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Review

Scope, limitations and classification of lactamases

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ABSTRACT

The hydrolysis of amide bonds is a ubiquitous process in nature and is catalyzed by various enzymes: Whereas *N*-unsubstituted amides are cleaved by amidases (EC 3.5.1.4), peptidases (EC 3.4.X.X) cleave peptide bonds in proteins and are involved in a number of vital physiological processes. Cyclic amides (lactams) are generally not hydrolyzed by proteases, but require specific lactamases. While the β-lactamase family (EC 3.5.2.6), acting on highly strained β-lactams, is constantly growing, lactamases able to hydrolyze γ- and δ-lactams are largely under-represented, owing to the lack of ring strain of 5- and 6-membered cyclic amides which accounts for their lower reactivity. To date, the only known substrate in which a 5- or 6-membered ring lactam is enzymatically cleaved is (±)-2-azabicyclo[2.2.1]hept-5-en-3-one (*rac*-Vince lactam), as well as four derivatives thereof. For these industrially relevant substrates, enantiocomplementary biocatalysts have been identified and their stereopreference was found to correlate with their amino acid sequence and protein structure: While (+)-lactamases belong to the amidase signature family, displaying the typical GGSS(S/G)GS motif in the center of the protein sequence and a conserved Ser-Ser-Lys catalytic triad, (−)-lactamase activity has been identified only among serine hydrolases, members of the α/β-hydrolase fold family, possessing a typical Ser-His-Asp catalytic triad. For larger 8- to 13-membered ring lactams, few active proteins have been identified, all are members of the amidase signature family. An enhanced partial C—N double bond character in the amide bond explains the lower reactivity of particularly chemically stable lactams.

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1. Introduction

Lactams represent a diverse set of cyclic amides that are typically classified according to the size of their ring. Four-membered ring β-lactams are most prominent due to their wide occurrence as constitutive motif in various antibiotics, accounting for more than 65% of the world antibiotic market (Elander, 2003). Their hydrolysis

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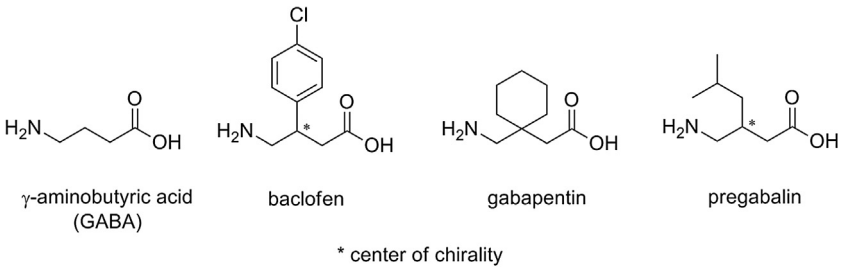

Fig. 1. γ-Aminobutyric acid analogues used as pharmacophores.

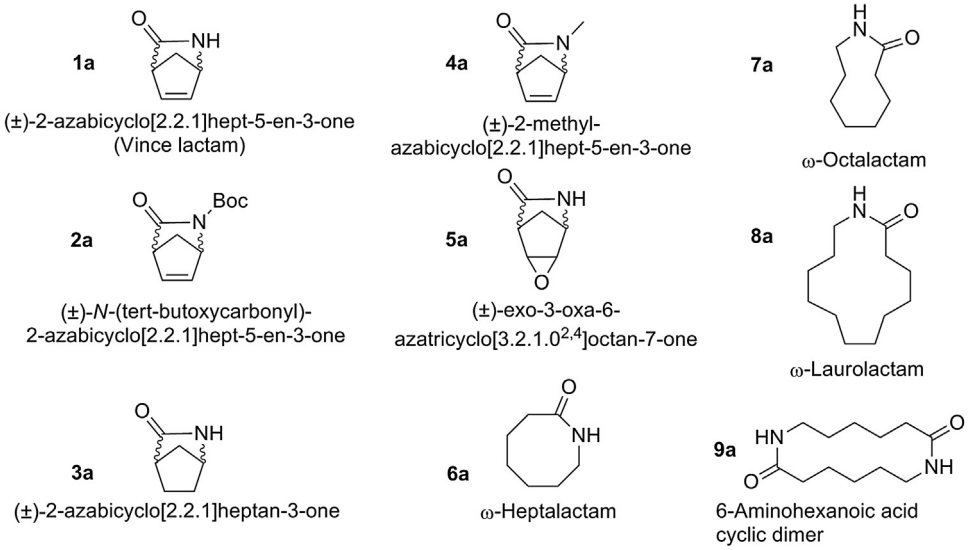

Fig. 2. Substrates discussed in this review.

is thermodynamically favored by an enhanced ring strain, exemplified by a widespread microbial β-lactamase activity, which has become a major clinical concern since β-lactam-resistant bacteria render any antimicrobial lactam-based treatment inefficient. Larger γ- and δ-lactam motifs appear in a broad range of natural products, e.g. alkaloids and oxindole derivatives (Aoki et al., 2006; Feling et al., 2003; Hartwig and Born, 1987; Jossang et al., 1991; Sebahar and Williams, 2000; Williams and Cox, 2003). They display only marginal reduced ring strain and are therefore less reactive. Consequently, their chemical hydrolysis requires harsh conditions, typically boiling in concentrated strong acid solution, which renders the presence of sensitive functional groups critical. In addition, subsequent neutralization generates large amounts of salts as undesired waste. Lactam hydrolysis is, however, synthetically very attractive as it leads to the formation of γ- and δ-amino acids. Derivatives of γ-aminobutyric acid (GABA), for instance, constitute an important class of drugs used as GABA receptor agonists (Fig. 1). Common examples include baclofen (muscle relaxant), gabapentin (antiepileptic) or pregabalin (used to reduce neuropathic pain) (Gajcy et al., 2010). Peptides containing γ-amino acids display very stable secondary structures and are resistant towards proteolytic cleavage compared to their 'natural' α-analogues, a feature that renders these molecules good targets as backbones for drug design (Bouillere et al., 2011; Frackenpohl et al., 2001). δ-Peptides are also gaining interest in the field of peptide, peptidomimetics and foldamer design (Baldauf et al., 2004; Vasudev et al., 2011).

Despite the profusion of β-lactamases (Bradford, 2001), up to now only few studies have reported on the enzymatic hydrolysis of γ- and δ-lactams, and most of them deal with the specific case of the enantioselective hydrolysis of derivatives of (±)-2-azabicyclo[2.2.1]hept-5-en-3-one (*rac*-Vince lactam, Fig. 2). This bicyclic lactam has been found to be the only substrate in a number of cases and the corresponding activity was classified as γ-lactamase activity, although the hydrolytic event may be regarded as taking place also within the 6-membered ring. True γ- and δ-lactamase activity on monocyclic lactams remains unreported. Few cases of activity on larger lactams (8- to 13-membered ring) have been reported.

The unusual chemical stability of the amide bond is due to resonance stabilization, which provides a partial double-bond character to the C—N bond, going in hand with restricted freedom of rotation and *cis-trans*-isomerism (Fig. 3) (Andrews, 1971). The latter causes the enhanced stability and rigidity of peptides and proteins as opposed to esters, which makes it obvious that Nature evolved its catalysts as poly*amides* and not as poly*esters*. Since resonance stabilization depends notably on steric factors, the bond strength of lactams strongly depends on the ring size, partially accounting for the trend observed in the enzymatic hydrolysis of lactams (Assaf et al., 2014).

This review surveys lactamase activities identified in various whole-cell microorganisms as well as isolated enzymes (all substrates listed in Fig. 2). Additionally, a classification of γ-lactamases is proposed based on mechanistic considerations and enantiopreference pattern observed.

2. Lactamases and their substrate spectrum

2.1. γ-Lactams

Earlier work on lactamase activity screening was focused on microbial sources in search of hydrolytic activity on *rac*-Vince