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Cutting back on pro-protein convertases: the latest approaches to pharmacological inhibition

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The secretory pathway in cells possesses an elaborate set of endoproteolytic enzymes that carry out a crucial step in protein precursor maturation. This step is proteolytic activation by cleavage at specific pairs of basic residues. These enzymes, named pro-protein convertases (PCs), are responsible for generating bioactive peptides and activating several enzymes and growth factors that are implicated in many important physiological events. PCs have roles in several pathologies including viral infections and cancers and, thus, are promising targets for therapeutic applications. Recent structural and homology-modeling studies demonstrate more similarity than expected at the catalytic site of the seven PCs, which makes the development of selective drugs to target individual PCs frustrating. Based on this information, we review the latest strategies to inhibit PCs, which might lead to the development of specific compounds.

Physiological functions of pro-protein convertases
The biology of pro-protein convertases (PCs) is fundamental to the cell infrastructure and to the proper coordination of the entire mammalian physiology. Through their endoproteolytic actions on inactive precursor proteins in the secretory pathway, PCs generate essential bioactive peptides, including hormones and neuropeptides (e.g. adrenocorticotropic hormone and insulin), that have vital roles in homeostatic balance and the regulation of life functions. They also activate growth factors and differentiation factors, proteins in the extracellular matrix and plasma, enzymes, receptors, viral-coat proteins and bacterial toxins. The PC family of enzymes is encoded by seven genes (see Box 1 and Table 1 for nomenclature), of which the archetype furin is the most studied (reviewed in [1,2]). Mammalian PCs are related to the yeast kexin and bacterial subtilisins, and Figure 1 presents the structural features of PCs. PCs are Ca$^{2+}$-dependent serine proteases that were identified originally based on their ability to cleave precursor proteins at the peptide bond C-terminal to paired basic residues, such as K-R$_1$ and R-R$_1$. However, the cleavage-recognition motifs are now known to extend N-terminal to the cleavage site and often include additional arginine residues (e.g. R-x-[K/R]-R$_1$).

During the past few years, several important pathologies have been linked with PC-like activity in the cell-secretory pathway and at the cell membrane. The aim is to develop inhibitors that target individual PCs because not every PC is implicated in a given disease state. In this article, we review the involvement of PCs in various pathologies, which justifies their targeting for therapeutic applications. Although increasing evidence of similarity between the various PCs raises the issue of whether specific compounds that target individual PCs can be developed, five major approaches that are described in this article appear to make this possible.

PCs as pharmacological targets
There is increasing interest in PCs as novel targets for drug design because they are proteolytically selective enzymes, the tissue and cellular localization patterns of which have been studied extensively [3]. In addition, they have well-defined cellular tasks and are crucial for the initiation and progression of many important diseases, most prominently in several viral infections and cancers. For example, furin and PC7 are required for the activation of HIV-1 viral coat protein glycoprotein 160 (gp160) to gp120 and gp41 to produce virions that are effective in receptor binding and membrane fusion during infection [4]. A similar PC-mediated processing requirement for viral spread and cytopathicity also occurs with the Epstein-Barr virus gB glycoprotein [5], the hepatitis B virus e protein, and the herpes simplex virus glycoprotein B glycoprotein [6]. There is a growing interest in the development of specific compounds to target individual PCs. The corresponding genes that encode these PCs are designated officially as PCSK type 1–PCSK type 7. Table 1 presents the names attributed to each PC and the corresponding official nomenclature.
antigen [6] and the severe acute respiratory syndrome coronavirus spike glycoprotein [7]. These roles make drugs that target PCs potential antiviral agents. In the area of cancer, ongoing work is clarifying the pivotal role of PCs in growth regulation and tumor progression by activating cancer-promoting factors such as stromelysin-3 [8], vascular endothelial growth factor c (VEGF-C) [9], insulin-like growth factor 1 receptor (IGFr1) [10,11], transforming growth factor β (TGF-β) [12] and membrane type 1 matrix metalloproteinase (MMP-1) [13]. In addition, PC-inhibition studies are beginning to provide direct evidence of their roles in tumorigenesis and invasiveness [13–15].

Evidence of the diverse roles of PCs in other pathologies is also accumulating. Bacterial pathogens such as Pseudomonas aeruginosa exotoxin A and the protective antigen of Bacillus anthracis tripartite toxin require furin-mediated cleavage for cytotoxicity, which can be reduced and blocked by PC inhibitors [16,17]. PCs have a role in neurodegenerative and aging maladies such as Alzheimer’s disease through the activation of pro-β site amyloid-precursor-protein-cleaving enzyme (BACE1) [18] and its novel binding partner BRI3 [19], and in arthritis through the processing of aggrecanase-1 [20] and tumor necrosis factor α-converting enzyme (TACE) [21]. Coordinated interplay between PC2 and PC1/3 has been uncovered recently in the production of peptides derived from cocaine and amphetamine-regulated transcript precursor (proCART) [22], which are believed to potentiate anorexia.

Thus, we conclude that the potential of PC inhibitors as novel therapeutic agents is considerable and warrants further analysis. To date, no PC-oriented therapies are available. To achieve this goal, new insights are needed into the molecular determinants that are responsible for the proteolytic activities of individual PCs.

**The rationale for the development of PC inhibitors**

Strategically, the mainstream of PC inhibitor development is to target their catalytic site. This is based on the fact that PCs are highly selective proteases that cleave an uncommon sequence motif in proteins (e.g. R-x-K-R). However, the most potent inhibitors known to date, which target the catalytic site, are not significantly specific for a single PC. Comparing inhibitors of PCs shows that many

| Gene designation (human) | Enzyme | Alternative enzyme names |
|--------------------------|--------|--------------------------|
| PCSK3                    | Furin  | PACE, PC1, SPC1          |
| PCSK2                    | PC2    | SPC2                     |
| PCSK1                    | PC1/3  | PC1, PC3, SPC3           |
| PCSK6                    | PACE4  | PACE4, SPC4              |
| PCSK4                    | PC4    | SPC5                     |
| PCSK5                    | PC5/6  | PC5, PC6, SPC6           |
| PCSK7                    | PC7    | SPC8, LPC, SPC7          |

Where: LPC, lymphoma pro-protein convertase; PACE, paired basic amino acids-converting enzyme; PCSK, pro-protein convertase subtilisin/kexin type; SPC, subtilisin-like (or subtilase-like) pro-protein convertase.

*(Abbreviations: LPC, lymphoma pro-protein convertase; PACE, paired basic amino acids-converting enzyme; PCSK, pro-protein convertase subtilisin/kexin type; SPC, subtilisin-like (or subtilase-like) pro-protein convertase.)*

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**Figure 1.** PC architecture. The PCs have a highly homologous architecture that is composed of: (i) an N-terminal signal peptide, which is responsible for directing proteins into the secretory pathway; (ii) a pro-domain, which acts as a putative intramolecular chaperone for facilitated transportation, folding and regulation of enzymatic activity; (iii) a catalytic domain, which contains an active site that is responsible for substrate-specific interactions and cleavage; (iv) a P domain, which is an independent folding moiety with the conformation of a barrel-like roll that is essential for structural cohesion of the enzyme and activity by regulating stability, and Ca²⁺- and pH-dependence; and (v) a divergent C-terminal domain that can contain membrane attachment sequences, Cys-rich regions and intracellular sorting signals. Additionally, furin contains two functional groups for internal ionic interactions (Ca²⁺-binding sites), one of which is beneath the active site and provides stability. D (aspartate), H (histidine) and S (serine) are part of the catalytic triad. N (asparagine) is located in the oxyanion hole and stabilizes, through its NH₂ group, the negative charge that is developed in the peptide bond as a result of the nucleophilic attack from the catalytic serine. At least two isoforms of PC5/6 (A and B) have been identified, both of which are encoded by the same gene.
have high potency but are not specific for one PC (Figure 2). In addition, PCs are often coexpressed within cells, so that all cells express a 'cocktail' of at least two PCs. Thus, poorly selective PC inhibitors will affect multiple cellular functions, and not only the pathological processes that are targeted.

The first approaches to developing specific PC inhibitors were based on our understanding of the molecular determinants of selectivity of cleavage by PCs. The cleavage sites of numerous furin substrates were aligned to assess these molecular determinants [23]. However, subsequent cleavage studies show that many substrates are also processed by other PCs. This has been emphasized recently by studies in transgenic inducible-knockout mice, which lack furin in the liver [24]. Known substrates of furin in the liver (e.g. albumin, α5 integrin, lipoprotein-receptor-related protein, vitronectin, α1-microglobulin and bikunin) are either processed or partially processed in these mice, which indicates redundancy with other PCs in the liver. These data also underline the potential undesirable effects of nonspecific PC inhibitors (that inhibit several PCs), which would block the processing of more proteins than desired if applied therapeutically.

Uniquely specific PC inhibitors have the potential advantage that they can be used in a combination therapy approach, should simultaneous blocking of two PCs be required.

Currently, the development of PC inhibitors is based on: (i) newly acquired structural and modeling information about PCs; (ii) peptides that are optimized by combinatorial methods; (iii) engineering polypeptide protease inhibitors that have not co-evolved with PCs but are used as stable scaffolds on which to graft the appropriate modifications to specifically target PCs; (iv) endogenous, co-evolutionary inhibitors; and (v) small non-peptidyl compounds and derivatives. Each of these approaches is discussed below.

The structural and molecular modeling approach

Understanding of the molecular basis of pro-protein processing by PCs and the design of specific inhibitors have both benefited from the recent elucidation of the three-dimensional conformation of the kinetically trapped furin [Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) entry 1P8J, molecule A] [25] and of the yeast homolog kexin (PDB entry 1ot5, chain A) [26]. The crystal structure of
furin provides crucial information on the spatial arrangement of the two domains that are implicated in PC activity: the catalytic domain, which contains the active site that is responsible for substrate-specific recognition and processing; and the P domain, which is responsible for enzyme stability (Figure 3a). The crystal structure of furin in a covalently bound complex with the suicide inhibitor decanoyl-RVKR-chloromethylketone (dec-RVKR-cmk) [25] has led to the identification of the reactive amino acids that make up the S1–S5 subsite pockets. This structural information permits modeling and docking analysis [27,28], which has led to the extrapolation of further subsites, including the S5, S6 and S1′ subsite pockets (Figure 3b, Table 2). Apart from S3 and S1′, all subsite pockets of the active site of furin contain several protophilic carboxylates and carboxamides, which explains the stringent preference for either arginyl or lysyl functionality groups in substrates and inhibitors (Table 2).

Based on homology modeling, the catalytic domains of the seven PCs are highly homologous (50–70%) [28] and the level of identity is even greater for the residues of the catalytic-cleft subsites (Table 2). For example, for all PCs, the S1 subsite that interacts with the P1 arginine residue of a substrate is composed of two acidic aspartic acid residues (Table 2) and three residues of the catalytic triad (aspartic acid, histidine and serine). In addition, nine additional amino acids that provide the steric environment to the P1 lateral chain are identical in all PCs, as illustrated by the comparison of furin and PC7 (Figure 3c,d).

The subsite identities go beyond the S1 subsite, and are also found from S2 to S5 (Table 2). However, differences

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**Figure 3.** Structure aspects of PCs. (a) Ribbon representation of the furin crystal structure complexed with the permanent inhibitor dec-RVKR-cmk showing the three-dimensional arrangement of the catalytic and P domains, which are essential for PC activity. Dec-RVKR-cmk is shown in ball and stick rendering where green is carbon, blue is nitrogen and red is oxygen. The catalytic triad (Asp153, His194 and Ser368) is shown in yellow. α-Helical regions are shown in red, β-sheets are shown in blue, and the two Ca2⁺-binding sites are shown as purple spheres. The image is rendered using IMol 0.3 (http://www.pirx.com/iMol/). (b) Representation of the electrostatic surface potential of the active site of furin with a modeled sequence of six residues from the C-terminal of the furin pro-domain (KRRTKR). White annotations indicate the inhibitor residues and black annotations show the catalytic triad and the subsite residues (S1–S6). (c,d) Representation of the S1 subsite with a P1 arginine residue for furin (c) and PC7 (d). The amino acids of the S1 subsite (furin: Asp258 and Asp306; and PC7: Asp292 and Asp340) and those in close proximity (within 3.2 Å) are shown in a ball-and-stick representation, whereas the P1 arginine is rendered as both ball and stick and a mesh surface. This comparison demonstrates the close identity of the S1 subsites, with the exception of Thr309 for furin and Ala343 for PC7. Similar analysis of the S1 subsite of all other PCs reveals perfect identity with furin (not shown).
increase away from the catalytic triad, at the S₆ subsite pocket and beyond. These observations lower expectations for the possible development of short peptide inhibitors of PCs (four–five residues) that are specific for individual PCs. However, the differences observed at the S₆ subsite indicate that specific, short-peptide inhibitors require at least six residues.

Beyond the S₆ subsite, further differences might be exploited to design specific inhibitors. As yet, these differences are not well identified but some attempts have been made. For example, a recent study demonstrates that exploiting the catalytic-site proximity of so-called ‘adventitious contacts’ can lead to more-specific inhibitors [29]. The mapping of proximal sites is also aided by solution-structure studies of the PC1/3 pro-domain [30] and subsequent docking analysis with PCs. Unlike dec-RVKR-cmk, which maps only four subsite positions [28], pro-domain-docking analysis demonstrates distinctions between furin and the other PCs at the S₅ subsite and S₆ subsite pockets (Table 2). Further structural studies of the crystallization of furin and other PCs complexed with different inhibitors are needed to provide a better understanding of the catalytic site of PCs.

The combinatorial approach

The inhibitory properties of a small-peptide inhibitor can be optimized in vitro using positional scanning of synthetic peptide combinatorial libraries (PS-SPCLs) [31,32]. An optimal sequence, determined by screening PS-SPCLs can then be embedded into polypeptides to achieve higher specificity and potency. Such combinatorial libraries of degenerate mixtures of small-peptide inhibitors have been used successfully to compile the most potent sequence for inhibiting furin (nona-arginine) [33], and to identify a previously unknown sequence, LLRVKR, which potently inhibits PC1/3 [31,34]. This latter sequence was identified in a novel, endogenous protein precursor, proSAAS, that has a tussles distribution pattern that resembles closely that of PC1/3 [35,36]. The development of nona-arginine and LLRVKR as PC inhibitors demonstrates the strength of PS-SPCLs in identifying potent inhibitory peptide sequences.

In the case of the polyarginine sequence, peptides ranging from four to nine residues are potent inhibitors of furin in both enantiomeric configurations [33]. The most potent polyarginine peptide is D-nona-arginine (Kᵢ = 1 nM) [27]. Interestingly, all L- and D-polyarginine peptides are also potent inhibitors of PC5/6 and PC7 (M. Fugère et al., unpublished), which highlights the difficulty in achieving substantial specificity using the active site exclusively. From a therapeutic perspective, D-polyarginines are interesting as lead compounds because they cannot be cleaved by PCs and are thus more stable. D-Peptide therapeutics are also less prone to other proteolytic activities such as carboxypeptidases. D-hexa-arginine blocks the activation of P. aeruginosa exotoxin A [16] and D-nona-arginine partially protects macrophages and mice against anthrax toxemia [17,27]. L-Hexa-arginine is an efficient inhibitor of the processing of gp160 (envelope) of the REKR551 sequence in vitro and ex vivo, and effectively suppresses HIV-1 infection of T cells and macrophages [4]. Both furin and PC7, the two principal PCs that are expressed by HIV-host cells, are the best candidates for processing the precursor of HIV-1 gp160 [37]. If polyarginine peptides do not exhibit undesirable interactions with other biological processes they might become key components for a combined therapy that targets furin, PC5/6 and PC7 simultaneously. Conversely, introducing these polyarginine peptide sequences into engineered polypeptides might achieve greater specificity while retaining high potency.

The protein-bioengineering approach

Astute bioengineering of protease inhibitors that did not co-evolve with PCs has also led to the development of potent PC inhibitors. The first successful example is α₁-antitrypsin, which has been transformed into a potent furin inhibitor by the insertion of a furin-like recognition sequence (Arg355-Ile-Pro-Arg358) into its reactive-site loop. This bioengineered protein, named antitrypsin-1-antitrypsin (α₁-AT-PDX), inhibits furin and PC5/6 without altering its serpin-like mechanism of inhibition (reviewed in [32]). The potential therapeutic value of α₁-AT-PDX is demonstrated by its ability, following exogenous application, to prevent processing of HIV type 1 gp160 and maturation ex vivo of pro-gB, the precursor of human cytomegalovirus envelope glycoprotein of β-herpesviridae. More recently, α₁-AT-PDX has been tested in cancer-related applications. Whereas overexpression of furin and other PCs is associated with a significant increase in metastatic spreading potential and tumor-cell proliferation [8,38], α₁-AT-PDX decreases the invasiveness of head and neck squamous-cell carcinoma by 50% in vitro, and by up to 80% when inoculated in tracheal xenotransplants [15]. Expression of α₁-AT-PDX from a DNA vector in transfectant tumor xenotransplants of astrocytoma cells also suppresses efficiently tumorigenicity and invasiveness [13]. Although α₁-AT-PDX represents a strong

Table 2. Important PC subsite residues

| PC   | S₁    | S₂    | S₃    | S₄    | S₅    | S₆    | Triad |
|------|-------|-------|-------|-------|-------|-------|-------|
| Furin| E230, D233 | E257, D264 | E236, D264 | L227, T254 | D154, D191 | D258, D306 | D163, H194, S368 | R193, R197, H364 |
| PC2  | P245, D249 | T273, D280 | E252, D280 | L242, W270 | D169, F206 | D274, D321 | D168, H209, S385 | S208, R212, H381 |
| PC1/3| D244, D247 | N271, E278 | E250, E278 | L241, W268 | D168, E205 | D272, D320 | D167, H208, S382 | K207, R211, H378 |
| PAC4 | D283, D286 | D310, D317 | E289, D317 | L280, W307 | D207, D244 | D311, D359 | D206, H247, S421 | K256, R250, H417 |
| PC7  | P264, D267 | D291, D298 | E270, D298 | L261, W288 | D188, G225 | D292, D340 | D187, H228, S406 | H227, R231, H402 |

*Numbering of the amino acids starts at the first amino acid (methionine) of the signal peptide sequence. Triad residues and S₁−S₄ subsite residues are extrapolated by sequence-alignment analysis and confirmed by homology modeling. Residues in bold and underlined contrast with the active site of furin. Refer to the footnote for subsite nomenclature.
case for targeting PCs in pathophysiological processes, the large size of this protein (45 kDa) limits its practical application. Nonetheless, z1-AT-PDX might be used effectively to block processing events that occur at the outer surface of the cell membrane.

A second example of a successful bioengineering approach has been achieved using eglin c, a potent inhibitor of elastase, cathepsin G and degradative subtilisins. Initially, arginine residues were inserted into the reactive site loop of eglin c to target furin and kexin [39]. Then, randomization of strategic eglin c residues that make contact with PC residues outside the catalytic cleft, namely ‘adventitious contacts’, proved extremely efficient in designing two variants of eglin c that target either furin or PC7 with significant specificity [29]. These two variants, Val33-Arg42-Arg45-Asp49-eglin c (K1 = 270 pM for furin and 11 nM for PC7) and Arg42-Arg45-Trp49-eglin c (K1 = 10 nM for furin and 470 pM for PC7) demonstrate that optimizing flanking contacts by in vitro molecular evolution provides a powerful technique to generate specific inhibitors for each PC. These two derivatives of eglin c show that differences outside but near the active site of PCs favor both binding affinity and specificity of the inhibitor. These eglin c variants might serve as biotechnological tools to dissect the functions of furin and PC7 in the cell and at the membrane, for co-crystallization processes, and as lead compounds to design potent, specific and smaller therapeutic drugs.

The endogenous approach

Endogenous molecules that inhibit PCs in vivo represent a major lead for designing pharmacological inhibitors of PCs. Although some are large polypeptides, core inhibitory domains can be identified and used as lead compounds. Examples of endogenous inhibitors are PC pro-domains, the neuroendocrine 7B2 protein, the pro-SAAS precursor and the proteinase inhibitor 8 (reviewed in [32]). Each contains a PC-recognition sequence and they act as substrate analogs, real substrates or ‘false baits’ for PCs. Recently, the inhibitory effects on PCs of two new candidate endogenous inhibitors, the cystatin-related epididymal spermatogenic (CRES) protein [40] and the serine-protease inhibitor 4 SPN4A/4.1 [41–43], have been described.

PC pro-domains are autoprocessed in cis by their cognate PC, but remain bound to the active site through their C-terminal PC-recognition sequence until the complex reaches the compartment of zymogen activation. Thus, pro-domains are dual-function molecules, being the first substrate and first inhibitor encountered by PCs in cells. Evaluation in vitro of the inhibitory capacity of the seven complete pro-domains and of derived, short C-terminal dodecapeptides demonstrates their nanomolar potency and some specificity [44]. However, none is a specific inhibitor of a unique PC. The lack of pro-domain specificity is also observed in inhibition experiments in cell culture. Recently, overexpression in trans in Chinese hamster ovary (CHO) cells of the wild-type PC5/6 pro-domain and a selection of key residue mutants efficiently inhibited the processing of an overexpressed substrate, proVEGF-C [45]. However, maturation of proVEGF-C is also decreased significantly by expression of the pro-domains of furin, PACE4 and PC7 in CHO cells. To date, there is no indication that specificity of inhibition can be achieved without significant structural modification of pro-domains. Nevertheless, because pro-domains have multiple interactions with PCs outside the catalytic site, bioengineering might result in more specific pro-domains. Co-crystallization of a pro-domain–PC complex might provide the information required to map these interactions.

Another endogenous candidate is CRES, a protein that has divergent functions. CRES is related to the family 2 cystatins, a family of cysteine-protease inhibitors of the larger cystatin superfamily. The inability of CRES to inhibit C1-type cysteine proteases and the presence of two accessible paired Lys-Lys basic-residue sites has prompted the assumption that CRES might inhibit PC2. Assays in vitro demonstrate that CRES inhibits PC2 but not furin and PC1/3 [40]. Inhibition of PC2 occurs at low nanomolar concentrations and is competitive, which indicates interaction with the catalytic site.

Last, the Spn4 gene in Drosophila melanogaster encodes the serpin SPN4A/4.1. SPN4A/4.1 has a consensus serpin sequence for the hinge region of the reactive-site loop (RSL), which indicates that it functions as a serpin, and includes a natural PC-recognition sequence (ArgP4-Arg-Lys-ArgP1) in its RSL. In vitro, SPN4A/4.1 inactivates human furin (K1 = 13 pM) and PC2 (K1 = 0.92 nM) by a slow-binding mechanism and the formation of a SDS-stable acyl–enzyme complex [41]. This kinetic trapping is typical of the serpin clade. That SPN4A/4.1 exerts potent inhibition on both furin and PC2 indicates a profound lack of selectivity between PCs in general. However, the removal of one amino acid in the RSL (AlaP6) generates a reduction of inhibition potency against PC2 (i.e. ID50 is 27-fold greater) but has no effect on furin. This change in specificity indicates that the RSL of SPN4A/4.1 is more amendable to modifications than other serpins such as z1-AT-PDX.

The chemical approach

Small non-peptidyl compounds represent a poorly explored area of PC inhibition. Because the activity of PCs is affected by metal ions, the first inhibitory chemical compounds investigated were 4′-(p-tolyl)-2,2′;6′,2″-terpyridine (TTP)-chelated metal salts such as Zn(TTP)Cl2 and Cu(TTP)Cl2. These bind slowly and competitively, and are irreversible inhibitors with low micromolar inhibition constants (IC50 = 5–10 μM), possibly by a chelate-coordinated interaction of the metal with the catalytic histidine of PCs [46] and Kex2 [47]. Although the free chelate alone is not inhibitory, the chelated metal salts are more potent than the solvated metal ions. Thus, the chelation of metal-based inhibitors to generate a prodrug serves to coordinate the delivery of the drug (either Zn2+ or Cu2+) to the active site of PCs. Modification and adjustment of the chelate might lead to greater inhibitory affinity and specificity for PCs. Also, as suggested by the authors, these metallo-drugs might be developed further as wide-spectrum PC blockers for rapid intervention in aggressive viral infections and/or for bacterial detoxification.
Improving the specificity of inhibition of small chemical molecules that target the active site might prove difficult given the highly conserved amino acids of PC subtypes. However, small chemical molecules, possibly obtained through library screening, might lead to the identification of compounds that can induce allosteric conformational changes that selectively affect PC activity. Presently, the only known PC allosteric sites are Ca2+-binding sites, but these appear to be unlikely sites for the design of specific PC inhibitors.

Allosteric inhibition of PCs might be a future avenue of research. It is mostly unexplored because of the difficulty in mapping allosteric sites and identifying ligands that cause conformational changes. However, screening large libraries of thiol-containing compounds has identified a regulatory site away from the active site for caspase-3 and caspase-7 that immobilizes the enzyme conformation when it forms a reversible disulfide bond with an accessible cysteine [48]. The link between this outer site and the active site is such that binding of the specific compounds blocks the interaction with substrates. We suggest that such strategies are also possible for inhibition of PCs.

Future perspectives
The design of unique, specific PC inhibitors represents a major challenge in light of the homology between the active sites of each family member. PC inhibitors have the potential to become important therapeutic agents, particularly in viral diseases and, possibly, in cancer. Additionally, studies to design specific PC inhibitors should: (i) identify the molecular determinants of selectivity between PCs; (ii) provide new pharmacological tools to probe normal PC functions; and (iii) define the true nature of compensating functions between PCs. The challenge is to identify small, but significant, differences away from the core active site and to exploit these through innovative and classical pharmacological drug design.

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