AAC 471-2012

Screen for Agents that Induce Autolysis in *Bacillus subtilis*

Christopher J. Lacriola, Shaun P. Falk, and Bernard Weisblum*

Department of Medicine, University of Wisconsin School of Medicine and Public Health Madison, Wisconsin 53706

Running title: Autolysis induction in *Bacillus subtilis*

Key words: *Bacillus subtilis*, autolysis, high throughput screen, reporter

*Corresponding author

Department of Medicine
University of Wisconsin School of Medicine and Public Health
1300 University Avenue
Madison, Wisconsin 53706

608-262-0972 (desk)
608-263-2490 (lab)
weisblum@wisc.edu (email)
ABSTRACT

The growing prevalence of antibiotic-resistant infections underscores the need to discover new antibiotics and to use them with maximum effectiveness. In response to these needs, we describe a screening protocol for the discovery of autolysis-inducing agents that uses two *Bacillus subtilis* reporter strains, SH-536, and BAU-102. To screen chemical libraries, autolysis-inducing agents were first identified with a BAU-102-based screen and then subdivided with SH-536 into two major groups - those that induce autolysis by their direct action on the cell membrane, and those that induce autolysis secondary to inhibition of cell wall synthesis. SH-536 distinguishes between the two groups of autolysis-inducing agents by synthesizing and then releasing β-Gal in late stationary phase at a time that cells have nearly stopped growing and are therefore tolerant of cell wall synthesis inhibitors. Four hits, named “compound 2”, “compound 3”, “compound 5”, and “compound 24”, obtained previously as inducers of autolysis by screening a 10,080 compound discovery library with BAU-102, were probed with SH-536 and found to release β-Gal, indicating that their mode of action was to permeabilize the *B. subtilis* cell membrane. The four primary hits inhibited growth in *Staphylococcus aureus*, *Enterococcus faecium*, *Bacillus subtilis*, and *Bacillus anthracis* with minimum inhibitory concentration (MIC) in the 12.5 - 25 µg/mL (20 - 60 µM) range. The four primary hits were further used to probe *B. subtilis* and their action was partially characterized with respect to the dependence of induced autolysis on specific autolysins.
INTRODUCTION

Bacterial resistance has rendered antibiotics ineffective against many infectious diseases that were previously curable. Attempts to cope with emergent resistance have stimulated the search for new targets and tools with which to probe them. Autolysis is a major mechanism by which antibiotics exert their bactericidal effect; however, autolysis, as a target for screening to discover new anti-infective agents has been neglected.

During the 1960’s, the β-lactam and glycopeptide antibiotics were found to inhibit bacterial cell wall synthesis. For a review, see [15]. The absence, in mammals, of genes that are either orthologous or paralogous to those genes that encode the cell wall synthesis pathway in bacteria, fed expectations that the cell wall synthesis pathway would offer a rich source of valid targets for antimicrobial drug discovery, and that such targets would be associated with low toxicity, based on mechanism of action. For reviews describing the range of potential cell wall-specific targets and the screening assays with which to discover them, see [8, 22, 24, 25, 26, 27].

Of significance for the present studies was the discovery [14, 32] that the bactericidal action of penicillin was actually due to induction of autolysis and that cell wall synthesis inhibition was an intermediate step in the process. The autolytic pathway should be useful, therefore, as a broader target for the discovery of new anti-microbial agents, but that potential has not yet been realized. The autolysis and cell wall synthesis pathways work in concert, and bacterial cells die when cell wall degradation mediated by the autolytic enzyme pathway outpaces cell wall synthesis. A direct link between cell wall damage and activation of dormant autolysins is an alternative model.
One area of intense research activity has been the attempt to replace the growing list of antibiotics that have become ineffective with cationic antimicrobial peptides (CAMPs) - naturally occurring host defense peptides [2, 6, 20]. Resistance to CAMPs develops slowly, and they appear to induce autolysis, though other mechanisms of action have been proposed, e.g., that CAMPs may act on unspecified intracellular targets [6].

Work of Tomasz on penicillin [14, 32], and of Rogers on vancomycin [21], reported that penicillin and vancomycin, long thought to inhibit cell wall synthesis as their primary mode of action, induced autolysis, suggesting autolysis per se as an alternative target for antibiotic screening. The recombinant strain, *B. subtilis* SH-536, constructed by Hoover et al. [10] for use in gene expression studies, appeared to incorporate the attributes needed in a screening strain that would enable a positive identification of membrane-active autolysis inducers based on their ability to synthesize β-Gal during the late-exponential to stationary phase of the growth cycle. The construction of SH-536 incorporated *Escherichia coli lacZ* transcriptionally fused to the *B. subtilis* catalase promoter, PkatA, which was integrated into the *B. subtilis* chromosome at the amyE locus. In the present studies we describe the results of using SH-563 and BAU-102 sequentially to discover members of the amphiphile-surfactant family to serve as probes of bacterial autolysis and possibly also as useful drug leads. Our main goal in this study is to demonstrate and validate the use of *B. subtilis* SH-536 as a reporter whose action is complementary to that of BAU-102, and that will assist in identifying the inducers of autolysis whose action targets the bacterial cell membrane directly.
MATERIALS AND METHODS

Strains. Bacterial strains used in this work are listed in Table 1.

Chemical libraries. A discovery library assembled at Southern Research Institute was screened in our previous studies using *B. subtilis* BAU-102, [3, 4]. Four hits from the discovery library studied in detail in the present work were purchased from InterBioScreen (Moscow, RF). 4-Methylumbelliferyl-β-D-galactopyranoside (MUG) was purchased from Sigma Chemical Company (St. Louis, MO). Stock solutions of test compounds were prepared at 2 mg/mL in DMSO.

Growth-phase-dependent β-Gal expression. Overnight cultures of the reporter strains *B. subtilis* SH-536 or BAU-102 were diluted 1:100 in LB medium. The BAU-102 culture was supplemented with 20% w/v sucrose. Cultures were incubated at 37°C on a rotary shaker at 200 RPM. Two hours after inoculation, 250 µL samples were collected as indicated, and absorbance at 650 nm was recorded using a 96 well Molecular Devices Emax microtiter plate reader. Parallel samples were taken; pellets and supernatant fractions obtained by centrifugation were stored at -20°C. On the following day, samples were thawed and the resuspended pellet and supernatant volumes were adjusted to 250 µL with PBS. Cell-associated β-Gal activity was measured in a microtiter plate by mixing 50 µL of cell suspension with a 50 µL mix consisting of Triton X-100 (2 mg/ml) and MUG, 80 µg/mL in LB medium. Fluorescence emission at 450 nm (F450) with excitation at 385 nm, was recorded after 1 hr incubation at 37°C. Activities were plotted as fluorescence emission relative to cell density, F450/A650, as a function of time and corrected for MUG background fluorescence.

Measurement of induced β-Gal release in SH-536 and BAU-102. Cultures were grown as described above. Upon reaching A660 of either 30 Klett Units (early exponential growth,
for both reporter strains) or 145 or 240 Klett Units (stationary phase for BAU-102 or SH-536, respectively), MUG (4 mg/mL) was diluted 100-fold by addition directly to the culture. A total reaction volume of 100 µL containing 95 µL MUG-supplemented culture and 5 µL test compound stock solution (2 mg/mL), was dispensed into wells. Microtiter plates were incubated at 37°C, and MUG fluorescence emission was measured at 450 nm, with excitation at 385nm, at the times indicated. To plot the data, the fold induction (F450 for MUG-supplemented culture with added test compound)/ (F450 for MUG-supplemented culture alone) - both corrected for background, was calculated, and the resultant ratio used as the abscissa coordinate. The same calculation was made for stationary phase cells and the resultant ratio used to calculate the ordinate value.

**Measurement of antibacterial activity of primary hits.** *B. subtilis* 168, *S. aureus* 1206, *E. faecium* A634 and *B. anthracis* (Sterne) were tested for susceptibility to primary hit compounds 2, 3, 5, and 24. Overnight cultures of the four strains in LB medium were diluted 1:100 in LB medium and 100 µL samples supplemented with the primary hit compounds at the concentrations specified were incubated at 37°C for 18 hours. Absorbance of the test culture at 650 nm was measured using a 96 well microtiter plate in the Molecular Devices Emax microtiter plate reader.

**Autolytic response of Lyt-deficient *B. subtilis* mutants to primary hit compounds.** *B. subtilis* strains 168 (wild type), L16611 (ΔlytABC), L16628 (ΔlytABCDEFG) [17] were grown overnight in LB medium with vigorous aeration, diluted 30-fold into fresh LB medium and grown to A660 = 1.3 in 24-well plastic microtiter plates, 1.5 mL culture volume. 100µL samples were withdrawn, transferred to a 96-well plate, and A650 was read in a Molecular Devices Emax plate reader at times indicated.
RESULTS

Reporter strains to detect membrane-active test compounds. Our previous screen of a 10,080 compound discovery library with a reporter strain, *B. subtilis* BAU-102 yielded 90 primary hits [3], of which four are further analyzed in detail below. We performed a secondary screen to subdivide the hits into those that induce autolysis by membrane disruption and those that induce autolysis secondary to inhibiting cell wall biosynthesis.

The rationale for this procedure is based on the use of a recombinant *B. subtilis* strain SH-536, constructed by Hoover et al. [10], that expresses *E. coli lacZ* transcriptionally fused to, and under control of, the late-acting *B. subtilis* catalase promoter *P_{katA}*. As shown in Fig. 1 (upper panel), β-Gal levels in SH-536 were maximal in late exponential growth, making SH-536 tolerant of cell wall synthesis inhibitors and responsive selectively to the membrane-active group of compounds active throughout the growth cycle. In contrast, BAU-102, expresses β-Gal maximally in early exponential growth, falling off 90% by the time the culture reaches stationary phase, Fig. 1 (lower panel).

Differential responses of *B. subtilis* SH-536 and BAU-102 to autolysis-inducing agents. The responses of *B. subtilis* SH-536 and BAU-102, respectively, to a collection of 33 known bio-actives was tested. Based on two bacterial reporter strains and two experimental time conditions, namely, early exponential and late stationary stages of growth, each test compound generated two pairs of data points, which were sorted in ascending order and listed as shown in Table 2, with the corresponding graphical plots shown in Fig. 2. The samples tested included the four primary hit compounds 2, 3, 5, and 24, obtained as described previously [3]. The response of SH-536 cells to membrane-active compounds, shown in Fig. 2 (upper panel), has a dynamic range of 0.90 - 16.0, whereas the response to cell wall synthesis inhibitors is weak, with a dynamic range of 1.15 - 1.91.
Judging by the relative areas populated by blue circles (membrane-active) and red circles (cell wall synthesis inhibitors), the membrane-active compounds are enriched to 90% of the total. The four hits from our primary screen co-distributed well within the membrane-active group.

In contrast, Fig. 2 (lower panel), BAU-102 showed a dynamic range of 0.86 - 6.39 for abscissa (early exponential) values and a dynamic range of 1.13 - 3.31 for the ordinate (late stationary) values. The BAU-102 early exponential response, however, did not allow us to distinguish between membrane active compounds shown in blue, and cell wall synthesis inhibitors, shown in red, despite the relatively bigger dynamic range. The main function of BAU-102 was to perform the primary identification of autolysis inducers in the library.

The structures and respective structure identity numbers (SID) of compounds 2, 3, 5, and 24 are shown in Fig. 3. Interestingly, compound 2 recapitulates the pattern of a pharmacophore derivatized through an aliphatic chain ester or amide linkage, in this case octanoyl, partially resembling daptomycin (with a decanoyl side chain) and telavancin (with a decylamino side chain). The four compounds were examined in further detail with respect to their antibacterial activity and their ability to induce autolysis in Lyt-deficient mutants.

Antibacterial activity of primary hit compounds. The autolysis-inducing activity of compounds 2, 3, 5, and 24 against B. subtilis 168, E. faecium A463, S. aureus 1206, and B. anthracis (Sterne) was tested. Results, shown in Fig. 4 indicate a MIC of 12.5 - 25 µg/mL (20-60 µM) for compounds 2, 3, 5, and 24.

Correlation between lyt mutations and cell survival in response to primary hit compounds. The availability of Lyt-deficient mutants [17] obtained by targeted insertional inactivation, allows us to try to learn Lyt enzyme requirements for autolysis induced by test
compounds. Two *B. subtilis* recombinant constructs were selected for our initial analysis, L16611 (LytABC-deficient), and L16628 (LytABCDEF-deficient). The two strains were incubated with the probe indicated, following which autolysis was measured by monitoring turbidity A650. Results shown in Fig. 5 indicate that autolysis in the LytABC-deficient strain was delayed in onset and occurred at a reduced rate compared to the wild-type strain. In contrast the LytABCDEF-deficient strain probed similarly showed minimal or delayed autolysis, at best. Thus it may be possible to use the Lyt-knockout strains to identify autolysins specifically needed for the activity of individual hits.
DISCUSSION

The use of high throughput screening techniques has played a major role in modern drug discovery. Despite the potential of this approach, its application does not appear to have generated the large number of drugs expected, particularly new-chemical-entity antibacterial agents that are needed to cope with the ever-increasing number of emergent resistant strains. Besides the lack of financial incentives to industry, candidates for specific bottlenecks limiting the discovery of new anti-infective drugs include, insufficient complex targets, insufficient library diversity, inappropriate match between screening library and target. In the studies described above, we report the development of a screen based disruption of the cell membrane, which, in turn, triggers autolysis.

The diversity of compounds we have studied that induce autolysis, summarized in Fig. 2 and Table 2, underscores the question why a primary autolysis pathway-based screen was not developed sooner. Rather, high throughput assay development efforts have concentrated on developing screens to discover inhibitors of individual enzymes of the cell wall synthesis pathway. The variety of cell wall synthesis pathway enzymes proposed as target, has been reviewed by Green [8] and by Silver [24, 25, 26, 27].

The goal of discovering membrane-active inducers of autolysis is an important one to pursue because the window of opportunity for the most effective use of cell wall synthesis inhibitors is limited, as shown by Tuomanen et al. [36], to the period of the most rapid rate of bacterial growth; non-growing (stationary) cells become tolerant of cell wall synthesis inhibitors, which is of importance in treating patients with bacterial endocarditis or with implanted prosthetic devices that become infected. Daptomycin kills stationary phase cells of S. aureus, as reported by Mascio et al. [18].
In its most recent form, the screen we have developed has two major objectives: (a) the discovery of probes that will be useful in forward and reverse genetic studies of autolysis irrespective of their clinical application, and (b) the identification and characterization of probes with potential anti-infective activity, primarily those that act directly on the cell membrane.

Concentration used for screening. The MICs for compounds 2, 3, 5, and 24 were shown to be in the 12.5 - 25 µg/mL range. Depending on the information sought, different concentrations have to be used in experimental studies. For example, 100 µg/mL used in these studies provided the most consistently interpretable set of responses with known bioactives. Testing BAU-102 for β-Gal release at (lower) probe concentrations around the MIC (20 µg/ml) gave erratic results (data not shown). Screening at higher concentrations, however, can also produce unexpected results. Feng et al. [5] have analyzed “promiscuous” inhibitors, compounds that are inactive at low concentrations, but become active owing to the formation of aggregates at high drug concentrations. In contrast, daptomycin provides an instructive example in which screening compounds at a high working concentration is favored on experimentally justified grounds. Kelleher et al. [13], have noted that daptomycin micelles, formed at 100-1000 µg/mL that “can be used therapeutically”, as the preferred embodiment of their commercial preparation of daptomycin. To avoid false positive or false negatives obtained as a concentration artefact, Inglese et al. [11] have proposed “quantitative high throughput screening”, (qHTS), in which each library test compound is tested as many as 30 times - 10 concentrations, each tested in triplicate.

Can inducers and autolysins can be functionally paired? The availability of numerous autolysis-inducing agents described in published studies, [23, 33, 34, 35], acting on as many as 36 potential autolysins [1, 29, 30], prompts us to inquire as to whether it is possible to match up cognate combinations of autolysins and inducers. A possible interpretation of
the observations shown in Fig. 5a is that some combination of LytA, B, or C together with some combination of LytD, E, or F mediate autolysis induced by Triton X-100. In contrast, cephalixin and compound 24, Fig. 5b,c, show less dependence on LytD, E, or F, since LytABC and LytABCDEF are closer in their response to the two antibiotics than they are in response to Triton X-100. These results suggest that it may be possible to associate autolysis inducers with individual autolysins, or combinations thereof.

Membrane-active compounds such as the polyene antibiotics have been thought to act by, (a) insertion into the lipid membrane, (b) aggregation within the membrane and rearrangement to form pores, and (c) leakage of a vital cell constituent, notably K+, through the pores, and (d) collapse of the electrical gradient leading to cell death. For a recent exposition in the context of amphotericinB-membrane interaction, see Neumann et al. [19]. Silverman et al. [28] proposed a model based on a similar set of steps in which daptomycin interaction with the bacterial cell membrane forms pores through which cellular potassium leaks, leading to collapse of the electric gradient and cell death [16].

Membrane-active compounds may likewise induce autolysis by a mechanism resembling that of the method by which phage escape from a bacterial cell upon the completion of maturation. Gründling et al. [9] have postulated that lambda phage induced bacteriolysis occurs as a consequence of the escape of phage-induced lysozyme through holes in the cell membrane that allow the lysozyme to access and digest the bacterial cell wall. According to the model, holes in the cell membrane occur depending on the pattern of aggregation of phage-induced membrane proteins, holins. The aggregation of holins in the membrane, in turn, is favored by a decrease in the energized state of the membrane. It will be interesting to learn the extent to which the behavior of chemical inducers of autolysis is similar to that proposed for holins.
ACKNOWLEDGEMENTS

These studies were supported in part by Grant R03-AI079638 from the US National Institutes of Health

LITERATURE CITED


Fig. 1. ß-Gal activity in *B. subtilis* strains BAU-102 and SH-536 as a function of the stage of growth. Variations in ß-Gal as a function of the stage of growth, was measured in *B. subtilis* BAU-102 and SH-536. Samples were taken hourly after inoculation, as indicated, and centrifuged to separate cells from growth medium. ß-Gal activity was measured fluorimetrically with added 4-methyl umbelliferyl galactoside following addition of Triton X-100. (upper panel) *B. subtilis* BAU-102, (lower panel) *B. subtilis* SH-536.

Fig. 2. ß-Gal release induced by a selection of known bio-actives in *B. subtilis* strains SH-536 and BAU-102. ß-Gal release in *B. subtilis* strains SH-536 and BAU-102 in response to a set of antibacterial compounds at a concentration of 100 µg/mL was compared as a function of the growth stage during the test. Data points for cell wall synthesis inhibitors are colored red. Membrane-actives are colored blue. The fold induction of ß-Gal, \( \frac{[F450 \text{ of (culture + MUG + test compound)}]}{[F450 \text{ of (culture + MUG)}]} \) was determined for early exponential cells (abscissa) and late exponential cells (ordinate) in BAU-102 and SH-536, respectively. Incubation time was 90 min. (upper panel) *B. subtilis* BAU-102, (lower panel) *B. subtilis* SH-536.

Fig. 3. Chemical structures of four selected primary hits. Four primary hits, designated as “compound 2, 3, 5, and 24”, respectively were obtained in a BAU-102 screen of 10,080 compounds and were retested as shown in Fig. 1 [3, Supplementary data]. Their respective chemical structures and Pubchem Structural Identifiers are: compound 2, SID: 26659846, compound 3, SID: 26667283, compound 5: SID: 26663912, and compound 24, SID: 26726160.
Fig. 4. Susceptibility of gram-positives to compounds 2, 3, and 5. Minimum inhibitory concentration of compounds 2, 3, and 5, discovered with the aid of the reporter strain *B. subtilis* BAU-102, was determined for (a) *B. subtilis* 168, (b) *E. faecium* A643, (c) *S. aureus* 1206, and (d) *B. anthracis* (Sterne).

Fig. 5. Lyt requirement for induced autolysis. Dependence of autolysis on multiple autolysins. Induction of autolysis in two autolysin knock-out mutants (*lytABC*) and (*lytABCDEF*), was tested using, (a) Triton X-100, (b) Cephalexin, and (c) Compound 24 - all at 100 µg/mL. (♦) *lytABCDEF*, (■) *lytABC*, (▲) wt
TABLE LEGENDS

Table 1. Strains used in these studies.

Table 2. β-Gal release induced by known bio-actives. The data plotted in Fig. 2 are collated in Table 2, above. See Fig. 2 legend for experimental details. Numerical data are organized and plotted as pairs which express the fold induction of β-Gal release during the early exponential and late stationary phases, respectively, screening with SH-536 (left panel) and BAU-102 (right panel). To see the dynamic range of induction values for the known bio-actives, fold-induction data were sorted as pairs in ascending order - (a) the late stationary data points as the basis for sorting with SH-536 (left panel), and (b) the early exponential data points as the basis for sorting with BAU-102 (right panel). Cell wall synthesis inhibitor names are bolded.
Fig 1

Relative β-Galactosidase activity (F450/A650) over time for SH-536 and BAU-102. The graphs show the growth, pellet, and supernatant profiles over the time period of 0 to 8 hours. SH-536 shows a higher peak in A650 (Klett Units) compared to BAU-102. The relative activity is depicted in the graphs with black and blue markers for SH-536 and BAU-102, respectively.
FIG. 2

Stationary cells, fold induction of β-Gal

Early exponential cells, fold induction of β-Gal
**Figure 4**

(a) *B. subtilis*

(b) *E. faecium*

(c) *S. aureus*

(d) *B. anthracis*

Absorbance (660 nm) vs. Compound Concentration (µg/ml) for different bacterial strains treated with control and various compounds (Cpd2, Cpd3, Cpd3/5, Cpd4, Cpd24).
Fig 5

(a) Triton X-100

(b) Cephalexin

(c) Compound 24
<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Reference or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis 168</td>
<td>wild type trypC2 met</td>
<td>Bacillus Genetic Stock Center</td>
</tr>
<tr>
<td>B. subtilis BAU-102</td>
<td>vanSR, PvanH::lacZ</td>
<td>[37]</td>
</tr>
<tr>
<td>B. subtilis SH-536</td>
<td>P\text{katA}::lacZ</td>
<td>[10]</td>
</tr>
<tr>
<td>B. subtilis L16611</td>
<td>ΔlytABC</td>
<td>[17]</td>
</tr>
<tr>
<td>B. subtilis L16628</td>
<td>ΔlytABC lytD lytE lytF</td>
<td>[17]</td>
</tr>
<tr>
<td>E. faecium A643</td>
<td>vanA inducibly resistant</td>
<td>[7]</td>
</tr>
<tr>
<td>S. aureus 1206</td>
<td>ermA, erythromycin-inducible</td>
<td>[38]</td>
</tr>
<tr>
<td>B. anthracis Sterne</td>
<td>pXO2-deficient, non-pathogenic</td>
<td>[12]</td>
</tr>
</tbody>
</table>
Table 2  β-Gal release induced by known bio-actives

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Late</th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6-Aminopenicillanic acid</td>
<td>1.15</td>
<td>0.90</td>
<td>Moenomycin</td>
</tr>
<tr>
<td>2</td>
<td>Telomycin</td>
<td>1.31</td>
<td>0.91</td>
<td>Polymixin nonapeptide</td>
</tr>
<tr>
<td>3</td>
<td>Cycloserine</td>
<td>1.21</td>
<td>0.94</td>
<td>Circulin</td>
</tr>
<tr>
<td>4</td>
<td>Cloxacillin</td>
<td>1.38</td>
<td>0.97</td>
<td>Antimycin</td>
</tr>
<tr>
<td>5</td>
<td>Phosphomycin</td>
<td>1.17</td>
<td>0.98</td>
<td>6-aminoopenicillanic acid</td>
</tr>
<tr>
<td>6</td>
<td>Gramicidin D</td>
<td>1.22</td>
<td>0.99</td>
<td>Phosphomycin</td>
</tr>
<tr>
<td>7</td>
<td>Circulin</td>
<td>0.99</td>
<td>1.00</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>8</td>
<td>Moenomycin</td>
<td>1.20</td>
<td>1.03</td>
<td>Clofazamine</td>
</tr>
<tr>
<td>9</td>
<td>Amoxicillin</td>
<td>1.29</td>
<td>1.05</td>
<td>Ristomycin</td>
</tr>
<tr>
<td>10</td>
<td>Amphotomycin</td>
<td>1.13</td>
<td>1.08</td>
<td>Bacitracin</td>
</tr>
<tr>
<td>11</td>
<td>Polymixin nonapeptide</td>
<td>0.99</td>
<td>1.12</td>
<td>Telomycin</td>
</tr>
<tr>
<td>12</td>
<td>Aspatocin</td>
<td>1.13</td>
<td>1.13</td>
<td>Cycloserine</td>
</tr>
<tr>
<td>13</td>
<td>Ampicillin</td>
<td>1.76</td>
<td>1.13</td>
<td>Cloxacillin</td>
</tr>
<tr>
<td>14</td>
<td>Vancomycin</td>
<td>1.09</td>
<td>1.17</td>
<td>Amoxacillin</td>
</tr>
<tr>
<td>15</td>
<td>Cephalexin</td>
<td>1.94</td>
<td>1.26</td>
<td>Gramicidin D</td>
</tr>
<tr>
<td>16</td>
<td>Telavancin</td>
<td>1.94</td>
<td>1.48</td>
<td>Amphotomycin</td>
</tr>
<tr>
<td>17</td>
<td>Ristomycin</td>
<td>1.08</td>
<td>1.66</td>
<td>Aspatocin</td>
</tr>
<tr>
<td>18</td>
<td>Clofazamine</td>
<td>1.12</td>
<td>2.00</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>19</td>
<td>Bacitracin</td>
<td>1.14</td>
<td>2.37</td>
<td>Cephalexin</td>
</tr>
<tr>
<td>20</td>
<td>Antimycin</td>
<td>1.57</td>
<td>2.66</td>
<td>Daptomycin</td>
</tr>
<tr>
<td>21</td>
<td>Nisin</td>
<td>1.81</td>
<td>4.18</td>
<td>Nigericin</td>
</tr>
<tr>
<td>22</td>
<td>Nigericin</td>
<td>2.05</td>
<td>4.27</td>
<td>Telavancin</td>
</tr>
<tr>
<td>23</td>
<td>Compound 24</td>
<td>1.58</td>
<td>5.45</td>
<td>Compound 24</td>
</tr>
<tr>
<td>24</td>
<td>Glycerol monolaurate</td>
<td>2.20</td>
<td>7.86</td>
<td>Compound 3</td>
</tr>
<tr>
<td>25</td>
<td>Daptomycin</td>
<td>1.68</td>
<td>8.41</td>
<td>Nisin</td>
</tr>
<tr>
<td>26</td>
<td>Compound 2</td>
<td>1.57</td>
<td>8.41</td>
<td>Triton X-100</td>
</tr>
<tr>
<td>27</td>
<td>Compound 5</td>
<td>1.88</td>
<td>10.15</td>
<td>Miconazole</td>
</tr>
<tr>
<td>28</td>
<td>Triton X-100</td>
<td>1.94</td>
<td>13.54</td>
<td>Na docecyl sulfonate</td>
</tr>
<tr>
<td>29</td>
<td>Compound 3</td>
<td>1.80</td>
<td>13.83</td>
<td>Compound 5</td>
</tr>
<tr>
<td></td>
<td>Na doceyl sulfonate</td>
<td></td>
<td>Glycerol monolaurate</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>---------------------</td>
<td>-------</td>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>1.24</td>
<td>14.71</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Tyrothricin</td>
<td>1.54</td>
<td>14.82</td>
<td>Miltefosine</td>
</tr>
<tr>
<td>32</td>
<td>Miconazole</td>
<td>1.95</td>
<td>15.14</td>
<td>Tyrothricin</td>
</tr>
<tr>
<td>33</td>
<td>Miltefosine</td>
<td>1.91</td>
<td>16.03</td>
<td>Compound 2</td>
</tr>
</tbody>
</table>