doi:10.2533/chimia.2022.402 Chimia 76 (2022) 402–408

Targeting Extracellular Bacterial Proteases for the Development of Novel Antivirulence Agents

Cansu Kaya and Anna K. H. Hirsch*

Abstract: As resistance to clinically available antibiotics persistently increases, applying new strategies to target pathogenic bacteria are paramount to design effective drugs. Bacterial proteases play vital roles in cell viability and stress response, contributing to the pathogenicity of the resistant bacteria. Targeting these extracellular enzymes by antivirulence therapy is a prominent strategy in combating multi-drug resistant bacteria. By preventing the colonization and infiltration of the host, this method can lower the selection pressure and reduce resistance development significantly. Here, we review the role of bacterial proteases, the rise of antivirulence therapy and we report on the development of novel antivirulence agents targeting two key virulence factors: elastase B (LasB) from *Pseudomonas aeruginosa* and collagenase H (ColH) from *Clostridium histolyticum*.

Keywords: Anti-virulence therapy · Collagenase · Elastase · Proteases

Cansu Kaya studied chemistry at Bilkent University, Ankara, Turkey, where she also obtained her MSci in Chemistry. She joined Prof. Anna Hirsch's laboratory as a PhD Student in 2017 at the Helmholtz Institute for Pharmaceutical Research Saarland, University of Saarland, where she worked on the design and synthesis of novel antivirulence agents against multi-resistant bacteria. Cansu is currently working as an Associate Publishing Editor at Springer Nature.

Prof. Anna K. H. Hirsch obtained her MSci from the University of Cambridge and her PhD from the ETH Zurich in 2008 under the supervision of Prof. F. Diederich. After a postdoc in Prof. J.-M. Lehn's group, she became assistant professor at the University of Groningen in 2010 and associate professor in 2015. In 2017, she became head of the department 'Drug Design and Optimization' at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and full professor at Saarland University. She focuses on target-based anti-infective drug discovery, recognized by numerous prizes such as the Innovation Prize for Medicinal Chemistry of the GdCh/DPhG.

1. Introduction: The Role of Proteases in Drug Discovery

Proteases are important signaling enzymes catalyzing the breakdown of proteins by hydrolyzing peptide bonds.[1] They control numerous key physiological processes such as cell-cycle progression, cell proliferation, cell death and DNA replication as well as processing of hormones and biologically active peptides. They are able to cleave protein substrates either from the N or C termini (aminopeptidases and carboxypeptidases, respectively) and/or in the middle of the molecule (endopeptidases).[2]

Fig. 1 illustrates the substrate binding to a protease where the structure of the active site of the protease determines the substrate specificity.[3,4]

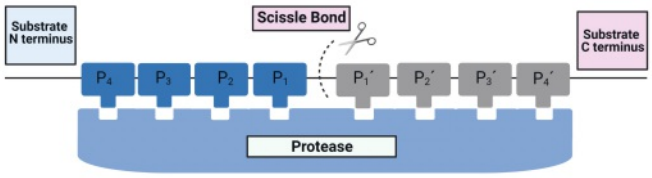

Fig. 1. Schematic representation of the substrate cleavage sites in proteases. Non-primed binding sites are located toward the N terminus, whereas the primed binding sites are toward the C terminus. The figure was adapted from Schechter and Berger and recreated by BioRender.com.[3]

A great variety of proteases exists that differ in size and structural composition.[5] Based on their mechanism of catalysis, proteases are classified into six classes; aspartic, glutamic, cysteine, serine and threonine proteases, as well as metalloproteases.[6] For cysteine, serine and threonine, catalysis involves formation of an acyl-enzyme complex followed by the release of both carboxylate and amine products.[3] In the case of aspartic, glutamic and metalloproteases, an activated water molecule acts as a nucleophile and attacks a carbonyl group to hydrolyze the peptide substrate.[7]

As a result of their crucial roles in almost all important biological pathways, proteases constitute attractive target proteins for the treatment of various diseases.[1] The common strategy for targeting proteases is to identify the active site and to design inhibitors that are able to block it.[7] Most of the proteases are sequence-specific and therefore, the designed active structures mimic the transition state of the substrate, assuring not to be processed by the enzyme.[8,9]

*Correspondence: Prof. A. K. H. Hirsch, E-mail: anna.hirsch@helmholtz-hips.de, Helmholtz Institute for Pharmaceutical Research, University of Saarland, Campus E8.1, 66123, Saarbrücken, Germany

Designing inhibitors with selectivity toward a single protease can be challenging if the targeted protease shares a similar catalytic mechanism and substrate specificity with other proteases, but is functionally completely different.[10] For the discovery of selective protease inhibitors, alternative methods like structure-based drug design or targeting allosteric binding sites are also applied.[11,12]

There are many successful protease inhibitors on the market (Fig. 2), for the treatment of various diseases such as angiotensin-converting enzyme (ACE) inhibitors (*e.g.* captopril, **1**)[11] used for hypertension, and thrombin (serine) protease inhibitors for treating blood coagulation (*e.g.* dabigatran, **2**).[13] Clinically approved protease inhibitors are also used for the treatment of diseases caused by viruses like HIV (*e.g*. ritonavir, **3**)[6] or hepatitis C.[14,15]

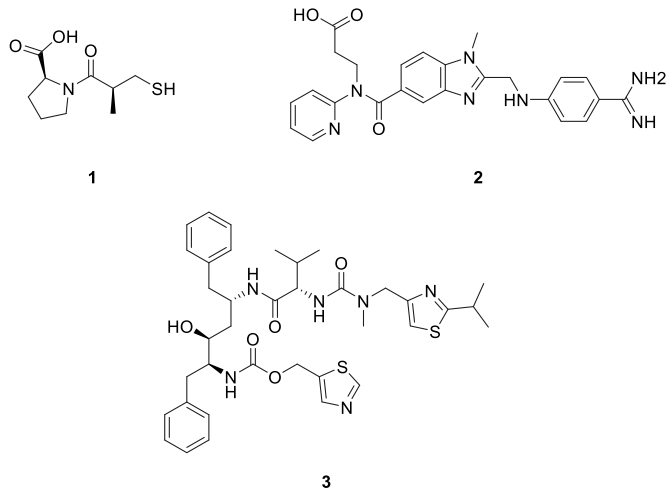

Fig. 2. Structures of the protease inhibitors in clinical use: captopril **1**,[11] dabigatran **2**,[13] and ritonavir **3**.[6]

Considering their versatile functions and the threat imposed by antibiotic-resistant bacteria, proteases are promising targets to develop new antibacterial agents.[6] Here, we focus on two exemplary ones from a representative Gram-negative and -positive pathogen, namely LasB from *Pseudomonas aeruginosa* and ColH from *Clostridium histolyticum.*

2. Antibiotic Resistance and Antivirulence Therapy

The discovery of antibiotics, starting with penicillin, has saved millions of lives during the 20th century.[16–18] The term 'antibiotic' initially referred to the natural secondary metabolites that were able to inhibit the growth of microorganisms.[17] Later, its context has been extended to synthetic and semi-synthetic antibacterial agents.[17] Antibiotics were not only successful in treating serious infections but also played pivotal roles in decreasing morbidity and increasing life expectancy.[19,20]

As early as the late 1930s, starting with resistance toward sulfonamides, antibiotic-resistant bacteria emerged as a serious threat for the treatment of bacterial infections.[16] Addition of new classes of antibiotics to the market was not successful in solving this problem as resistance has developed in all cases regardless of the chemical classes that were introduced over the years.[19,21]

Factors like patient compliance, *i.e.* stopping the treatment too early or overprescription of antibiotics can activate mutations or gene transfer among bacteria for developing resistance.[20,22] Moreover, the excessive use of antibiotics in livestock kills the susceptible bacteria causing the rise of more resistant strains.[16] These strains are transferred to humans by the food supply, leading to serious infections.[16] All these aspects contribute to the evolution of different ways to maintain or develop resistance.[23,24]

Several bacteria that are insensitive to multiple drugs have been listed as critical pathogens on the WHO priority list, as they pose a serious threat, especially for immune-compromised patients.[21,25,26] These are not only responsible for the great share of nosocomial infections but they also represent a reference for possible resistance mechanisms.[27] Among these pathogens, Gram-negative bacteria such as *P. aeruginosa* and *Acinetobacter baumanii* have been under a special focus since the permeability issues associated with the Gram-negative cell wall represent a particular challenge.[25,27,28]

In contrast to the rapid emergence of resistant strains witnessed over the past decade, the translation of antibiotics with novel mode of action into clinical practice has not been efficient.[21,29–31] The efforts for developing novel antibiotics have decreased significantly with most of the newly approved drugs being derivatives of existing classes.[19] As the current antibiotics target mainly the vital functions in bacteria, this results in a high selection pressure, facilitating more resistance.[32]

Due to all aforementioned reasons, a 'post-antibiotic era' is on the horizon, where the treatment options for many bacterial infections are highly limited.[33] To combat this issue, an innovative approach should be taken, which requires not only a strong oversight of currently developed drugs but also a consideration of alternative strategies to treat infections.

Antivirulence therapy has emerged as such an alternative strategy, which aims to disarm the bacteria by inhibiting their virulence factors.[34–36] This method, with a novel mode of action, aims to reduce and reverse the selective pressure, leading to a significant decrease in bacterial resistance (Fig. 3).[37,38]

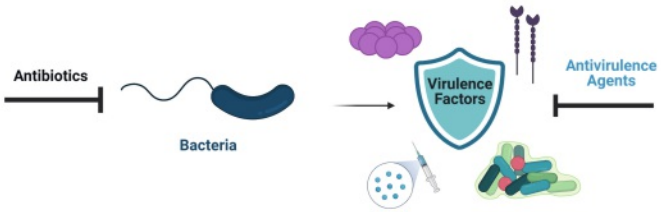

Fig. 3. Schematic representation of the mode of action of conventional antibiotics versus inhibition of virulence factors by antivirulence agents. The figure was adapted from Heras *et al.* and created with Biorender.com.[40]

Virulence factors are produced by bacteria in order to invade the host cell and to evade the host-immune system.[33] These include proteases, adhesins, regulators, toxins and siderophores.[37,39,40] The purpose of targeting virulence factors with so-called 'pathoblockers' is to reduce the pathogenicity, thereby enabling the cellular immune response of the host cell to eliminate the bacteria.[34] This concept is also successful for a vast number of other potential drug targets like key components of quorum-sensing (QS) networks.[41] Contrary to traditional antibiotics, potential antivirulence drugs can lead to a more effective use of the antibiotics and preserve the host commensal flora.[42]

Given that each bacterium has its own unique virulence mechanisms, it is likely that the developed antivirulence drugs have a narrow spectrum, which might potentially be a setback.[40] Furthermore, whether a virulence factor is beneficial for the bacteria or not plays a substantial role in the development of resistance to antivirulence drugs, *i.e.* the selection of the right target in combination with the right treatment is important for maintaining the efficacy of these drugs.[43]

The use of antivirulence drugs is in slow progress as most of them are still in pre-clinical development and only a few have made it to the clinic.[34] Some of these FDA-approved inhibitors

are targeting immunoglobulins.[34,44] One recent example reaching the market is the antibody drug bezlotoxumab used as a toxin B neutralizer in the treatment of *C. difficile* infections.[45]

The lack of clinically approved small-molecule drugs proves the urgent need for designing novel inhibitors of virulence factors secreted by pathogenic bacteria. In view of this, zinc-containing metalloproteases, present both in Gram-negative and Gram-positive organisms, have emerged for designing successful inhibitor profiles.[46]

2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium, frequently involved in chronic infections of immune-suppressed patients.[47] It can be isolated from numerous environments including soil, plants and mammalian tissue.[48] The World Health Organization (WHO) places carbapenem-resistant *P. aeruginosa* among the most critical pathogens as it is responsible for 10% of hospital-acquired infections, and has a frequent occurrence among cystic-fibrosis (CF) patients.[26,49,50] It is also responsible for urinary-tract, cornea and wound infections especially in patients with predisposing factors.[51]

Various mechanisms of resistance are reported for *P. aeruginosa*, including target mutations, disabling compound efflux and inactivation of β-lactam antibiotics by β-lactamases.[52–55] Another important factor contributing to resistance is the formation of biofilms.[38,56] Biofilms consist of different components such as extracellular proteins, polysaccharides and extracellular DNA (eDNA) that constitute an effective protection against the host-immune response.[57–59] *P. aeruginosa* uses QS mechanisms, some of which are partially responsible for the formation and maturation of these biofilms.[38,58] QS allows bacteria to communicate and coordinate, which adds up to the bacterial infection progress.[60] Formation of biofilms further favors the adherence to medical devices leading to the rise of nosocomial infections.[61] It has also been demonstrated that biofilm-like microcolonies are formed in the lungs of CF patients by *P. aeruginosa* exacerbating the effect of infection.[62,63]

P. aeruginosa produces numerous virulence factors responsible for its pathogenicity.[51] These can be distinguished by their location either as cell-associated virulence factors, *i.e.* lectins, flagella and biofilms or extracellular virulence factors such as proteases, hemolysins, cytotoxin or pyocyanin.[62] Cell-associated virulence factors play a crucial role in adhesion and colonization of the bacteria.[63] For example, lectin inhibitors hinder host-cell invasion and in particular biofilm formation.[64] As presented previously, quorum sensing is a way for bacteria to manipulate the host immune response by regulation of virulence factors.[65] Although targeting this network represents a challenge due to the intracellular nature of the QS cascade, recent progress on targeting its key elements, namely the las, rhl and pqs systems, yielded successful inhibitors with favorable pharmacokinetic properties for pulmonary application.[66–68]

Consequently, extracellular virulence factors represent more attractive targets, as there is no need for crossing the Gram-negative cell wall, which is a highly challenging task due to the presence of two membranes.[38] Elastases belonging to the protease family are such extracellular targets playing a pivotal role in invasion and evasion of the host immune response, leading to a faster disease progression.[69–71]

2.2 *Elastase (LasB)*

LasB is a zinc metalloprotease secreted by *P. aeruginosa*.[72] Also known as pseudolysin, LasB is encoded by the *lasB* gene and has a mature mass of 33 kDa.[73,74] The N-terminal domain of the protease consists of antiparallel β-strands, whereas the C-terminal part is predominantly α-helical. The active site is located in between these two domains (Fig. 4).[75] The overall tertiary structure of LasB is highly similar to thermolysin from *Bacillus thermoproteolyticus*, which makes it a part of the thermolysin family of enzymes.[76,77] Accordingly, conserved binding site residues are zinc-coordinating His-140, His-144, and Glu-164, as well as Glu-141, Tyr-155 and His-223.[76]

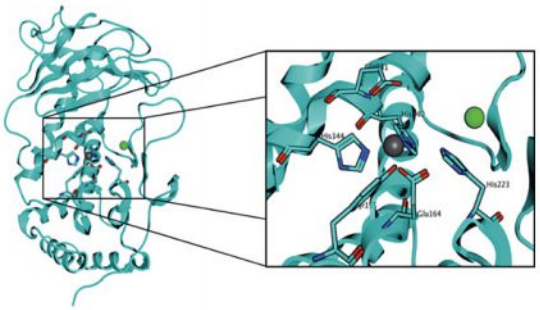

Fig. 4. Apo structure of LasB (PDB code : 1EZM) shown in standard orientation.[75]

Target-validation experiments have demonstrated LasB as the most abundant extracellular enzyme present in *P. aeruginosa* supernatant with the highest endopeptidase activity, therefore making it a critical anti-infective target.[78,79] LasB is able to degrade elastin, fibrin and collagen, which are important components of tissue cells and blood vessels, facilitating host colonization.[72] Moreover, it can degrade surfactant proteins in the lung and it is also involved in the inactivation of human immunoglobulins A and G, cytokines gamma-interferon and tumor necrosis factor alpha.[80–85]

In addition to these mechanisms of evading host immune response, LasB is also a central player in the formation of biofilms.[86,87] To date, two mechanisms have been reported for the formation of biofilms that are regulated by LasB.[86,88] The first one describes a mechanism where LasB activates nucleoside diphosphate kinase (NDK), an enzyme generating guanosine triphosphate (GTP) for the formation of alginate, which is a crucial constituent of the biofilm. The other mechanism is related to the rhamnolipid-regulated biofilm formation, which is controlled by the *lasB* gene. Once they are formed, biofilms are highly resistant to immune response and antibiotics, creating an inflammatory response, which maintains the infection.[86] Last but not least, LasB is also widely recognized as the key virulence factor in the development of chronic infections in CF patients.[89]

P. aeruginosa secretes several other proteases, which are also responsible for the infection progress. Aeruginolysin, for example, can also contribute to tissue degradation but the proteolytic capacity of this enzyme is low compared to LasB.[90,91] LasA, another zinc-metalloprotease secreted by these bacteria with an elastolytic activity, makes elastin tissue more susceptible to LasB by breaking Gly–Gly bonds.[92,93]

All the aforementioned features of LasB make it an attractive anti-infective drug target, which is attracting more and more attention nowadays.[94]

2.3 *Inhibitors of Elastase (LasB)*

As a common trend for all zinc-containing enzymes, most of the inhibitors of LasB contain metal chelators.[95] In addition to known inhibitors such as ethylenediaminetetraacetic acid (EDTA) and phenanthroline, other chemical classes such as hydroxamates and thiols have also emerged as potent inhibitors of LasB.[72,96,97]

Cathcart *et al.* demonstrated that P1' amino acid residues in the active site of LasB are responsible for recognition of inhibitors

with a preference for nonpolar and aromatic amino acids.[68] This observation led to the thiol compound **4** (Fig. 5), which was able to reduce *P. aeruginosa*-induced biofilm formation.[98]

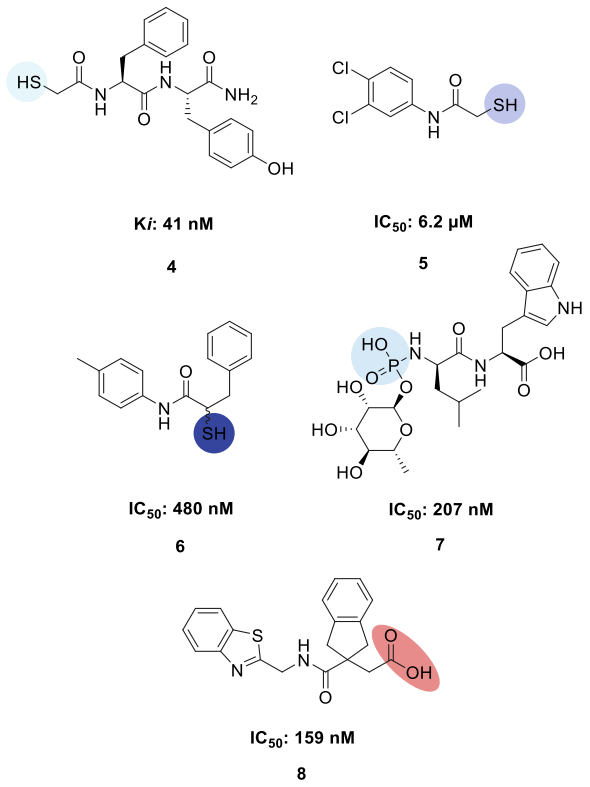

Fig. 5. Structures of selected LasB inhibitors: Peptidic mercaptoacetamide **4**,[68] *N*-aryl mercaptoacetamide **5**,[101] α-benzyl *N*-aryl mercaptoacetamide **6**,[102] phosphoramidon **7**,[103,104] and a virtual screening hit compound **8**.[105]

The first nonpeptidic inhibitors of LasB were designed as heterocyclic structures, followed by the introduction of the mercaptoacetamide class.[99,100] The discovery of the non-peptidic inhibitor compound **5** remains noteworthy as it demonstrated an *in vivo* effect in a *Galleria mellonella* infection model.[101] Substrate-inspired merging and fragment-growing strategies applied on this structure yielded compound **6** demonstrating a 12-fold increase in potency while maintaining the selectivity and *in vivo* activity, demonstrating the potential for this new scaffold.[102]

Nevertheless, nonpeptidic inhibitors continue to demonstrate low micromolar activities, whereas peptide-based compounds are active in the sub-micromolar range. A successful example for this observation is phosphoramidon (compound **7**, Fig. 5), a peptidic thermolysin inhibitor.[103,104]

Recent drug-discovery campaigns with rational approaches involving virtual screening and computer-aided drug design have yielded a series of non-peptidic LasB inhibitors like compound **8** with submicromolar activities and a good selectivity profile.[105]

The main drawback of zinc-chelating inhibitors of bacterial metalloproteases is the presence of matrix metalloproteases (MMPs) in the human cell, which are essential for important regulatory mechanisms.[106] These enzymes belong to the M10 family of peptidases and are responsible for degradation of extracellular matrix (ECM) components, regulation of apoptosis and inflammatory processes.[107–109] More than 20 different MMPs are present in humans, each classified based on their function and the depth of their S1' binding pocket.[107,110] On the other hand, dysfunction of these enzymes causes various diseases such as cancer, cardiovascular diseases and inflammation, which makes them attractive targets for treatment of these diseases.[107,108] Due to these diverse functions, MMPs are often regarded as both targets and anti-targets, creating a challenge for the design of novel, selective metalloprotease inhibitors.[111]

While designing inhibitors for extracellular metalloenzymes, screening against a series of representative MMPs is essential to assess the selectivity profile of the designed inhibitor. Mercaptoacetamides were reported to be selective over human proteases such as MMP-2 and histone deacetylases (HDACs).[100] Thiol-containing derivatives were also shown to be successful in terms of selectivity over several MMPs.[101] For hydroxamate-based inhibitors selectivity, however, remains an issue.[112,113]

3. Targeting of Clostridial Collagenases

3.1 *Introduction to Clostridia Genus*

Clostridia are rod-shaped Gram-positive bacteria that are obligate anaerobes and present in soil, waste water or human commensal flora.[114,115] Among more than 80 different species in this family, a few are known to cause severe diseases.[115] These include *C. perfrigens* and *C. histolyticum*, both causing gas gangrene, *C. tetani* causing tetanus and *C. botulinum* causing botulism.[115] The strain *C. difficile* is of particular interest as it is responsible for pseudomembranous colitis, which occurs as a consequence of antibiotic use.[42,116] It also causes severe nosocomial diarrhea.[115,117] The secretion of toxins and hydrolytic enzymes like collagenases contributes to the pathogenicity of clostridia.[118] Some of the secreted toxins are known biological warfare agents such as botulinum neurotoxin from *C. botulinum*, which is highly toxic.[119] In the meantime, collagenases are not only able to invade the host cell directly, but also cause infections by acquiring nutrients and toxin diffusion indirectly.[118]

An increasing resistance is observed among many strains of clostridia, representing a challenge for treatment of the infections caused by these pathogens.[120] As extracellular collagenases are becoming highly important antivirulence targets, the next part will focus on virulence factors from *C. histolyticum.*

3.2 *Clostridium histolyticum*

Clostridium histolyticum produces five different types of toxins.[115] Among these, β-toxins are the biggest contributors to its pathogenicity as collagenases are crucial for clostridial virulence.[115] This bacterium uses these extracellular metalloenzymes as a means to invade the host cell and acquire nutrients to evade the immune defense.[121] Collagenases can then effectively cleave the triple helix collagen into smaller oligopeptides, breaking the connective tissue.[117,122]

The two types of proteases secreted by this bacterium are encoded by *colH* or *colG* genes.[123] Collagenase H (ColH) and collagenase G (ColG) are classified as the M9 family of metalloproteases.[124] Structural studies performed on these enzymes have revealed a similar zinc-binding motif to elastase from *P. aeruginosa*.[125] A calcium binding site was also discovered close to the zinc-binding site, which proves the necessity of a calcium cation for the peptidolytic and collagenolytic activity.[126,127]

Contrary to the full collagenase unit ColG, ColH is not able to degrade collagen tissue as a result of bearing an activator and a peptidase domain.[127,128] Interestingly, the ColG unit shares a greater structural similarity to ColT from *C. tetani* and ColA from *C. perfringens* than to ColH.[128]

As collagenases play a detrimental role in the progress of bacterial infections caused by *C. histolyticum*, they are considered as essential targets for the development of alternative treatment options for antibiotic resistance.

3.3 *Inhibitors of Collagenase H (ColH)*

ColH inhibitors also require zinc-chelating groups to maintain their activity by coordination of a zinc cation.[126] Accordingly, thiols, phosphonamides and hydroxamate motifs with activities ranging from low micromolar to nanomolar are commonly reported for inhibition of ColH.[129–131] Phosphoramidon has been shown to inhibit ColH as well, but it is not as potent as for LasB.[129] Interestingly, modified natural coumarin derivatives isolated from *Viola yedonesis* are also potent inhibitors with a nanomolar activity (**9**, Fig. 6).[131]

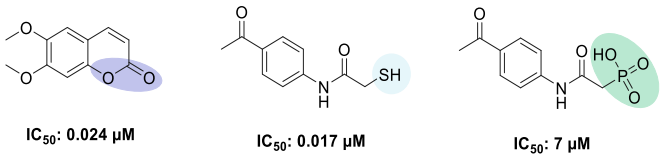

Fig. 6. Structures of selected ColH inhibitors: coumarin derivative **9**,[131] *N*-aryl mercaptoacetamide derivative **10**,[132] an alternative zinc binding motif phosphonate derivative **11**.[133]

Selectivity against human MMPs remains a challenge for ColH inhibitors as most of the existing structures are derived from the established MMP inhibitors.[134–136] Therefore, efforts for designing novel structual motifs are essential to obtain selective and potent inhibitors.

Schönauer *et al.* introduced *N*-aryl-mercaptoacetamide-based inhibitors that are quite successful in addressing the selectivity issue.[132] The inhibitor **10** (Fig. 6) with a low nanomolar activity displayed more than 1000-fold selectivity over human MMPs. Building on this exploration, we presented *N*-aryl-mercaptosuccinimide derivatives with an improved chemical stability and lower *in vivo* zebrafish cytotoxicity while demonstrating a significant reduction of collagen degradation in an *ex vivo* pig-skin model.[137]

The stability issues of thiols triggered the exploration of alternative zinc-binding motifs as inhibitors of ColH.[138] Inspired by the structure of compound **10**, phosphonate derivative compound **11** showed a low micromolar inhibition of ColH with a good selectivity profile, offering a new direction for inhibitors of this virulence factor.[132,133] Moreover, the similarity in the substitution pattern of these two structures has provided a better insight into the binding pocket of ColH, indicating a preference for polar and hydrogen-bonding substituents.[132,133,137]

4. Conclusions and Outlook

In this review, we highlighted the importance of targeting proteases in designing successful inhibitors for antivirulence therapy. We focused on two key bacterial proteases Las B and ColH secreted from the notorious pathogens *P. aeruginosa.* and *C. histolyticum*, respectively.

Many peptidic and non-peptidic drug-design inhibitors of LasB have been reported up to date, demonstrating the potential of this virulence factor. In some examples, application of different drug design strategies such as fragment merging/linking or computer-aided drug design accelerated the development of these inhibitors. Nevertheless, selectivity over human metalloproteins and stability of zinc-chelating motifs remain as great challenges that need to be tackled when aiming for inhibitors with a considerable *in vivo* activity.

ColH inhibition has gained more attention lately, as several different inhibitors with successful selectivity profiles and low micromolar activity have been reported. The discovery of inhibitors with different zinc-binding groups has shown that the efforts for designing and optimizing are not limited to certain scaffolds and there is room for improvement.

Overall, all these efforts underline the importance and the success of antivirulence therapy and various drug-design strategies in the fight against the antimicrobial resistance crisis and increasing the potential of inhibitors to move further into clinical development.

Received: April 11, 2022

[1] C. López-Otín, J. S. Bond, *J. Biol. Chem.* **2008**, *283*, 30433, https://doi.org/10.1074/jbc.R800035200.
[2] G. S. Salvesen, D. Marcin, *Nat. Rev. Drug Discov.* **2010**, *9*, 690, https://doi.org/10.1038/nrd3053.
[3] B. Turk, *Nat. Rev. Drug Discov.* **2006**, *5*, 785, https://doi.org/10.1038/nrd2092.
[4] E. Harper, A. Berger, *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1956, https://doi.org/10.1016/0006-291X(72)90076-9.
[5] J. S. Bond, *J. Biol. Chem.* **2019**, *294*, 1643, https://doi.org/10.1074/jbc.TM118.004156.
[6] E. Culp, G. D. Wright, *J. Antibiot. (Tokyo)* **2017**, *70*, 366, https://doi.org/10.1038/ja.2016.138.
[7] A. A. Agbowuro, W. M. Huston, A. B. Gamble, J. D. A. Tyndall, *Med. Res. Rev.* **2018**, *38*, 1295, https://doi.org/10.1002/med.21475.
[8] T. P. Smyth, *Bioorganic Med. Chem.* **2004**, *12*, 4081, https://doi.org/10.1016/j.bmc.2004.05.041.
[9] R. M. Raju, A. L. Goldberg, E. J. Rubin, *Nat. Rev. Drug Discov.* **2012**, *11*, 777, https://doi.org/10.1038/nrd3846.
[10] I. M. Verhamme, S. E. Leonard, R. C. Perkins, 'Proteases: Pivot points in functional proteomics', **2019**, https://doi.org/10.1007/978-1-4939-8814-3_20.
[11] G. Abbenante, D. Fairlie, *Med. Chem. (Los. Angeles)* **2006**, *1*, 71, https://doi.org/10.2174/1573406053402569.
[12] C. Jensen, P. Herold, H. R. Brunner, *Nat. Rev. Drug Discov.* **2008**, *7*, 399, https://doi.org/10.1038/nrd2550.
[13] W. G. Eisert, N. Hauel, J. Stangier, W. Wienen, A. Clemens, J. Van Ryn, *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 1885, https://doi.org/10.1161/ATVBAHA.110.203604.
[14] N. C. Marshall, B. B. Finlay, C. M. Overall, *Mol. Cell. Proteomics* **2017**, *16*, S161, https://doi.org/10.1074/mcp.O116.066456.
[15] D. Leung, G. Abbenante, D. P. Fairlie, *J. Med. Chem.* **2000**, *43*, 305, https://doi.org/10.1021/jm990412m.
[16] C. Lee Ventola, 'The Antibiotic Resistance Crisis Part 1: Causes and Threats', P T. **2015** Apr; 40(4): 277–283.
[17] S. Sengupta, M. K. Chattopadhyay, H. P. Grossart, *Front. Microbiol.* **2013**, *4*, 1, https://doi.org/10.3389/fmicb.2013.00047.
[18] A. Fleming, *Bull. World Health Organ.* **2001**, *79*, 780.
[19] D. Lyddiard, G. L. Jones, B. W. Greatrex, *FEMS Microbiol. Lett.* **2016**, *363*, 1, https://doi.org/10.1093/femsle/fnw084.
[20] D. I. Andersson, N. Q. Balaban, F. Baquero, P. Courvalin, P. Glaser, U. Gophna, R. Kishony, S. Molin, T. Tønjum, *FEMS Microbiol. Rev.* **2021**, *44*, 171, https://doi.org/10.1093/FEMSRE/FUAA001.
[21] 'The antibiotic alarm', *Nature* **2013**, *495*, 141, https://doi.org/10.1038/495141a.
[22] N. Van De Sande-Bruinsma, H. Grundmann, D. Verloo, E. Tiemersma, J. Monen, H. Goossens, M. Ferech, H. Mittermayer, S. Metz, W. Koller, E. Hendrickx, B. Markova, A. Tambic-Andrasevic, I. Francetic, S. Kalenic, D. Bagatzouni, P. Dvorak, P. Urbaskova, D. Monnet, A. Anker Nielsen, P. Naaber, P. Huovinen, P. Paakkari, O. Lyytikainen, A. Nissinen, P. Maugendre, D. Guillemot, B. Coignard, V. Jarlier, W. Kern, H. Schroeder, W. Witte, K. Heckenbach, H. Giamarellou, A. Antoniadou, A. Tsakris, A. Vatopoulos, G. Ternak, M. Fuzi, K. Kristinsson, E. Smyth, R. Cunney, D. Igoe, O. Murphy, R. Raz, G. Cornaglia, A. Pantosti, P. D'Ancona, S. Berzina, A. Balode, R. Valenteliene, J. Miciulevicience, R. Hemmer, M. Bruch, M. Borg, P. Zarb, R. Janknegt, M. Filius, H. De Neeling, E. Tiemermsa, J. Degener, H. Salvesen Blix, A. Hoiby, G. Simonsen, W. Hryniewicz, P. Grzesiowski, L. Caldeira, M. Canica, I. Codita, V. Foltan, T. Tesar, L. Langsadl, M. Cizman, M. Mueller-Premru, J. Kolman, J. Campos, F. Baquero, O. Cars, G. Skoog, B. Liljequist, G. Kahlmeter, S. Unal, D. Gür, P. Davey, A. Johnson, R. Hill, H. Hughes, M. Coyne, *Emerg. Infect. Dis.* **2008**, *14*, 1722, https://doi.org/10.3201/eid1411.070467.
[23] W. C Reygaert, *AIMS Microbiol.* **2018**, *4*, 482, https://doi.org/10.3934/microbiol.2018.3.482.
[24] L. B. Rice, *J. Infect. Dis.* **2008**, *197*, 1079, https://doi.org/10.1086/533452.

[25] '2019 Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline. Geneva: World Health Organization; **2019**. Licence: CC BY-NC-SA 3.0 IGO. Cataloguing-in-Publication', 2019.
[26] S. R. Shrivastava, P. S. Shrivastava, J. Ramasamy, *JMS - J. Med. Soc*. **2018**, *32*, 76, https://doi.org/10.4103/jms.jms_25_17.
[27] Y. X. Ma, C. Y. Wang, Y. Y. Li, J. Li, Q. Q. Wan, J. H. Chen, F. R. Tay, L. N. Niu, *Adv. Sci*. **2020**, *7*, https://doi.org/10.1002/advs.201901872.
[28] World Health Organization, WHO Publ. **2012**, 1.
[29] M. Lomazzi, M. Moore, A. Johnson, M. Balasegaram, B. Borisch, *BMC Public Health* **2019**, *19*, 1, https://doi.org/10.1186/s12889-019-7173-7.
[30] K. Lewis, *Nature* **2012**, *485*, 439, DOI: 10.1038/485439a.
[31] L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen, K. Lewis, *Nature* **2015**, *517*, 455, https://doi.org/10.1038/nature14098.
[32] T. Oz, A. Guvenek, S. Yildiz, E. Karaboga, Y. T. Tamer, N. Mumcuyan, V. B. Ozan, G. H. Senturk, M. Cokol, P. Yeh, E. Toprak, *Mol. Biol. Evol*. **2014**, *31*, 2387, https://doi.org/10.1093/molbev/msu191.
[33] D. A. Rasko, V. Sperandio, *Nat. Rev. Drug Discov*. **2010**, *9*, 117, https://doi.org/10.1038/nrd3013.
[34] S. W. DIckey, G. Y. C. Cheung, M. Otto, *Nat. Rev. Drug Discov*. **2017**, *16*, 457, https://doi.org/10.1038/nrd.2017.23.
[35] O. F. Martínez, M. H. Cardoso, S. M. Ribeiro, O. L. Franco, *Front. Cell. Infect. Microbiol*. **2019**, *9*, 1, https://doi.org/10.3389/fcimb.2019.00074.
[36] O. Pacios, L. Blasco, I. Bleriot, L. Fernandez-Garcia, A. Bardanca, Mónica González Ambroa, M. López, G. Bou, M. Tomas, *Antibiotics* **2020**, *9*, 1, https://doi.org/10.3390/antibiotics9020065.
[37] A. E. Clatworthy, E. Pierson, D. T. Hung, *Nat. Chem. Biol.* **2007**, *3*, 541, https://doi.org/10.1038/nchembio.2007.24.
[38] S. Wagner, R. Sommer, S. Hinsberger, C. Lu, R. W. Hartmann, M. Empting, A. Titz, *J. Med. Chem*. **2016**, *59*, 5929, https://doi.org/10.1021/acs.jmedchem.5b01698.
[39] B. B. Finlay, S. Falkow, *Microbiol. Mol. Biol. Rev*. **1997**, *61*, 136, https://doi.org/10.1128/mmbr.61.2.136-169.1997.
[40] B. Heras, M. J. Scanlon, J. L. Martin, *Br. J. Clin. Pharmacol*. **2015**, *79*, 208, https://doi.org/10.1111/bcp.12356.
[41] P. Piewngam, J. Chiou, P. Chatterjee, M. Otto, *Expert Rev. Anti. Infect. Ther.* **2020**, *18*, 499, https://doi.org/10.1080/14787210.2020.1750951.
[42] G. Taubes, *Science* **2008**, *321*, 356, https://doi.org/10.1126/science.321.5887.356.
[43] R. C. Allen, R. Popat, S. P. Diggle, S. P. Brown, *Nat. Rev. Microbiol*. **2014**, *12*, 300, https://doi.org/10.1038/nrmicro3232.
[44] M. Hentzer, H. Wu, J. B. Andersen, K. Riedel, T. B. Rasmussen, N. Bagge, N. Kumar, M. A. Schembri, Z. Song, P. Kristoffersen, M. Manefield, J. W. Costerton, S. Molin, L. Eberl, P. Steinberg, S. Kjelleberg, N. Høiby, M. Givskov, *EMBO J*. **2003**, *22*, 3803, https://doi.org/10.1093/emboj/cdg366.
[45] M. H. Wilcox, D. N. Gerding, I. R. Poxton, C. Kelly, R. Nathan, T. Birch, O. A. Cornely, G. Rahav, E. Bouza, C. Lee, G. Jenkin, W. Jensen, Y.-S. Kim, J. Yoshida, L. Gabryelski, A. Pedley, K. Eves, R. Tipping, D. Guris, N. Kartsonis, M.-B. Dorr, *N. Engl. J. Med.* **2017,** *376,* 305, DOI: 10.1056/NEJMoa1602615.
[46] H. Nagase, *Curr. Protoc. Protein Sci.* **2001**, *24,* https://doi.org/10.1002/0471140864.ps2104s24.
[47] M. D. Obritsch, D. N. Fish, R. MacLaren, R. Jung, *Antimicrob. Agents Chemother*. **2004**, *48*, 4606, https://doi.org/10.1128/AAC.48.12.4606-4610.2004.
[48] C. K. Stover, X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. L. Brinkman, W. O. Hufnagle, D. J. Kowallk, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. S. Wong, Z. Wu, I. T. Paulsen, J. Relzer, M. H. Saler, R. E. W. Hancock, S. Lory, M. V. Olson, *Nature* **2000**, *406*, 959, https://doi.org/10.1038/35023079.
[49] W. D. Smith, E. Bardin, L. Cameron, C. L. Edmondson, K. V. Farrant, I. Martin, R. A. Murphy, O. Soren, A. R. Turnbull, N. Wierre-Gore, E. W. Alton, J. G. Bundy, A. Bush, G. J. Connett, S. N. Faust, A. Filloux, P. S. Freemont, A. L. Jones, Z. Takats, J. S. Webb, H. D. Williams, J. C. Davies, *FEMS Microbiol. Lett*. **2017**, *364*, 1, https://doi.org/10.1093/femsle/fnx121.
[50] G. Raman, E. E. Avendano, J. Chan, S. Merchant, L. Puzniak, *Antimicrob. Resist. Infect. Control* **2018**, *7*, 1, https://doi.org/10.1186/s13756-018-0370-9.
[51] A. W. Musser, P. R. Beamer, *J. Indiana State Med. Assoc.* **1961**, *54*, 1627.
[52] P. A. Lambert, *J. R. Soc. Med.* **2002**, *95 Suppl 4*, 22.
[53] Z. Pang, R. Raudonis, B. R. Glick, T. J. Lin, Z. Cheng, *Biotechnol. Adv.* **2019**, *37*, 177, https://doi.org/10.1016/j.biotechadv.2018.11.013.
[54] J. Botelho, F. Grosso, L. Peixe, *Drug Resist. Updat*. **2019**, *44*, 100640, https://doi.org/10.1016/j.drup.2019.07.002.
[55] W. Ullah, M. Qasim, H. Rahman, Y. Jie, N. Muhammad, *J. Chinese Med. Assoc*. **2017**, *80*, 173, https://doi.org/10.1016/j.jcma.2016.08.011.
[56] P. K. Taylor, A. T. Y. Yeung, R. E. W. Hancock, *J. Biotechnol.* **2014**, *191*, 121, https://doi.org/10.1016/j.jbiotec.2014.09.003.
[57] M. S. Blackledge, R. J. Worthington, C. Melander, *Curr. Opin. Pharmacol.* **2013**, *13*, 699, https://doi.org/10.1016/j.coph.2013.07.004.
[58] T. Rasamiravaka, Q. Labtani, P. Duez, M. El Jaziri, *Biomed Res. Int*. **2015**, 759348, https://doi.org/10.1155/2015/759348.
[59] H. C. Flemming, J. Wingender, *Nat. Rev. Microbiol*. **2010**, *8*, 623, https://doi.org/10.1038/nrmicro2415.
[60] M. Moses, P. K. Singh, A. L. Schaefer, M. R. Parsek, T. O. Moninger, M. J. Welsh, E. P. Greenberg, *Nature* **2000**, *407*, 762.
[61] R. Mittal, S. Aggarwal, S. Sharma, S. Chhibber, K. Harjai, *J. Infect. Public Health* **2009**, *2*, 101, https://doi.org/10.1016/j.jiph.2009.08.003.
[62] T. Strateva, I. Mitov, *Ann. Microbiol*. **2011**, *61*, 717, https://doi.org/10.1007/s13213-011-0273-y.
[63] M. Pollack, *Clin. Infect. Dis*. **1984**, *6*, S617, https://doi.org/10.1093/clinids/6.Supplement_3.S617.
[64] R. Sommer, S. Wagner, K. Rox, A. Varrot, D. Hauck, E. C. Wamhoff, J. Schreiber, T. Ryckmans, T. Brunner, C. Rademacher, R. W. Hartmann, M. Brönstrup, A. Imberty, A. Titz, *J. Am. Chem. Soc*. **2018**, *140*, 2537, https://doi.org/10.1021/jacs.7b11133.
[65] K. P. Rumbaugh, A. Armstrong, 'The Role of Quorum Sensing in Biofilm Development', in 'Antibiofilm Agents', Eds. K. Rumbaugh, I. Ahmad, Springer Series on Biofilms, vol 8. Springer, Berlin, Heidelberg, **2014**, pp. 97, https://doi.org/10.1007/978-3-642-53833-9_6.
[66] T. Bjarnsholt, M. Givskov, *Anal. Bioanal. Chem*. **2007**, *387*, 409, https://doi.org/10.1007/s00216-006-0774-x.
[67] C. Schütz, D. K. Ho, M. M. Hamed, A. S. Abdelsamie, T. Röhrig, C. Herr, A. M. Kany, K. Rox, S. Schmelz, L. Siebenbürger, M. Wirth, C. Börger, S. Yahiaoui, R. Bals, A. Scrima, W. Blankenfeldt, J. C. Horstmann, R. Christmann, X. Murgia, M. Koch, A. Berwanger, B. Loretz, A. K. H. Hirsch, R. W. Hartmann, C. M. Lehr, M. Empting, *Adv. Sci.* **2021**, *2004369*, 1, https://doi.org/10.1002/advs.202004369.
[68] G. R. A. Cathcart, D. Quinn, B. Greer, P. Harriott, J. F. Lynas, B. F. Gilmore, B. Walker, *Antimicrob. Agents Chemother*. **2011**, *55*, 2670, https://doi.org/10.1128/AAC.00776-10.
[69] B. Wretlind, O. R. Pavlovskis, *Clin. Infect. Dis.* **1983**, *5*, S998, https://doi.org/10.1093/clinids/5.Supplement_5.S998.
[70] Y. Tamura, S. Suzuki, T. Sawada, *Microb. Pathog*. **1992**, *12*, 237, https://doi.org/10.1016/0882-4010(92)90058-V.
[71] C. Van Delden, B. H. Iglewski, *Emerg. Infect. Dis*. **1998**, *4*, 551, https://doi.org/10.3201/eid0404.980405.
[72] K. Morihara, H. Tsuzuki, T. Oka, H. Inoue, M. Ebata, *J. Biol. Chem.* **1965**, 240, 3295, DOI: 10.1016/s0021-9258(18)97217-0.
[73] E. Kessler, M. Safrin, J. K. Gustin, D. E. Ohman, *J. Biol. Chem.* **1998**, *273*, 30225, https://doi.org/10.1074/jbc.273.46.30225.
[74] E. Kessler, M. Safrin, *J. Bacteriol*. **1988**, *170*, 5241, https://doi.org/10.1128/jb.170.11.5241-5247.1988.
[75] F. X. Gomis-Rüth, T. O. Botelho, W. Bode, *Biochim. Biophys. Acta - Proteins Proteomics* **2012**, *1824*, 157, https://doi.org/10.1016/j.bbapap.2011.04.014.
[76] M. M. Thayer, K. M. Flaherty, D. B. McKay, *J. Biol. Chem*. **1991**, *266*, 2864, https://doi.org/10.1016/s0021-9258(18)49927-9.
[77] O. A. Adekoya, I. Sylte, *Chem. Biol. Drug Des*. **2009**, *73*, 7, https://doi.org/10.1111/j.1747-0285.2008.00757.x.
[78] F. Bastaert, S. Kheir, V. Saint-Criq, B. Villeret, P. M. C. Dang, J. El-Benna, J. C. Sirard, R. Voulhoux, J. M. Sallenave, *Front. Immunol*. **2018**, *9*, 1, https://doi.org/10.3389/fimmu.2018.01675.
[79] V. Saint-Criq, B. Villeret, F. Bastaert, S. Kheir, A. Hatton, A. Cazes, Z. Xing, I. Sermet-Gaudelus, I. Garcia-Verdugo, A. Edelman, J.-M. Sallenave, *Thorax* **2018**, *73*, 49, https://doi.org/10.1136/thoraxjnl-2017-210298.
[80] L. W. Heck, K. Morihara, W. B. McRae, E. J. Miller, *Infect. Immun.* **1986**, *51*, 115, https://doi.org/10.1128/iai.51.1.115-118.1986.
[81] L. W. Heck, P. G. Alarcon, R. M. Kulhavy, K. Morihara, M. W. Mestecky, J. F. Russell, *J. Immunol.* **1990**, *144*, 2253.
[82] I. A. Holder, R. Wheeler, *Can. J. Microbiol*. **1984**, *30*, 1118, https://doi.org/10.1139/m84-175.
[83] D. R. Galloway, *Mol. Microbiol*. **1991**, *5*, 2315, https://doi.org/10.1111/j.1365-2958.1991.tb02076.x.
[84] M. Parmely, A. Gale, M. Clabaugh, R. Horvat, W. Zhou, *Infect. Immun.* **1990**, *58*, 3009, https://doi.org/10.1128/iai.58.9.3009-3014.1990.
[85] W. I. Mariencheck, J. F. Alcorn, S. M. Palmer, J. R. Wright, *Am. J. Respir. Cell Mol. Biol.* **2003**, *28*, 528, https://doi.org/10.1165/rcmb.2002-0141OC.
[86] H. Yu, X. He, W. Xie, J. Xiong, H. Sheng, S. Guo, C. Huang, D. Zhang, K. Zhang, *Can. J. Microbiol*. **2014**, *60*, 227, https://doi.org/10.1139/cjm-2013-0667.
[87] P. Tielen, F. Rosenau, S. Wilhelm, K. E. Jaeger, H. C. Flemming, J. Wingender, *Microbiology* **2010**, *156*, 2239, https://doi.org/10.1099/mic.0.037036-0.

[88] S. Kamath, V. Kapatral, A. M. Chakrabarty, *Mol. Microbiol*. **1998**, *30*, 933, https://doi.org/10.1046/j.1365-2958.1998.01121.x.
[89] J. F. Poschet, J. C. Boucher, A. M. Firoved, V. Deretic, *Methods Enzymol*. **2001**, *336*, 65, https://doi.org/10.1016/S0076-6879(01)36579-5.
[90] E. Kessler, M. Safrin, 'Elastinolytic and Proteolytic Enzymes', in 'Pseudomonas Methods and Protocols. Methods in Molecular Biology (Methods and Protocols)', Eds: A. Filloux, J. L. Ramos, vol 1149, Humana Press, New York, NY, **2014**, pp. 135, https://doi.org/10.1007/978-1-4939-0473-0_13.
[91] A. Barrett, N. Rawlings, J. Woessner, 'Handbook of Proteolytic Enzymes, 3rd Edition', Elsevier, **2012**.
[92] E. Kessler, M. Safrin, W. R. Abrams, J. Rosenbloom, D. E. Ohman, *J. Biol. Chem.* **1997**, *272*, 9884, https://doi.org/10.1074/jbc.272.15.9884.
[93] E. Kessler, M. Safrin, J. C. Olson, D. E. Ohman, *J. Biol. Chem*. **1993**, *268*, 7503, https://doi.org/10.1016/s0021-9258(18)53203-8.
[94] M. J. Everett, D. T. Davies, *Drug Discov. Today* **2021**, *26*, 2108, https://doi.org/10.1016/j.drudis.2021.02.026.
[95] R. Ye, C. Tan, B. Chen, R. Li, Z. Mao, *Front. Chem*. **2020**, *8*, 1, https://doi.org/10.3389/fchem.2020.00402.
[96] J. A. Jacobsen, J. L. Major Jourden, M. T. Miller, S. M. Cohen, *Biochim. Biophys. Acta - Mol. Cell Res*. **2010**, *1803*, 72, https://doi.org/10.1016/j.bbamcr.2009.08.006.
[97] A. M. Kany, A. Sikandar, S. Yahiaoui, J. Haupenthal, I. Walter, M. Empting, J. Köhnke, R. W. Hartmann, *ACS Chem. Biol*. **2018**, *13*, 2449, https://doi.org/10.1021/acschembio.8b00257.
[98] G. R. Cathcart, B. F. Gilmore, B. Greer, P. Harriott, B. Walker, *Bioorganic Med. Chem. Lett*. **2009**, *19*, 6230, https://doi.org/10.1016/j.bmcl.2009.08.099.
[99] A. L. Garner, A. K. Struss, J. L. Fullagar, A. Agrawal, A. Y. Moreno, S. M. Cohen, K. D. Janda, *ACS Med. Chem. Lett*. **2012**, *3*, 668, https://doi.org/10.1021/ml300128f.
[100] J. Zhu, X. Cai, T. L. Harris, M. Gooyit, M. Wood, M. Lardy, K. D. Janda, *Chem. Biol*. **2015**, *22*, 483, https://doi.org/10.1016/j.chembiol.2015.03.012.
[101] A. M. Kany, A. Sikandar, J. Haupenthal, S. Yahiaoui, C. K. Maurer, E. Proschak, J. Köhnke, R. W. Hartmann, *ACS Infect. Dis*. **2018**, *4*, 988, https://doi.org/10.1021/acsinfecdis.8b00010.
[102] C. Kaya, I. Walter, S. Yahiaoui, A. Sikandar, A. Alhayek, J. Konstantinovic, A. M. Kany, J. Haupenthal, J. Köhnke, R. W. Hartmann, A. K. H. Hirsch, *Angew. Chemie Int. Ed.* **2021**, *61*, e202112295, https://doi.org/10.1002/anie.202112295.
[103] N. Nishino, J. C. Powers, *J. Biol. Chem.* **1979**, *255*, 3482.
[104] K. Morihara, H. Tsuzuki, *Jpn. J. Exp. Med*. **1978**, *48*, 81.
[105] S. Leiris, D. T. Davies, N. Sprynski, J. Castandet, L. Beyria, M. S. Bodnarchuk, J. M. Sutton, T. M. G. Mullins, M. W. Jones, A. K. Forrest, T. D. Pallin, P. Karunakar, S. K. Martha, B. Parusharamulu, R. Ramula, V. Kotha, N. Pottabathini, S. Pothukanuri, M. Lemonnier, M. Everett, *ACS Med. Chem. Lett*. **2021**, *12*, 217, https://doi.org/10.1021/acsmedchemlett.0c00554.
[106] P. G. Jobin, G. S. Butler, C. M. Overall, *Biochim. Biophys. Acta - Mol. Cell Res*. **2017**, *1864*, 2043, https://doi.org/10.1016/j.bbamcr.2017.05.013.
[107] D. Sbardella, G. F. Fasciglione, M. Gioia, C. Ciaccio, G. R. Tundo, S. Marini, M. Coletta, *Mol. Aspects Med*. **2012**, *33*, 119, https://doi.org/10.1016/j.mam.2011.10.015.
[108] H. Nagase, R. Visse, G. Murphy, *Cardiovasc. Res.* **2006**, *69*, 562, https://doi.org/10.1016/j.cardiores.2005.12.002.
[109] N. D. Rawlings, A. J. Barrett, A. Bateman, *Nucleic Acids Res*. **2009**, *38*, 227, https://doi.org/10.1093/nar/gkp971.
[110] H. I. Park, Y. Jin, D. R. Hurst, C. A. Monroe, S. Lee, M. A. Schwartz, Q. X. A. Sang, *J. Biol. Chem*. **2003**, *278*, 51646, https://doi.org/10.1074/jbc.M310109200.
[111] A. Dufour, C. M. Overall, *Trends Pharmacol. Sci*. **2013**, *34*, 233, https://doi.org/10.1016/j.tips.2013.02.004.
[112] S. Sjøli, E. Nuti, C. Camodeca, I. Bilto, A. Rossello, J. O. Winberg, I. Sylte, O. A. Adekoya, *Eur. J. Med. Chem*. **2016**, *108*, 141, https://doi.org/10.1016/j.ejmech.2015.11.019.
[113] O. A. Adekoya, S. Sjøli, Y. Wuxiuer, I. Bilto, S. M. Marques, M. A. Santos, E. Nuti, G. Cercignani, A. Rossello, J. O. Winberg, I. Sylte, *Eur. J. Med. Chem.* **2015**, *89*, 340, https://doi.org/10.1016/j.ejmech.2014.10.009.
[114] L. R. Lopetuso, F. Scaldaferri, V. Petito, A. Gasbarrini, *Gut Pathog*. **2013**, *5*, 1, https://doi.org/10.1186/1757-4749-5-23.
[115] C. L. Hatheway, *Clin. Microbiol. Rev*. **1990**, *3*, 66, https://doi.org/10.1128/cmr.3.1.66-98.1990.
[116] J. G. Bartlett, *Ann. N. Y. Acad. Sci*. **2010**, *1213*, 62, https://doi.org/10.1111/j.1749-6632.2010.05863.x.
[117] M. D. Bond, H. E. Van Wart, *Biochemistry* **1984**, *23*, 3077, https://doi.org/10.1021/bi00308a035.
[118] O. Matsushita, A. Okabe, *Toxicon* **2001**, *39*, 1769, https://doi.org/10.1016/S0041-0101(01)00163-5.
[119] G. A. Senisterra, B. S. Hong, H.-W. Park, M. Vedadi, *J. Biomol. Screening* **2008**, *13*, 337, https://doi.org/10.1177/1087057108317825.
[120] C. J. Alexander, D. M. Citron, J. S. Brazier, E. J. C. Goldstein, *J. Clin. Microbiol*. **1995**, *33*, 3209, https://doi.org/10.1128/jcm.33.12.3209-3215.1995.
[121] U. Eckhard, P. F. Huesgen, H. Brandstetter, C. M. Overall, *J. Proteomics* **2014**, *100*, 102, https://doi.org/10.1016/j.jprot.2013.10.004.
[122] O. Matsushita, C. M. Jung, J. Minami, S. Katayama, N. Nishi, A. Okabe, *J. Biol. Chem*. **1998**, *273*, 3643, https://doi.org/10.1074/jbc.273.6.3643.
[123] O. Matsushita, C. M. Jung, S. Katayama, J. Minami, Y. Takahashi, A. Okabe, *J. Bacteriol*. **1999**, *181*, 923, https://doi.org/10.1128/jb.181.3.923-933.1999.
[124] A. S. Duarte, A. Correia, A. C. Esteves, *Crit. Rev. Microbiol*. **2016**, *42*, 106, https://doi.org/10.3109/1040841X.2014.904270.
[125] M. D. Obritsch, D. N. Fish, R. MacLaren, R. Jung, *Pharmacotherapy* **2005**, *25*, 1353, https://doi.org/10.1592/phco.2005.25.10.1353.
[126] C. M. Jung, O. Matsushita, S. Katayama, J. Minami, J. Sakurai, A. Okabe, *J. Bacteriol.* **1999**, *181*, 2816, https://doi.org/10.1128/jb.181.9.2816-2822.1999.
[127] U. Eckhard, E. Schönauer, H. Brandstetter, *J. Biol. Chem.* **2013**, *288*, 20184, https://doi.org/10.1074/jbc.M112.448548.
[128] U. Eckhard, E. Schönauer, P. Ducka, P. Briza, D. Nüss, H. Brandstetter, *Biol. Chem.* **2009**, *390*, 11, https://doi.org/10.1515/BC.2009.004.
[129] A. Yiotakis, A. Hatgiyannacou, V. Dive, F. Toma, *Eur. J. Biochem*. **1988**, *172*, 761, https://doi.org/10.1111/j.1432-1033.1988.tb13954.x.
[130] V. Dive, A. Yiotakis, A. Nicolaou, F. Toma, *Eur. J. Biochem*. **1990**, *191*, 685, https://doi.org/10.1111/j.1432-1033.1990.tb19175.x.
[131] N. Oshima, Y. Narukawa, T. Takeda, F. Kiuchi, *J. Nat. Med*. **2013**, *67*, 240, https://doi.org/10.1007/s11418-012-0665-8.
[132] E. Schönauer, A. M. Kany, J. Haupenthal, K. Hüsecken, I. J. Hoppe, K. Voos, S. Yahiaoui, B. Elsässer, C. Ducho, H. Brandstetter, R. W. Hartmann, *J. Am. Chem. Soc*. **2017**, *139*, 12696, https://doi.org/10.1021/jacs.7b06935.
[133] K. Voos, E. Schönauer, A. Alhayek, J. Haupenthal, A. Andreas, R. Müller, R. W. Hartmann, H. Brandstetter, A. K. H. Hirsch, C. Ducho, *ChemMedChem* **2021**, *16*, 1257, https://doi.org/10.1002/cmdc.202000994.
[134] A. Scozzafava, C. T. Supuran, *Eur. J. Med. Chem*. **2000**, *35*, 299, https://doi.org/10.1016/S0223-5234(00)00127-6.
[135] M. A. Santos, S. Marques, M. Gil, M. Tegoni, A. Scozzafava, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2003**, *18*, 233, https://doi.org/10.1080/1475636031000081134.
[136] M. Ilies, M. D. Banciu, A. Scozzafava, M. A. Ilies, M. T. Caproiu, C. T. Supuran, *Bioorganic Med. Chem*. **2003**, *11*, 2227, https://doi.org/10.1016/S0968-0896(03)00113-5.
[137] J. Konstantinovic, S. Yahiaoui, A. Alhayek, J. Haupenthal, E. Schönauer, A. Andreas, A. M. Kany, R. Müller, J. Koehnke, F. K. Berger, M. Bischoff, R. W. Hartmann, H. Brandstetter, A. K. H. Hirsch, *J. Med. Chem*. **2020**, *63*, 8359, https://doi.org/10.1021/acs.jmedchem.0c00584.
[138] R. E. Galardy, D. Grobelny, *Biochemistry* **1983**, *22*, 4556, https://doi.org/10.1021/bi00288a032.

The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2022.402