protease research will be probably focused on new approaches in drug discovery, such as combinatorial chemistry and structure based design.

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