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MINIREVIEW – Physiology, Biochemistry & Genetics

A ban on BAM: an update on inhibitors of the β -barrel assembly machinery

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One sentence summary: In pursuit of new antibiotics, the current state of drug development targeting the BAM complex is reviewed.

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ABSTRACT

Gram-negative pathogens are a rapidly increasing threat to human health worldwide due to high rates of antibiotic resistance and the lack of development of novel antibiotics. The protective cell envelope of gram-negative bacteria is a major permeability barrier that contributes to the problem by restricting the uptake of antibiotics. On the other hand, its unique architecture also makes it a suitable target for antibiotic interference. In particular, essential multiprotein machines that are required for biogenesis of the outer membrane have attracted attention in antibacterial design strategies. Recently, significant progress has been made in the development of inhibitors of the β -barrel assembly machine (BAM) complex. Here, we summarize the current state of drug development efforts targeting the BAM complex in pursuit of new antibiotics.

Keywords: Antibiotics; *Escherichia coli*, potentiators; cell envelope stress; outer membrane; resistance

INTRODUCTION

Antibiotic resistance in human pathogens is of huge concern to public health worldwide, illustrated by the fact that some bacterial strains have acquired resistance to nearly all available antibiotics (Tacconelli *et al.* 2018). Infections due to gram-negative bacteria are particularly difficult to treat as newly developed antibiotics generally have to penetrate the outer membrane (OM) or both the OM and inner membrane (IM) depending on the location of their target (Tacconelli *et al.* 2018).

The OM functions as a selective barrier that protects bacteria against harmful substances in the environment. It is an asymmetric bilayer composed of lipopolysaccharides (LPS) in the outer leaflet and phospholipids in the inner leaflet. The OM also contains outer membrane proteins (OMPs) that mostly

comprise a β -barrel structure. An abundant class of trimeric β -barrel proteins form integral water-filled porins that allow diffusion of small hydrophilic nutrients and waste products with a molecular weight up to ~600 Da (Nikaido 2003).

Biogenesis of both the OM and composite OMPs is a complex process that requires multiple factors, including chaperones and integral membrane proteins/complexes to integrate the building blocks at the right place and time (Bos, Robert and Tomassen 2007; Noinaj *et al.* 2013; Rollauer *et al.* 2015). Interference with these assembly machineries is an attractive strategy not only to develop stand-alone antibiotics but also potentiators that increase the permeability of the OM for other drugs. No antibiotic presently in clinical use targets the OM assembly machineries and hence no pre-existing resistance to this type of drugs is known. Recently, several groups have reported on

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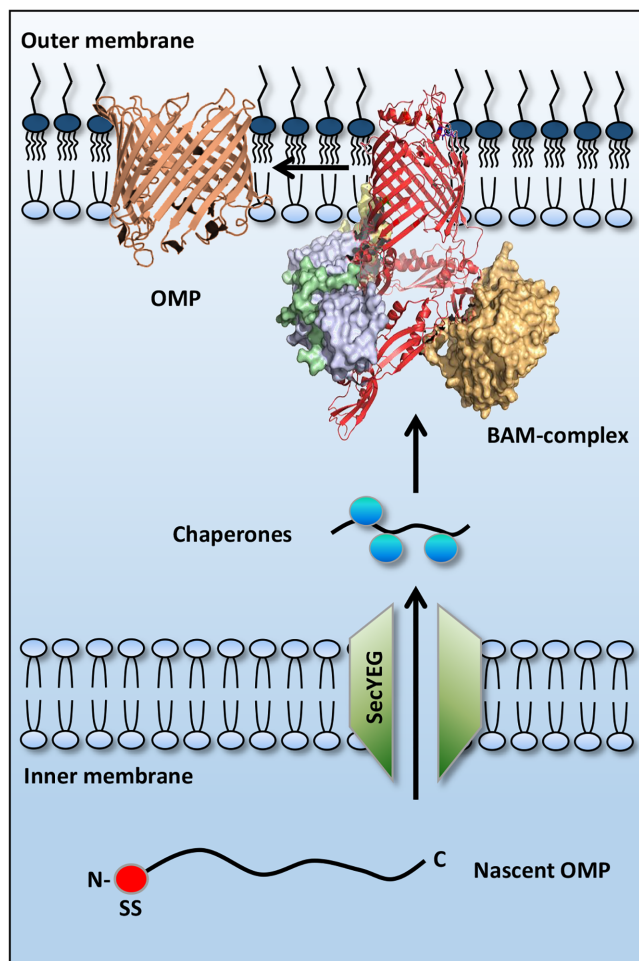


Figure 1. Schematic overview of OMP biogenesis. Nascent OMPs are synthesized in the cytosol and targeted to the SecYEG translocon using their signal sequence (SS). In the periplasm the newly formed unfolded OMP associates with chaperones that escorts the OMP to the BAM complex in the OM. Here, the BAM complex catalyzes insertion and folding of OMPs into a β -barrel structure. In addition, the BAM complex is directly involved in the secretion of autotransporters (not drawn here).

novel compounds that affect the gram-negative β -barrel assembly machine (BAM) complex (Hart et al. 2019; Imai et al. 2019; Steenhuis et al. 2019), an essential membrane protein complex involved in insertion and folding of β -barrel proteins into the OM. The goal of this review is to provide an overview of the current state of BAM complex inhibitors, the potential of this protein complex as antibiotic target, and the discovery strategies that have proven successful in identifying these inhibitors.

ROLE OF BAM IN OMP ASSEMBLY

The BAM complex in the OM is required for OMP assembly, the key steps of which have been investigated in detail (Fig. 1) (Konovalova, Kahne and Silhavy 2017; Silhavy and Ricci 2019). OMPs are synthesized in the cytosol as precursor proteins with an N-terminal signal sequence to trigger transfer into the periplasm via the SecYEG-translocon (Denks et al. 2014). Periplasmic chaperones, such as SurA and Skp interact with the nascent OMP that emerges from the SecYEG-translocon in a vulnerable unfolded conformation. DegP is the third main player in the periplasmic quality control network having both chaperone and protease activity, the latter being dominant at higher temperatures. While SurA is believed to be the primary chaperone involved in biogenesis of OMPs, Skp and DegP function to rescue OMPs

that have deviated from the SurA pathway (Plummer and Fleming 2016; Soltes et al. 2017). Chaperone-bound OMPs then transit across the periplasm to the inner leaflet of the OM. Here, the nascent OMPs are handed over to the BAM complex for folding and insertion into the lipid bilayer as a β -barrel structure (Noinaj et al. 2014; Noinaj, Rollauer and Buchanan 2015; Lee et al. 2016; Schiffrin et al. 2017; Wu et al. 2020).

In *E. coli* the BAM complex consists of the essential integral membrane subunit BamA, that actually catalyzes membrane insertion of nascent OMPs and four associated lipoproteins, BamB, BamC, BamD and BamE. The lipoproteins are anchored in the inner leaflet of the OM and fulfill accessory functions in the reception and transfer of nascent OMPs and in the modulation of BamA activity. With the exception of BamD, they are not essential (Wu et al. 2005; Sklar et al. 2007; Kahne 2020). BamA, the most conserved component, consists of two characteristic regions: a sequence of five polypeptide transport associated (POTRA) domains that extend into the periplasm and a C-terminal integral β -barrel domain with surface exposed loops. Although various structures of the BAM complex and its individual subunits have been reported (Noinaj, Fairman and Buchanan 2012; Noinaj et al. 2013; Han et al. 2016; Iadanza et al. 2016; Tomasek et al. 2020), the mechanism by which it facilitates β -barrel folding remains to be fully elucidated. Current thinking regarding the

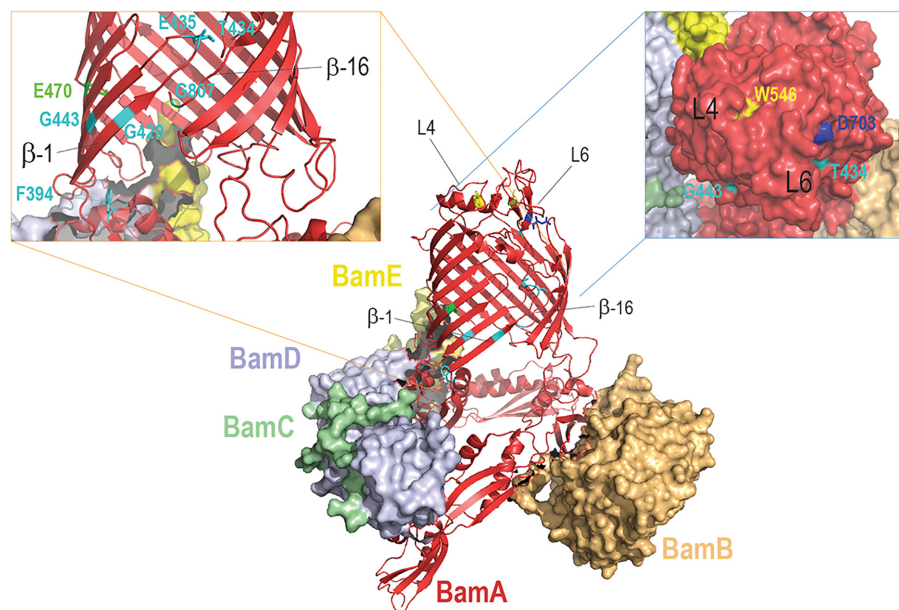


Figure 2. Mutations conferring resistance to BAM inhibitors mapped upon the structure of BamA of *E. coli* in complex with subunits BamB-E. In the middle a cartoon representation of the BamA crystal structure is shown in red. For reference the BamB-E subunits are shown in surface view. Indicated are the positions of strands β -1 and β -16, which form the lateral gate that in this case is closed, and loops 4 and 6 that cap the β -barrel pore at the extracellular surface. Mutations conferring resistance to the compounds are displayed in sticks with the colors green, cyan, yellow and blue representing the inhibiting compounds as indicated in Table 2. The insert in the left panel shows a close-up of the lateral gate and the positions of residues that were mutated to confer resistance to darobactin (cyan) and MRL-494 (green). The insert in the right panel shows a top view of BamA in surface representation, which shows the positions of residues that were found to confer resistance to peptide 8 (blue), LlpA (yellow) and darobactin (cyan). Of note, these residues are accessible from the extracellular milieu. The figure was compiled using Pymol from the structure of the BAM complex from *E. coli* (PDB 5AYW, (Han et al. 2016)).

mechanism(s) by which BamA fulfills its role centers around the ‘assisted’ and ‘budding’ models (Noinaj et al. 2014; Lee et al. 2016, 2019; Schiffriin et al. 2017; Doyle and Bernstein 2019; Wu et al. 2020). In the ‘assisted’ model OMPs insert in a folded or partially folded conformation into the membrane and BamA only facilitates this by locally thinning and destabilizing the membrane, providing an energetically favorable entry point for OMP insertion. However, more recent data point towards the ‘budding’ model, where OMP maturation is catalyzed in a stepwise fashion. The first β -strand of the BamA β -barrel is only weakly connected to the last β -strand forming the so-called ‘lateral gate’ (see Fig. 2). This lateral gate has been shown to exist in an open and closed state and this conformational switching appears necessary for β -barrel insertion into the OM (Noinaj et al. 2014; Noinaj, Rollauer and Buchanan 2015). The gate is targeted by the so-called ‘ β -signal’ that comprises the C-terminal strand of the nascent OMP (Konovalova, Kahne and Silhavy 2017) and a recent BAM complex structure illustrates how this β -signal aligns with the first strand of the lateral gate (Xiao et al. 2021). Subsequently, a nascent barrel grows as each added strand nucleates formation of the next strand until the new β -barrel is complete and buds from BamA into the membrane.

BAM INHIBITORS

Considering the crucial role of the BAM complex in OMP assembly and the fact that BamA and BamD are essential for growth, BAM is increasingly recognized as a promising target for antibacterials. Importantly, most of the activity and mass of the BAM complex is located at the periplasmic side of the OM and hence accessible to relatively smaller compounds. On top of that, BamA is partly surface exposed, allowing compounds to directly bind BamA without the need to cross the OM. This has incited

recent development of the following BAM complex inhibitors that affect growth and/or virulence (Hart et al. 2019; Imai et al. 2019; Luther et al. 2019; Steenhuis et al. 2019; Li et al. 2020) (Table 1).

MRL-494

Hart and coworkers identified the synthetic compound MRL-494 as an unintended byproduct formed during the synthesis of an unrelated compound in a screen to find antibacterial compounds that do not need to cross the OM to exert their effect (Hart et al. 2019). MRL-494 was shown to impair the biogenesis of OMPs and exhibits moderate potency against gram-negative bacteria, including *K. pneumoniae* and *P. aeruginosa*. Strikingly, MRL-494 also affects gram-positive organisms, presumably via a destabilizing effect on the cytoplasmic membrane. Mutations in *bamA* were found to confer resistance to MRL-494 in *E. coli* (Table 2 and Fig. 2), inferring the BamA complex as potential target. A direct or proximal interaction of MRL-494 with BamA was further substantiated using a cellular thermostability assay. However, where MRL-494 binds to BamA remains to be determined as well as the efficacy and toxicity of MRL-494 in animal models.

Darobactin

Darobactin was found by Imai and coworkers when they screened extracts of *Photobacterium* and *Xenorhabdus* species that reside in the gut of entomopathogenic nematodes for antibacterial activity against *E. coli* (Imai et al. 2019). Mass spectrometry and NMR revealed the structure of the active compound as a modified heptapeptide characterized by a unique fused bicyclic

Table 1. Overview of BAM complex inhibitors including their structural formula and molecular weight.

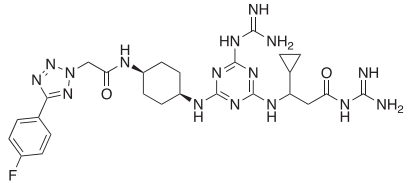
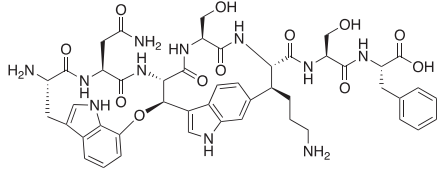
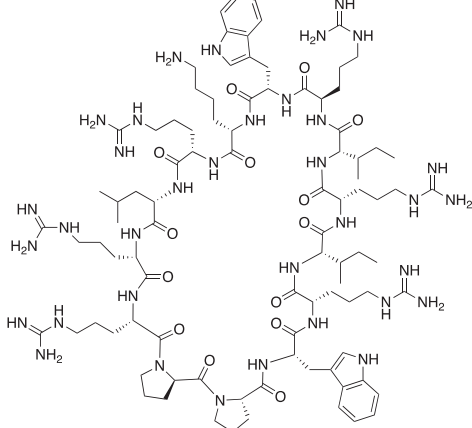
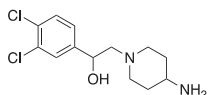
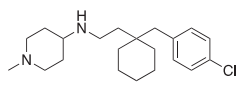
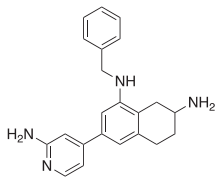
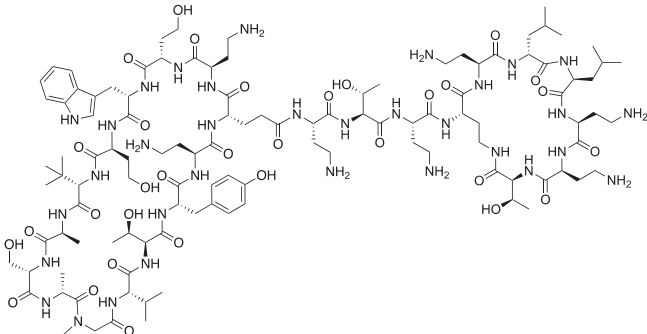
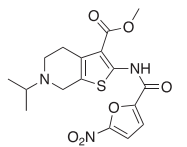
Name	Structural formula	MW (Da)	References
MRL-494		622.67	(Hart et al. 2019)
Darobactin		966.02	(Imai et al. 2019)
JB-95		1971.45	(Urfer et al. 2015)
VUF15259		289.20	(Steenhuis et al. 2019)
Compound 2		348.96	(Steenhuis et al. 2020, submitted)
Compound 14		344.46	(Steenhuis et al. 2020, submitted)
Peptide 3		2531.95	(Luther et al. 2019)
IMB-H4		393.41	(Li et al. 2020)

Table 2. Overview of mutations in *E. coli* *bamA* that confer resistance to the indicated compounds.

Compound name	Mutations in <i>bamA</i>	Strain used	Color in fig. 2	References
MRL-494 Darobactin	E470K	<i>E. coli</i>	Green	(Hart et al. 2019)
	E435K	<i>E. coli</i>	Cyan	(Imai et al. 2019)
	T434A			
	G443D			
	Q445P			
	F394V			
	A705T			
	G429V			
Peptide 8 LlpA	D703Y*	<i>K. pneumoniae</i>	Blue	(Luther et al. 2019)
	T663P (V673)**	<i>P. aeruginos</i>	Yellow	(Ghequire et al. 2018)
	G540D (W546)	<i>a</i>		

*The D at position 703, as well as the surrounding sequence is similar in *E. coli* and *K. pneumoniae*.

**The sequence of BamA of *Pseudomonas* was aligned with that of *E. coli* K12. The equivalent amino acid in *E. coli* is given in between brackets.

peptide core that is formed post-translationally by dedicated tailoring enzymes. Darobactin is expressed as a propeptide from *darA*, which is located within the *dar* operon that also encodes an ABC-type transenvelope exporter of darobactin. Under laboratory conditions the *dar* operon is silent and darobactin production is low.

Several direct lines of evidence indicate that darobactin targets the central core subunit of the BAM complex: BamA. First, resistant strains were generated that harbor mutations in *bamA*, which were shown to be solely responsible for darobactin resistance (Table 2 and Fig. 2). Second, darobactin inhibited folding of the protease OmpT in an *in vitro* folding assay that makes use of proteoliposomes in which the BAM complex has been reconstituted. Third, using isothermal titration calorimetry, darobactin was shown to interact with BamA with a *K_d* of 1.2 μ M. These observations were corroborated by recent cryo-EM and crystal structures of the BAM complex interacting with darobactin (Kaur et al. 2021). Strikingly, darobactin binds with high affinity to the first β -strand of the BamA lateral gate, at the position where also the β -signal was found (Xiao et al. 2021). The interaction appeared further stabilized by interactions of darobactin with OM lipids. The structure suggests that darobactin competes effectively with the β -signal of nascent OMPs, thereby blocking their entry and the movement of the lateral gate.

Darobactin exhibits promising activity with MIC values ranging from 2 to 16 μ g/mL against specifically those gram-negative species that are found on the WHO list of priority pathogens (*A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*). Of note, a single dose of darobactin protected mice infected intraperitoneally with *E. coli*, *K. pneumoniae* and *P. aeruginosa* against septicemia. Combined with its low toxicity in several eukaryotic cell lines, these findings point to the potential of darobactin as a lead compound for further development.

Murepavadin and polymyxin B chimeras

A relatively new approach to construct novel antibiotics is by making chimeras of existing compounds to provide intrinsic synergy. Luther and coworkers used this strategy to combine the activities of the antibacterial peptides murepavadin and polymyxin B (Luther et al. 2019). Murepavadin is a macrocyclic peptidomimetic, originally identified as an inhibitor of the essential β -barrel OMP LptD that functions in the assembly of LPS at the surface of the OM. It has strong but narrow

antibiotic activity against *P. aeruginosa*. In an attempt to increase the spectrum of target organisms, fragments of polymyxin B, a clinically used antibiotic known to bind to the lipid A part of LPS, were fused to murepavadin-like peptides. Excitingly, one of the chimeras, peptide 3, showed potent activity towards a wide range of clinically relevant Gram-negative pathogens *in vitro* and in a mouse infection model for septicemia and peritonitis (Luther et al. 2019). Importantly, no kidney failure was reported, which is in contrast to what was found in a clinical evaluation of murepavadin on its own.

Mechanistic studies further indicated that peptide 3 also permeabilized and perturbed the *E. coli* cell envelope. Noticeably, binding studies using peptide 3 with bound photoprobes identified not only LptE, part of a complex with LptD (Botos et al. 2016), but also members of the BAM complex, especially BamA, as binding partners. Furthermore, by using the analog peptide 8 with a more stabilized hairpin structure a mutation was found in *bamA*, in the region encoding the external loop L6 of BamA, which resulted in a large increase in MIC for *K. pneumoniae* (Table 2 and Fig. 2). In addition, resistance-conferring mutations were found in genes involved in the biogenesis of lipid A, confirming that these chimeric molecules have a complex mode of action that involves binding to LptE and BamA as well as targeting LPS. More studies are needed to determine how binding to BamA and LptE causes the downstream bactericidal activity. Interestingly, NMR studies indicated interactions of peptide 3 with external loops L4, L6 and L7 of BamA resulting in an apparent stabilization of the lateral gate in a closed conformation.

JB-95

Analogous to murepavadin, the β -hairpin macrocyclic peptidomimetic JB-95 was identified as an inhibitor of BamA and LptD (Urfer et al. 2015). JB-95 was synthesized as a part of a family of peptidomimetic antibiotics based on the antimicrobial peptide protegrin I. Although it shares some similarity to murepavadin, there are notable differences in structure and cellular activity. JB-95 showed antimicrobial activity against a panel of gram-negative bacteria, in particular *E. coli* with a MIC of only 0.25 μ g/ml. Exposure of *E. coli* cells to JB-95 impaired OM integrity, decreased abundance of OMPs and induced cell envelope stress. Photolabeling experiments identified BamA and LptD as prime interaction partners of JB-95. Similar to MRL-494, JB-95 also affected cell viability of gram-positive cells, lacking an

OM, presumably reflecting a second mechanism of action that involves the cytoplasmic membrane.

VUF15259, compound 2 and 14

Our group recently reported the development of a phenotypic fluorescence-based assay that reports on activation of the σ^E and Rcs cell envelope stress response in *E. coli*. By using the σ^E assay as primary screen, sensitized by expression of the auto-transporter haemoglobin protease (Hbp), a focused library of 1 600 fragment-based compounds (Steenhuis et al. 2019) and a larger library of ~320 000 compounds were screened (Steenhuis et al. 2020, submitted). This resulted in the identification of three compounds (compound 2, compound 14 and VUF15259) that decreased the abundance of OMPs, impaired secretion of autotransporters, synergized with OMP biogenesis mutants, and increased OM permeability, effects all indicative of BAM complex inhibition. Furthermore, compound 2 was shown to inhibit OmpT folding in the *in vitro* reconstituted folding assay described above. While these effects are indicative of BAM complex inhibition, future studies are needed to determine the exact target of these compounds and their *in vivo* efficacy and toxicity in animal models.

MAB1

In addition to small molecules, antibodies have been developed that target BamA or assembly of the BAM complex and have antibacterial effect. Storek and coworkers screened around 1 600 α -BamA IgG monoclonal antibodies and identified seven clones that completely inhibited *E. coli* growth, of which MAB1 α -BamA IgG was further characterized and shown to bind to the extracellular loop L4 (Storek et al. 2018). However, MAB1 falls short as therapeutic due to its limited access to the epitope and was only able to reach the BAM complex in an *E. coli* $\Delta waaD$ strain containing a truncated LPS layer. Nevertheless, these results do serve as proof of concept and point to the potential of developing antibodies or nanobodies that can more effectively access BamA, for example by conjugation to antibiotics that target LPS, as in the chimeras discussed above.

Lectin-like bacteriocins

BamA was recently identified as the prime target of lectin-like bacteriocins (LlpA), which are secreted by *Pseudomonas* strains (Ghequire et al. 2018). LlpAs are midsize bacteriotoxins of ~28 kDa that comprise a tandem of β -lectin domains followed by a short non-conserved carboxy-terminal extension. They have affinity for LPS, but LlpA-resistant mutants were identified to carry mutations in variable part of external loop L6 of BamA thus determining targeting selectivity (Table 2). It was further speculated that binding to this loop interferes with the dynamics of the lateral gate of BamA, leading to misfolded OMPs and the corresponding bactericidal effects.

Peptide 2 and IMB-H4

So far, much attention has been paid to BamA as target, because of its accessibility and central role in functioning of the BAM complex. However, the BAM lipoproteins also play an important role in substrate recognition and modulation of BamA activity (Noinaj, Fairman and Buchanan 2012; Noinaj et al. 2013; Misra, Stikeleather and Gabriele 2015; Han et al. 2016; Lee et al. 2018; Har et al. 2020). For instance, the essential lipoprotein BamD

binds nascent OMPs by recognizing the β -signal (Lee et al. 2018). Interestingly, Hagan et al. showed that expression of a peptide that resembles the β -signal in the periplasm invokes reduced growth, increased OM permeability, reduced levels of OMPs and induction of cell envelope stress (Hagan, Wzorek and Kahne 2015). Furthermore, the same peptide was shown to interact with BamD by photocrosslinking and inhibited assembly of β -barrel OMPs *in vitro*. Although the site of action is located in the periplasm, the combined data indicate that peptidomimetics deserve attention as a means to block early steps in β -barrel assembly, perhaps upon fusion to, or in combination with potentiators to permeabilize the OM.

Alternatively, small compounds may inhibit crucial interactions in the BAM complex in the periplasm. Yan Li et al., screened a library of 25 000 compounds (synthetic and natural products) by using a yeast two-hybrid system to monitor disruption of binding between BamA to BamD (Li et al. 2020). This strategy identified compound IMB-H4 that was found to impair OM integrity and decreased the abundance of OMPs, consistent with disturbed BamA-BamD binding although this has to be confirmed experimentally.

CONCLUDING REMARKS AND DISCUSSION

Traditionally, antibiotic drug discovery has been focused on compounds that inhibit bacterial growth. While the BAM complex performs an essential function in OM biogenesis and is required for cell viability, it is only in the last two years that potent BAM complex inhibitors were discovered. One of the reasons that BAM has, until recently, been overlooked as an antibiotic target is the relative ease with which bacteria can survive at low BAM levels under laboratory conditions. The *E. coli* *bamA101* knockdown strain, for example, in which BamA levels are reduced by ~90% compared to wild-type cells, can support *in vitro* growth, although OMP biogenesis is affected (Aoki et al. 2008). However, it is likely that in the host or environment an optimally functioning BAM complex is crucial given the challenging conditions with respect to nutrient availability that demand a high quality and quantity of OMPs. Furthermore, while subtle changes in the OM may be missed in standard *in vitro* MIC assays, *in vivo* the same OM disruption may serve to enhance the activity of the innate immune system including the action of the membrane attack complex and endogenous host-defense peptides (Lehrer and Lu 2012; Doorduyn, Rooijackers and Heesterbeek 2019). Finally, even a moderate effect on the BAM complex may reduce the secretion of virulence factors sufficiently to reduce the fitness of gram-negative pathogens *in vivo*. In this respect, it is notable that mutations in BamA that were selected to confer resistance to darobactin *in vitro*, appeared to be avirulent in a mouse infection model for pathogenic *E. coli* (Imai et al. 2019). Presumably, mutations in BamA come at a fitness costs and for this reason BAM complex inhibitors may be expected to result in low selection pressure to develop resistant mutations.

The finding that a number of OMPs are not essential for growth under laboratory conditions, but do have important roles in survival in environmental and pathogenic niches, should be considered in future drug screening programs. For BAM inhibitors this may require the use of BAM-cripple mutants that are more sensitive to live-death screening *in vitro*. Also, less straightforward but potentially more rewarding, are screening assays more closely approximating the *in vivo* setting, for example based on infected cells or model organisms such as Zebrafish embryos that can be conveniently grown in microtiter plates.

Experiences with the recently identified BAM inhibitors described in this review may also underpin alternative *in vitro* screening efforts. It is notable that the majority of BAM inhibitors identified display similar effects on Gram-negative cells: (i) induction of σ^E and/or Rcs cell envelope stress, (ii) synergy with mutations in the biogenesis pathway of β -barrel OMPs, (iii) reduction of *de novo* biogenesis and steady state levels of β -barrel OMPs, but not Lpp's and (iv) impaired OM integrity. For instance, stress-based assays can detect even moderate inhibition of BAM activity *in vitro* using reporters that are based on fluorescent or luminescent output (Steenhuis et al. 2019, 2020). This simple format has been shown robust and compatible with high throughput phenotypic screening as a primary selection of BAM inhibitors. Recently obtained lead compounds may also inspire target-based approaches focused on regions in BamA that appear accessible and particularly susceptible to intervention. As displayed in Fig. 2, the mutations identified that confer resistance to the BAM inhibitors discovered to date seem to cluster near the lateral gate and the external L6 loop, capping the β -barrel domain of BamA, indicating that these regions are particularly suitable as target for antibiotics. Similarly, interactions between the BAM subunits, such as the interaction between BamA and BamD and between BAM subunits and nascent substrate OMPs, may be interrogated in more depth using high throughput *in situ* interaction assays and two-hybrid analyses. Finally, the loss of OM integrity allows the passage of large-scaffold antibiotics that would normally not pass the OM. This potentiating effect could be explored in combination therapy but also experimentally in the development of new screening assays.

To date, all known small molecule BamA inhibitors have been found by screening either synthetic or natural product libraries. While such approaches can provide leads for dedicated medicinal chemistry campaigns, the lack of detailed structural insights in the form of co-crystal structures for most inhibitors bound to BamA, currently limit rational design strategies. Recent progress in determining the structures of BamA and the other members of the BAM complex, as well as the recent elucidation of the BAM-darobactin structure (Kaur et al. 2021), suggest we may expect more co-crystal structures in the near future. It is also interesting to compare the structures of the small molecule and peptide-based BamA inhibitors reported to date (Table 1). While there is significant diversity in these structures it is notable that all confirmed BamA targeting compounds (excluding IMB-H4) are highly positively charged and contain one or more aromatic moieties.

The importance of proper BAM functioning in its natural niche, combined with its relative accessibility, makes it an obvious target for bacterial warfare. Following this reasoning one would expect an abundance of BAM targeting compounds in nature. Indeed, the most potent inhibitors, darobactin and peptide 3, were derived from natural compounds. Importantly, darobactin is encoded by a biosynthetic gene cluster (BGCs) that, like many other BGCs, is silent *in vitro* suggesting a yet unexplored reservoir of BAM inhibitors that require the right conditions for expression and characterization.

In addition to its role in OM assembly and integrity, the BAM complex is essential for the secretion of important virulence factors via the type 5 secretion pathway, also known as auto-transport (Van Ulsen et al. 2018). Classical autotransporters have a conserved β -barrel domain at their C-terminus that together with BamA is required for translocation of the functional auto-transporter domain across the OM in a coordinated assembly/translocation mechanism that has yet to be fully elucidated.

Possibly, compounds can be found that specifically inhibit the autotransporter-related function of BAM rather than blocking β -barrel OMP assembly in general. Such compounds could be considered as anti-virulence drugs that may also offer distinct advantages over generic bactericidal antibiotics (Dickey, Cheung and Otto 2017; Martínez et al. 2019).

In conclusion, the BAM complex is an intriguing and under-explored target for the development of bactericidal and anti-virulence drugs from a variety of possible sources (synthetic small molecules, natural products, peptides, bacteriocins and antibodies) that may act alone or in combination with other drugs to attack the shield and Achilles' heel of gram-negative pathogens.

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