Potential Opportunity in the Development of New Therapeutic Agents Based on Endogenous and Exogenous Inhibitors of the Proprotein Convertases

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Abstract: The proprotein convertases (PCs) are responsible for the endoproteolytic processing of various protein precursors (e.g., growth factors, receptors, adhesion molecules, and matrix metalloproteinases) implicated in several diseases such as obesity, diabetes, atherosclerosis, cancer, and Alzheimer disease. The potential clinical and pharmacological role of the PCs has fostered the development of various PC-inhibitors. In this review we summarized the recent findings on PCs inhibitors, their mode of actions and potential use in the therapy of various diseases. © 2006 Wiley Periodicals, Inc. Med Res Rev, 27, No. 5, 631–648, 2007

Key words: protein maturation; convertases; drug development

1. INTRODUCTION

The proprotein convertases (PCs) are serine proteases that bellow to the kexin subfamily of subtilases enzymes responsible for the processing and the activation of multiple polypeptide precursors. These secretory precursors are usually cleaved at the general motif (K/R)-(X)n-(K/R)↓, where X is any amino acid (except C), n = 0, 2, 4, or 6, and ↓ represents the cleavage site where the peptide is hydrolyzed.[1–6] To date, seven basic amino acids (AA)-specific PCs, serine proteases belonging to the kexin subfamily of subtilases, were reported to be involved in these processes.[1–6] These include

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Furin, PC1 (also called PC3), PC2, PC4, PACE4, PC5 (also called PC6), and PC7 (also called PC8, LPC, or SPC7).[1–6] Recently, other two nonbasic-AA-specific convertases, SKI-1[7,8] and NARC-1[9] were identified. These convertases belong to the Pyrolysin and Proteinase K subfamily of subtilases, respectively. While, SKI-1 was found to exhibit a cleavage specificity for the motif (R/K)-X-(hydrophobic)-(L,T)↓, based on its autocatalytic site, NARC-1 seems to prefer the V-F-A-Q↓ motif.[10] In this review, the role of the proprotein convertases in the mediation of some diseases will be briefly summarized, the mode of action of their natural and exogenous inhibitors will be described and their potential use as new targets for the treatment of various diseases will be discussed.

2. PROPROTEIN CONVERTASES AND DISEASES

A. Convertases in Neurodegenerative Pathology

Recently PCs have been linked to some neurodegenerative disorders via their direct or indirect roles in the production of amyloidogenic peptides. In Alzheimer's disease the amyloid-β (Aβ) is the principal component of senile plaques. The latter is generated by proteolytic cleavage of its precursor by β- and γ-secretases. Recently, the PCs were found to process the zymogens of both α- and β-secretases, suggesting the implicating of the PCs in this disease.[11,12]

B. Convertases and Cancer

The involvement of proprotein convertases in tumorigenesis has been extensively reviewed.[2,13,14] Some of the cleaved protein precursors by the PCs, such as matrix metalloproteases, adhesion molecules, growth factors, and growth factor receptors are directly or indirectly involved in tumorigenesis and metastasis by regulating either degradation of extra-cellular matrix and/or modulation of cell growth and survival.[2,13,14] Using different tumor cells with invasive/metastatic phenotypes, the inhibition of PC-activity was found to provoke dramatic changes in several phenotypes that impact on the metastatic potential of tumor cells.[2,13,14] Similarly, using various site-directed mutagenesis, we found that the inhibition of the processing of several PC substrates such as PDGF-A,[15] and VEGF-C[16] reduced significantly their ability to induce tumor development and angiogenesis, respectively.[15,16] This data highlighted the importance of PCs in the activation of these growth factors during tumor progression and angiogenesis.[15,16]

C. Bacterial Toxins Activation by the PCs

Three different classes of bacterial toxins were described to be activated by the PCs. The toxins of the first class are synthesized as single polypeptide chains that group the toxic subunit and the target binding subunit. The toxin precursors are cleaved during their interaction with the target cell surface or in the endosomal compartment by the PCs.[17–20] Of the toxins that belong to this class and were reported to be activated by the PCs are the *Diptheria* toxin,[19] *Pseudomonas aeruginosa* exotoxin A (PEA),[17,18] *Botulinum* neurotoxin, and *Bordetella* dermonecrotic toxin.[20] The second class of toxins such as Anthrax are synthesized as separate polypeptide chains and usually assemble on the target cell surface to form the active toxin following activation of the binding subunit by the PCs.[21,22] The third class groups the pore-forming toxins such as the aerolysin. These toxins are produced and secreted as a dimer that bind on target cells to the glycosylphosphatidyl inositol anchors of membrane proteins. Usually the cleavage of these toxins by the PCs occurred on the surface of the target cell during their binding. This process seems to be crucial for the association of the toxin dimers into heptamer pore complex and causes cell lysis.[23]

D. Convertases and Viral Infections

Previously, data on various infectious viruses revealed that the cleavage of their envelope glycoprotein precursors by one or more PC is a required step for the acquisition of the infectious

capacity of viral particles. Indeed, various studies demonstrated the capacity of the PCs to correctly cleave a variety of viral surface glycoproteins. These include the HIV-1 gp160[24,25] and surface glycoproteins of Hong Kong, Ebola virus, and the severe acute respiratory syndrome coronavirus.[26,27] In parallel, other studies revealed that the inhibition of processing of these viral surface glycoproteins by the PC inhibitors such as dec-R-V-K-R-CMK completely abrogated the virus-induced cellular cytopathicity. Recently, the surface glycoproteins of other viruses, particularly the hemorrhagic fever viruses (Arenaviridae family) such as Lassa,[28,29] Crimean Congo hemorrhagic fever,[30] and lymphocytic choriomeningitis[31] were shown to be cleaved by the convertase SKI-1. Similarly, blockade of SKI-1 activity by specific inhibitor were also shown to affect the processing and the stability of the glycoproteins of these viruses.[32]

3. PROPROTEIN CONVERTASES INHIBITORS

Since the discovery of Furin, the growing evidence of PCs implication in various pathological processes made these enzymes important potential therapeutic targets. Thereby various attempts have been made to develop specific and potent inhibitors to target these enzymes. All the inhibitors that were found or developed so far are grouped into natural endogenous or exogenous PC inhibitors.

A. Natural Endogenous Inhibitors of PCs

1. Prosegments or Propeptides of the PCs

To date the only naturally occurring intracellular PC inhibitors found in the constitutive secretory pathway are PCs own propeptides or prosegments.[33,34] Previously, it was reported that many proteins use their propeptides as intramolecular chaperones for their correct folding, transport, and/or secretion.[35] In addition to these prosegment functions, these enzyme fragments were also reported to act as inhibitors for various enzymes including the PCs.[2,33,34] Like their substrates the PCs are synthesized as inactive proenzymes and are auto-catalytically activated.[1–6,36] Following their signal sequence removal and endoplasmic reticulum (ER) folding events, PCs undergo auto-proteolytic cleavage of their prosegment at R107 (Fig. 1). The prosegment, however, remains associated with the mature domain of the enzyme and functions as a potent auto-inhibitor during transport to the late secretory pathway. After this step, the inactive complex transits to the late *trans*-Golgi network (TGN) where the relatively acidic pH permits a second autoproteolytic cleavage of the prosegment at R75 and activates the convertase (Fig. 1).[6,35] Using these inhibitors, we were able to inhibit the processing and the function of various PC substrates such as PDGF-A,[15] PDGF-B,[37] VEGF-C,[16] and IGF-1 receptor[2] (Fig. 2). Recently, the Furin inhibition by its pro-segment proFurin was reported as feasible approach to reduce and/or abolish the malignant phenotype of various malignancies.[38] Indeed, the expression of the complete proFurin cDNA sequence in various human head and neck squamous cell carcinoma cell lines was found to reduce dramatically their proliferation, tumorigenicity, and invasiveness *in vitro* and *in vivo* as well.[38] These proFurin effects were directly linked to the inhibition of Furin-mediated activation of various crucial cancer-related substrates, such as TGF-β, VEGF-C, IGF-1 receptor, and MT1-MMP.[38]

2. 7B2; the Naturally Occurring Inhibitor of PC2

Little is known about the cellular function of 7B2. Nevertheless, Braks and Martens were the firsts to show that 7B2 act as a chaperone for proPC2 and be able to bound to the latter in the early compartment of the secretory pathway and dissociates from it in the latter ones.[39] Other studies proposed that 7B2 may also facilitates proPC2 transport from the endoplasmic reticulum to the secretory granules and participates in the generation of fully active PC2.[40] Following their secretion,

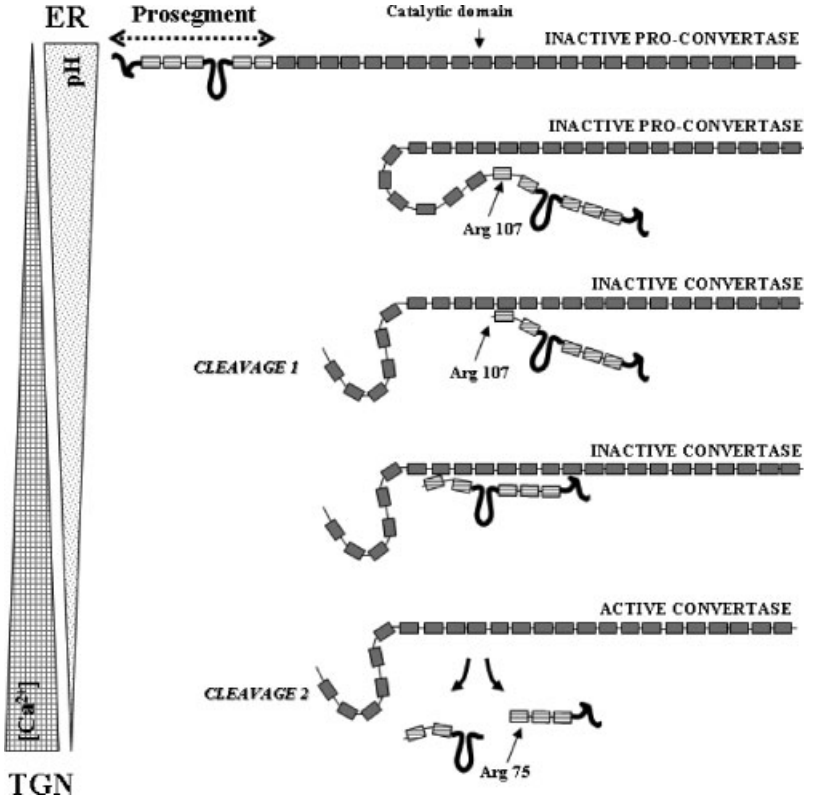

Figure 1. Processes of Furin activation. At the endoplasmic reticulum (ER) emplacement, the Furin propeptide acts as an intramolecular chaperone to facilitate the folding of the catalytic domain into the active conformation. During transport to the late secretory pathway, Furin undergoes autoproteolytic intramolecular cleavage of its propeptide at R107. The latter remains associated with the mature fragment of the Furin and functions as a potent autoinhibitor. At low pH and higher [Ca^{2+}], the propeptide is cleaved a second time at its R75 leading to a rapid dissociation of the propeptide fragments and Furin activation.

pro7B2 and proPC2 interact in the ER in the presence of an alkaline pH and form an inactive complex (pro7B2-proPC2). During its progression through the TGN in the presence of decreased pH and increased [Ca^{2+}] the pro7B2 is cleaved by the PCs and released a *C*-terminal fragment with inhibitory function on PC2.[41,42] In the secretory granules, additional pH decreases and [Ca^{2+}] increases permits proPC2 self activation and liberates the prodomain of PC2 that provides a fully active PC2 (Fig. 3). Subsequently, the *N*-terminal domain of PC2 and *C*-terminal domain of 7B2 are rapidly degraded by PC2 and carboxypeptidase E (Fig. 3).[40–42]

3. ProSAAS; the Naturally Occurring Inhibitor of PC1

Like 7B2, ProSAAS, contains an *N*-terminal and a *C*-terminal domain that are separated by a PC cleavage sites. The sequence responsible for the inhibitory potency of PC1 was previously pointed to the hexapeptide, L-L-R-V-K-R, located in the proSAAS *C*-terminal domain.[43] This peptide was identified by combinatorial library peptide screening as a tight binding site for PC1.[44,45] Like PC2, PC1 is inactive in the endoplasmic reticulum and Golgi apparatus due to the neutral pH and relatively low Ca^{2+} levels in addition to its interaction with proSAAS.[46] Following its progression through the TGN, proSAAS is cleaved into two peptides designed as PEN and LEN fragments that remove the inhibition of PC1 by proSAAS (Fig. 4).

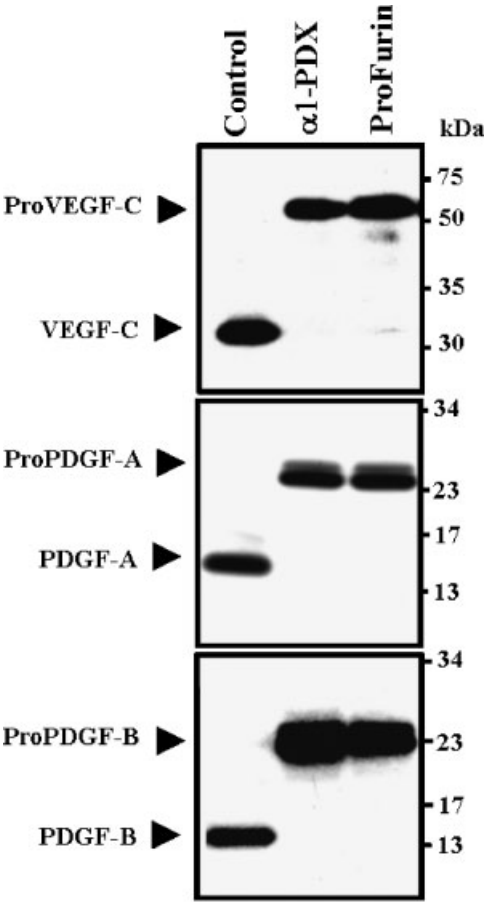

Figure 2. Inhibition of proVEGF-C, proPDGF-A, and proPDGF-B processing by PC inhibitors. The processing of proVEGF-C, proPDGF-A, and proPDGF-B was analyzed by Western blotting in HEK 293 cells transiently transfected with the empty vector (control) or with the same vector that expresses the endogenous Furin inhibitor (proFurin) or the exogenous PC inhibitor (α1-PDX).

4. Inter-Alpha-Inhibitor Protein (IalphaIp)

Inter-alpha-inhibitor protein (IalphaIp) is an abundant endogenous serine protease inhibitor initially isolated from human plasma.[47–50] IalphaIp consists of three polypeptides: two heavy chains and one light chain called bikunin. Analysis of the bikunin structure revealed the presence of two protease domains of the Kunitz type carrying the antiproteolytic function of the IalphaIp. Bikunin was found to be effective against a broad range of enzymes that includes trypsin, chymotrypsin, plasmin, and leukocyte elastase, as well as cathepsins B and H.[46–49] Although crystal structure analysis of bikunin revealed direct interaction between trypsin and the two domains of bikunin, the inhibitory mechanism of IalphaIp remain unknown.[48] Recently, the IalphaIp was also proposed as a good inhibitor for the Furin to prevent the formation of active anthrax lethal toxin.[51] Indeed, this inhibitor was able to provide significant protection against cytotoxicity for murine peritoneal macrophages exposed to high doses of the anthrax lethal toxin[51] (Fig. 5).

5. Human Proteinase Inhibitor 8

Initial studies indicated that the serpin proteinase inhibitor 8 (PI8) was able to inhibit a variety of proteinases through different mechanisms. This inhibitor was shown to inactivate the porcine trypsin,

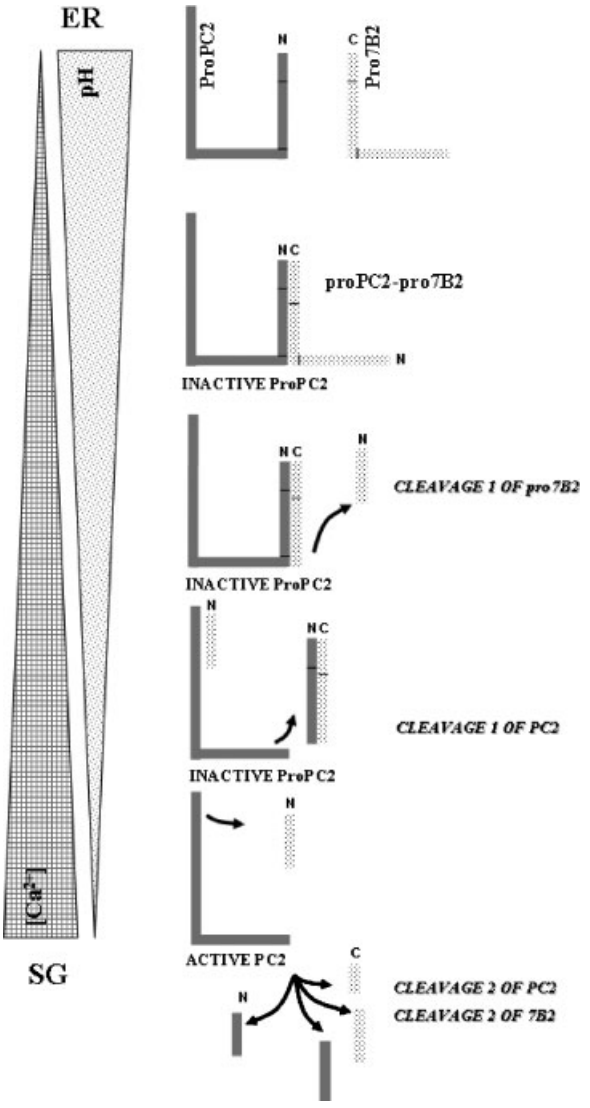

Figure 3. Inhibition and activation of PC2. In the presence of alkaline pH and a poor environment, the free ProPC2 interact with Pro7B2 to form an inactive complex pro7B2-proPC2. Through its progression in the *trans*-Golgi network (TGN) where the pH is decreased and the [Ca^{2+}] is increased, pro7B2 is cleaved by the PC and the *C*-terminal fragment of 7B2 remained attached to proPC2. In the secretory granule (SG), where the pH is lower and the [Ca^{2+}] is higher, the proPC2 is cleaved auto-catalytically to liberate the prodomain fragment. The *N*-terminal domain of PC2 and the *C*-terminal and *N*-terminal domains of 7B2 are rapidly degraded by PC2 and carboxypeptidase E and generates a fully activated PC2.

human thrombin, human coagulation factor Xa, and the *Bacillus subtilis* dibasic endoproteinase subtilisin A.[52] This 45-kDa serpin was reported to form a SDS-stable complex with human Furin and provoke the inhibition of the enzyme. The PI8-mediated Furin inhibition is due to the presence in the reactive site domain of the inhibitor a PC cleavage sites namely R-N-S-R339 and R-C-S-R342.[53]

B. Exogenous Inhibitors of the PCs

Most exogenous inhibitors of the proprotein convertases were generated to act in a competitive fashion. Most of these inhibitors contain the general cleavage motif of the PCs (K/R) – $(X)_n$ – (K/R)↓.

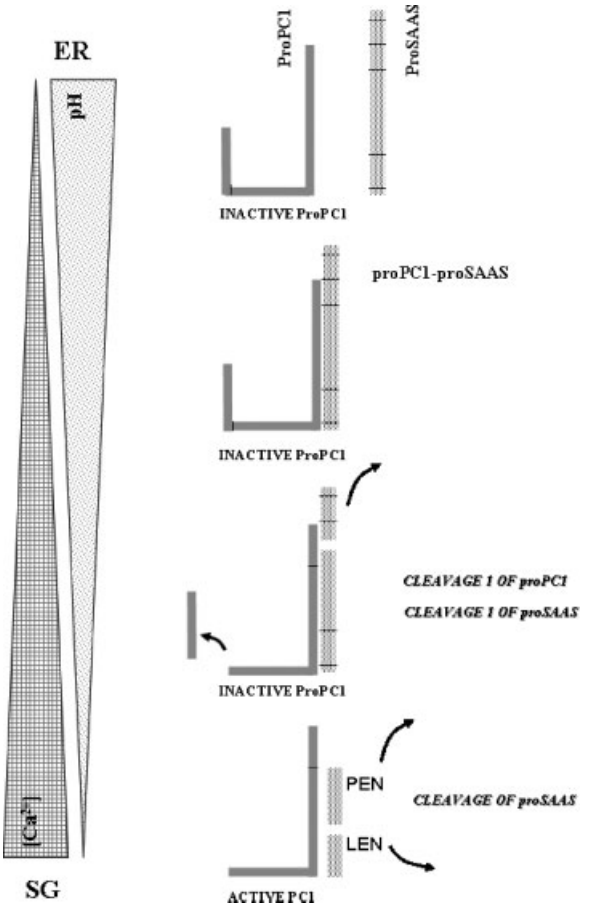

Figure 4. Inhibition and activation of PC1. Similarly to PC2, in the ER the free proPC1 interact with ProSAAS to form the inactive complex proPC1-ProSAAS. The progression of this complex through the *trans*-Golgi network (TGN) resulted in the first cleavages of ProSAAS and ProPC2. At this step, the *C*-terminal fragment of ProSAAS is attached to/and inhibits PC1. In the secretory granules (SG) the proSAAS is cleaved into the two peptides PEN and LEN that removes the inhibition of PC1 by proSAAS.

1. Acyl-Peptidyl-Chloromethyl Ketones

These synthetic inhibitors contain in their structures an acyl moiety that allows them to enter into the cells and bind to the active site of the PCs through its peptidyl group.[54] They were the first compounds that were demonstrated to inhibit the PCs.[55] Of the members of this family the derivative decanoyl-R-V-L-R-chloromethylketone was found to inhibit various PCs substrates ranging from growth factors to viral glycoproteins.[55] This inhibitor was previously used to inhibit the activity of various MMPs and tumor cell invasion processes.[56,57] Similarly, treatment of a prostate cancer cell line with this reagent was found to inhibit the processing of prostate-derived factor (PDF) and other members of the TGF-β superfamily that was associated with a loss of prostate cancer cell differentiation.[58]

2. Poly-Arginines

Recently the poly-arginines were also described as potent inhibitors of the PCs.[59–61] Based on the reported structure of mouse Furin the active site of the enzyme seems to contain an extended

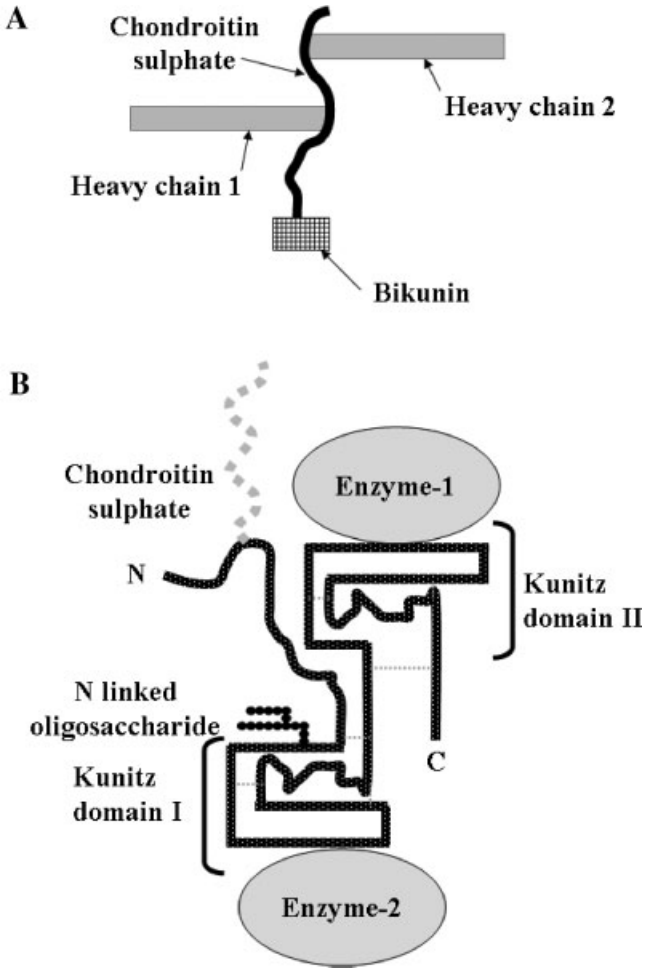

Figure 5. Schematic representation of the interalpha inhibitor. The interalpha inhibitor consists of two heavy chain covalently linked to a light chain (bikunin) by a chondroitin sulphate chain (**A**). Bikunin contains two protease inhibitor domains of the Kunitz type able to interact with two enzymes (**B**). Adapted from Acta Biochim Pol. 2003;50(3):735–42.

substrate-binding groove that is lined with many negatively charged residues.[60] Thereby the highly acidic character of the substrate-binding groove explains the high-inhibitory potency of positively charged polyarginine-containing peptides.[59–61] Recently, the hexa-D-arginine amide was found to inhibit significantly the *Pseudomonas aeruginosa* exotoxin A (PEA) processing and PEA-induced toxicity in mice.[61] Also the polyarginine inhibitors were reported to be able to inhibit the processing of the human immunodeficiency virus-1 gp160 and the replication of the virus as well.[62]

3. Turkey Ovomucoid Mutant

Turkey ovomucoid third domain with normal reactive site is known as a potent inhibitor of various serine proteinases including subtilisins, chymotrypsins, and elastases.[63] Mutation of this inhibitor at L18K in its reactive site made it a strong inhibitor of trypsin and its mutation at the same site into L18E made it a strong inhibitor of Glu-specific streptomyces griseus proteinase (GluSGP)[64] (Fig. 6). In parallel, the introduction of a proprotein convertases site its structure made it a moderate Furin inhibitor[65] (Fig. 6).

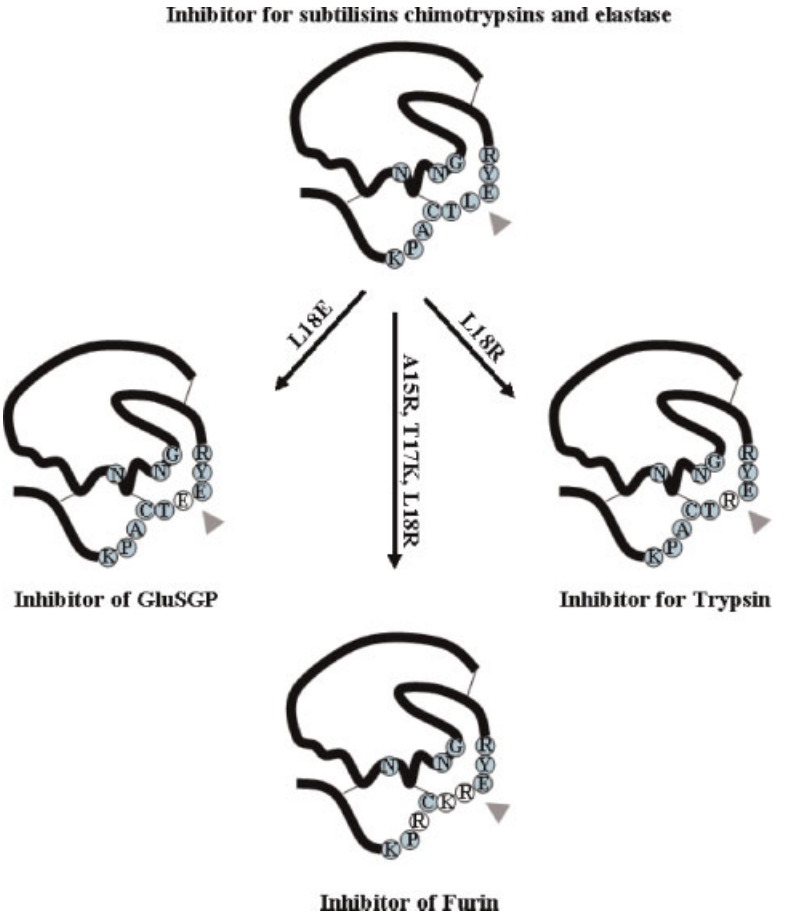

Figure 6. Schematic representation of turkey ovomucoid the third domain. Indicated are the different mutations that were introduced in the turkey ovomucoid the third domain to generate a Glu-specific *Streptomyces griseus* proteinase (GluSGP) inhibitor, trypsin inhibitor, or Furin inhibitor. Arrowhead indicates the reactive site peptide bond. The 12 AA forming the consensus enzyme inhibitor contact set are also named.

4. Eglin C Mutant

Eglin C is a proteinase inhibitor that strongly inhibits human leukocyte elastase, cathepsin G, a-chymotrypsin, and substilisin.[66] This inhibitor was initially isolated from the leech *Hirudo medicinalis* and belongs to the potato I inhibitor family.[67] Previously, it was reported that its inhibitory specificity could be changed and inhibits trypsin by a point mutation at its reactive site L45R. Subsequently, substitution of residues at each position P1, P2, and P4 of eglin C with a basic residue made it a very strong inhibitor for Furin.[68] Recently, the generation of the three-dimensional complex structures of Furin-eglin C mutant interaction by a modeller program provided crucial information on the interaction between the Furin and this inhibitor.[69] The modellation of this interaction allowed the calculation of the electrostatic interaction energies between the Furin and eglin C mutant.[69] The results that were obtained from this study highlighted the importance of the charge–charge interactions in the binding of Furin to its inhibitors, suggesting the roles of the electrostatic interactions in the inhibitory activity of eglin C mutant toward Furin.[69] Further analysis revealed that the mutation of R48D (P3′ residue) in eglin C seems to increase the inhibitory action of the eglin C mutant due to the electrostatic interactions of D48 with R86 and R90 of Furin (Fig. 7).

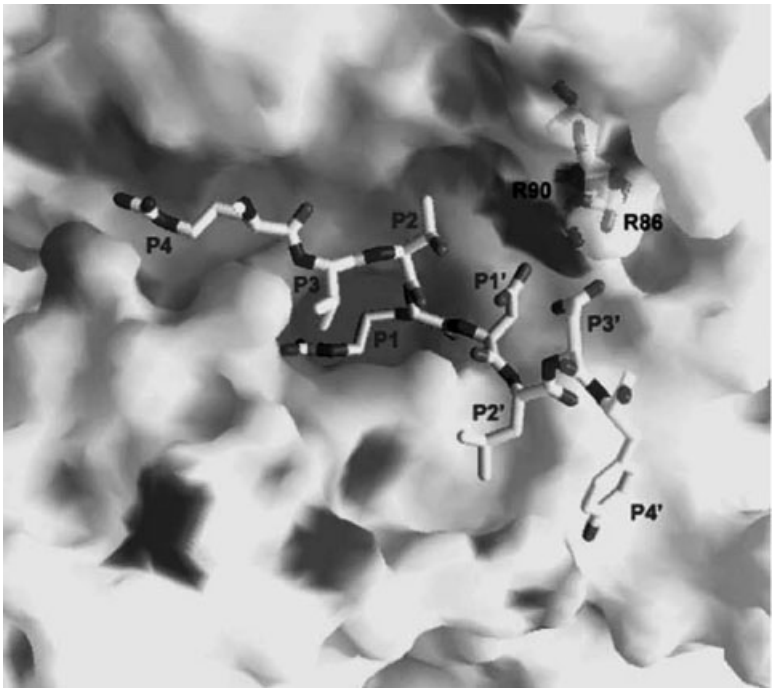

Figure 7. Model of three-dimensional complex structures of Furin and its inhibitor the eglin C mutant interaction. The mutation of R48D in eglin C seems to interact with R86 and R90 of Furin.

5. α1-Anti-Trypsin Variant or α1-Anti-Trypsin Portland (α1-PDX)

The discovery of this inhibitor was initially based on the observation previously reported for a patient with a mutation in its α1-antitrypsin.[70] This patient was shown to be unable to cleave the pro-albumin at the Furin consensus site.[71] This variant of α1-antitrypsin, called α1-anti-trypsin Pittsburgh (PIT), has a replacement of the reactive-site M358 residue by R358 residue. Subsequently, the group of G. Thomas developed another variant of α1-antitrypsin, called α1-anti-trypsin Portland (α1-PDX), in which the reactive-site A-I-P-M has been replaced by R-I-P-R. This serpin was revealed to inhibit Furin with a Ki of 600 pM, three times lower than the PIT inhibitor.[72] Subsequently, kinetic analysis showed that a portion of bound α1-PDX operates as a suicide inhibitor (Fig. 8). Once bound to Furin's active site, α1-PDX can either undergo proteolysis by Furin or form a kinetically trapped SDS-stable complex with the enzyme.[73] Furthermore, when expressed in cells, α1-PDX was shown to be a potent inhibitor of Furin-mediated cleavage of HIV gp 160,[72] and subsequently demonstrated to inhibit all PCs involved in processing within the constitutive secretory pathway. *In vitro* experiments revealed also its ability to block the processing of various proteins related tumor progression and metastasis such as several growth factors (Fig. 2), receptors, various MMPs and adhesion molecules.[1–6,13,15,16]

6. Mini-PDX Peptides

These synthetic peptides were designed and developed from the reactive site loop of the PC inhibitor α1-PDX in a way to contain the PC cleavage motif R-I-P-R382.[74] To make a circular peptide a Cys residue was inserted at each terminal residue of several mini-PDX peptides. *In vitro* digestion analysis in the presence of various synthetic PC substrates revealed that the mini-PDX is able to inhibit *in vitro* Furin activity in a slow tight-binding manner. Contrary to the PCs inhibitor α1-PDX, these synthetic

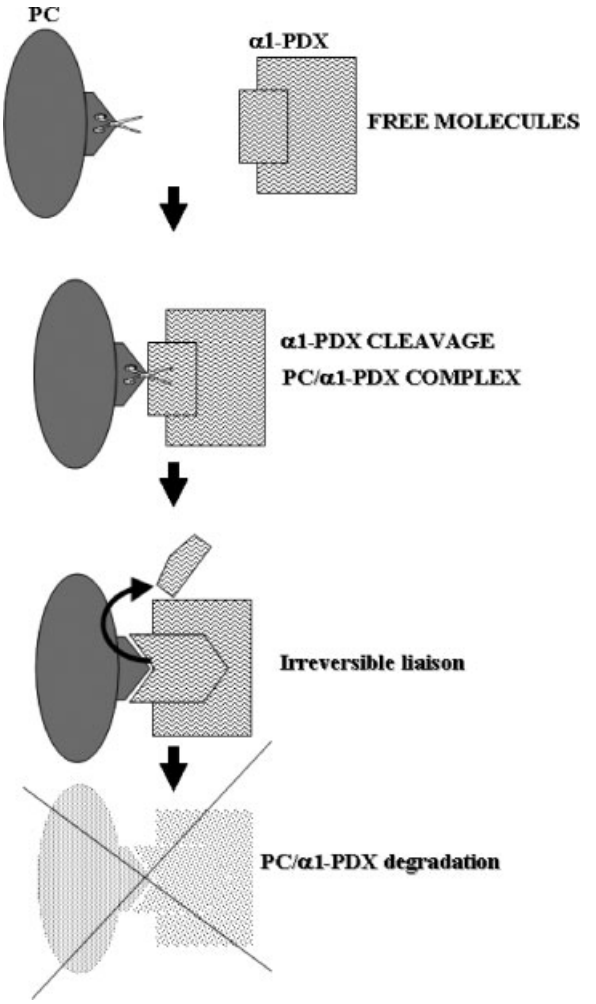

Figure 8. Inhibition of PCs by (1-PDX. The interaction between α1-PDX and PC resulted in the formation of complexes where the α1-PDX seems to act as a suicide substrate.

peptides seem to inhibit Furin via a different mechanistic pathway that required further investigations.[74]

7. α2-Macroglobulin-Furin

Human α2-macroglobulin is a homotetrameric glycoprotein present at high concentrations in the blood. Each monomeric subunit contains an internal S-ester (ISE). α2-macroglobulin inhibits a wide range of proteases by a unique mechanism.[75] Inhibition is initiated by cleavage of a flexible and surface-accessible peptide stretch called the bait region (Fig. 9). This cleavage triggers the hydrolysis of the ISEs, followed by a major conformational change. The protease becomes ''trapped'' by the inhibitor and is thus sterically shielded from its substrate.[75] By introducing a Furin recognition sequence in the bait region of α2-macroglobulin, Van Rompaey et al., have generated a potent PC inhibitor as revealed by its ability to inhibit the processing of von Willebrand factor, TGF-β1, and the HIV-1 glycoprotein gp160.[76] Lately, it was revealed that the mutation introduced in the bait region of α2-macroglobulin did not interfere neither with folding, neither on tetramerization of the inhibitor.

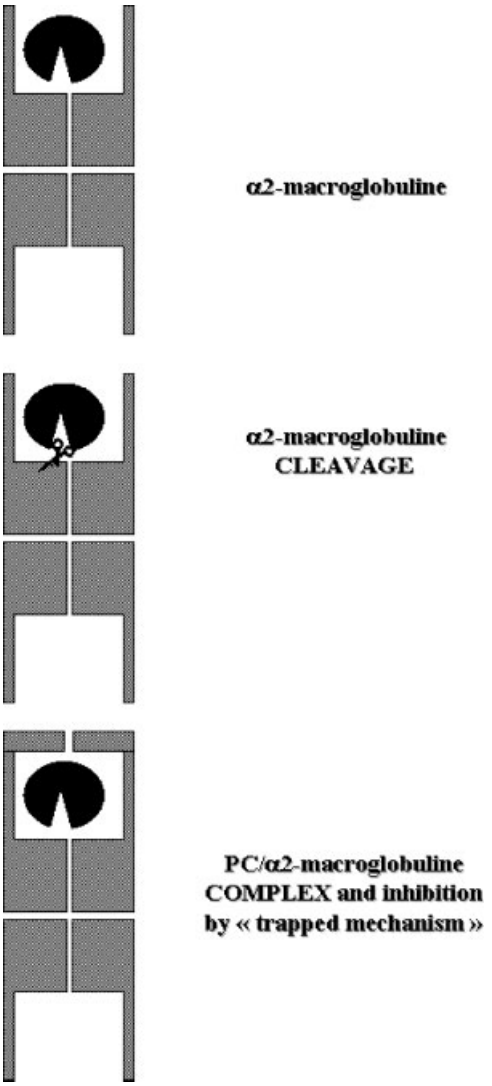

Figure 9. Inhibition of PCs by α2-macroglobulin-Furin. The introduction of Furin recognition sequence in the bait region of α2-macroglobulin generates a PC inhibitor. Like the inhibition of the other enzymes by the α2-macroglobulin, the α2-macroglobulin mutant use a trap mechanism to capture and inhibit the Furin.

Also the Furin inhibition mechanism by this α2-macroglobulin mutant was found to be similar to those used for the inhibition of other proteases by α2-macroglobulin[76] (Fig. 9).

8. Diterpines of the Labdane Family

Diterpines are the first reported nonprotein inhibitor of Furin.[77] These neoandrographolide, are extracted from the medicinally active plant *Andrographis paniculata*, and are succinoyl ester derivatives[77] (Fig. 10). The actual mechanism by which these diterpines exert their inhibitory effects against PC is not clearly understood. Nevertheless, these molecules contain a very reactive five-membered lactone ring that was found in several elastases inhibitors, suggesting the potential role of this lactone ring in the Furin activity inhibition. Although the *in vitro* inhibition is relatively weak,

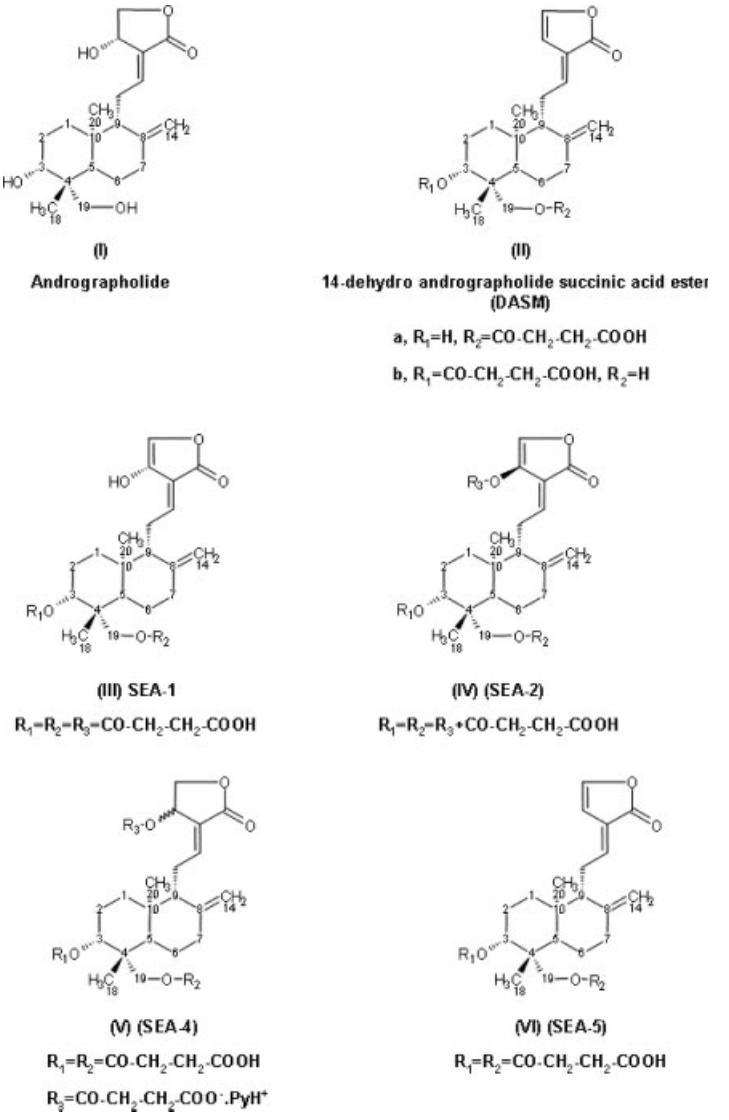

Figure 10. Chemical structures of andrographolide, dehydroandrographolide succinic acid monoester (DASM), and various succinoyl ester of andrographolide (SEA) fractions.

these compounds seem to penetrate more easily in the cell and might enhance their inhibitory potential *in vivo*.

9. Copper and Zinc Chelate Compounds

These compounds were recently shown to have an interesting degree of convertases selectivity.[78] This new class of nonpeptide inhibitor consists of an ion Cu^{2+} or Zn^{2+} coupled with a chelator compound (Fig. 11). The inhibition of the Furin by these compounds is irreversible and the inhibitor binds at the enzyme active site of the enzyme. Indeed, analysis of Furin sequence revealed the presence in its active site residues being able to bind divalent zinc and copper. This includes the catalytic H194, C198, and H364.[78]

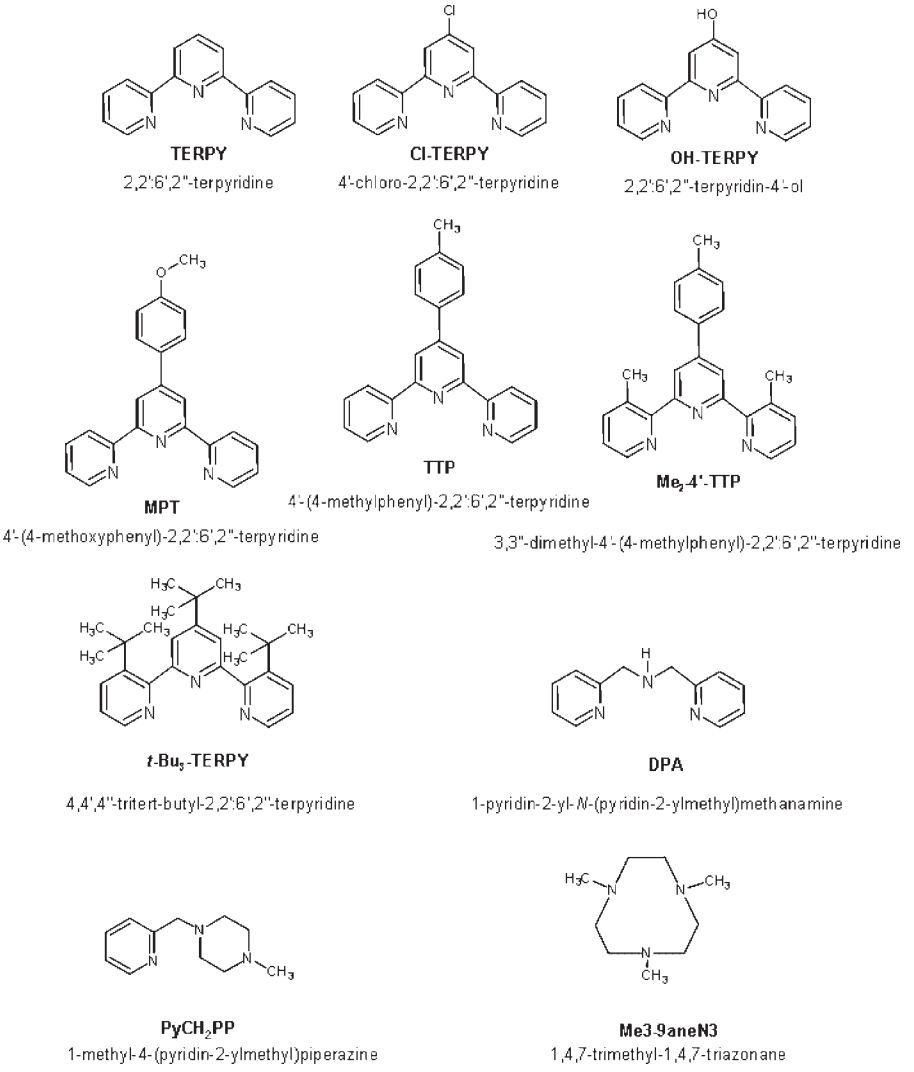

Figure 11. Structures of various chelators used for Furin inhibition.

4. CONCLUSIONS

Over the last 15 years, the cumulative knowledge revealed the implication of the proprotein convertases in various disorders including diabetes, atherosclerosis, cancer, familial hypercholesterolemia, viral infections, and Alzheimer disease. Thereby, the use of general PC inhibitors is now suggested to be advantageous and could be a promising therapeutic strategy. However, in some cases it may be necessary to target only one member of the PC family. This is feasible, as was demonstrated previously for pro-SAAS and 7B2. In addition the recently published crystal structures of the Furin will undoubtedly help for the search, design, and the development of specific and potent inhibitor for each PC. Indeed, the availability of the crystal structures of the Furin has recently revealed precious knowledge on the characteristics of the other PCs through modellation of their structures.[60] Based on these studies, the arrangement of the catalytic and P domains, and the architecture of the substrate binding clefts of the Furin seems to be more similar to those of PC4, PACE4, and PC5/6, and less

similar to those of PC1/3, PC2, and PC7.[60] Following their development these specific inhibitor could be used alone or in combination to target PC-mediated diseases. Recent studies revealed that small molecule proprotein convertases inhibitors are the most attractive potential therapeutic agents. However, only the diterpene and several Cu and Zn chelators where reported as nonpeptide PC inhibitors. Although these inhibitors were able to inhibit the activity of the PCs *in vitro*, their ability to block the processing of various PC substrates *in vivo* is not yet tested. Similarly, to improve the specificity and the efficacy of such inhibitors, chemical modifications of their structures followed by structure-activity studies are required. In the long term, the potential developed specific and potent PC inhibitors may provide a rationale for testing this family of compounds as therapeutic agents or in conjunction with standard therapy in clinical settings.

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