RX-P873, a novel protein synthesis inhibitor, accumulates in human THP-1 monocytes and is active against intracellular infections by Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria.

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**Running title**: Intracellular activity of RX-P873

**Keywords**: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, monocytes, intracellular, Hill equation, RX-P873

**Paper metrics**

Abstract word count: 248

Text word count: 3079

References: 44

Number of figures: 6

Number of Tables: 1

Supplemental material: none

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ABSTRACT

The pyrrolocytosine RX-P873, a new broad-spectrum antibiotic in preclinical development, inhibits protein synthesis at the translation step. The aims of this work were to study RX-P873’s ability to accumulate in eukaryotic cells, together with its activity against extracellular and intracellular forms of infections by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using a pharmacodynamic approach allowing the determination of maximal relative efficacies [E\text{max}] and bacteriostatic concentrations [C_s] based on Hill equations of concentration-response curves. RX-P873’s apparent concentration in human THP-1 monocytes was about 6-fold higher than the extracellular one. In broth, MICs ranged from 0.125 to 0.5 mg/L (*S. aureus*) and 2 to 8 mg/L (*P. aeruginosa*), with no significant shift in these values against strains resistant to currently-used antibiotics. In concentration-dependent experiments, the pharmacodynamic profile of RX-P873 was not influenced by the resistance phenotype of the strains. E\text{max} values (expressed as CFU decrease from the initial inoculum) against *S. aureus* and *P. aeruginosa* reached more than 4 log and 5 log in broth, respectively, and 0.7 log and 2.7 log in infected THP-1 cells, respectively, after 24 h. C_s values remained close to the MIC in all cases, making RX-P873 more potent than antibiotics to which the strains were resistant (moxifloxacin, vancomycin, daptomycin for *S. aureus*; ciprofloxacin, ceftazidime for *P. aeruginosa*). Kill curves in broth showed that RX-P873 was more rapidly bactericidal against *P. aeruginosa* than against *S. aureus*. Taken together, these data suggest that RX-P873 may constitute a useful alternative for infections involving intracellular bacteria, especially Gram-negative species.
INTRODUCTION

Bacterial resistance is spreading worldwide, which makes therapeutic options scarce in many circumstances and can lead to therapeutic failures. In this context, the discovery and development of antibiotics acting on novel, still unexploited targets is a priority, and is recognized as such by both European and American agencies or by scientific societies (1-3).

The pyrrolocytosine RX-P873 (see calculated ionization and octanol-water partition coefficients in Figure 1) is a new antibiotic in preclinical development that shows an innovative mode of action, inspired by the analysis of the crystal structure of the translation inhibitor blasticidin (4) in interaction with peptidyl-tRNA. RX-P873 inhibits bacterial protein synthesis at the translation step by stabilizing a distorted binding conformer of peptidyl-tRNA (5). Preliminary data with this compound or others in the series report (a) a broad spectrum of activity, including multidrug resistant Gram-positive or Gram-negative organisms as well as biodefense pathogens (6-9) and (b) a bactericidal activity (10,11), which is classically not observed for inhibitors of protein synthesis, except aminoglycosides.

Intracellular survival is clearly part of the life cycle of biodefense pathogens like Bacillus anthracis (12), Yersinia pestis (13), Francisella tularensis (14), and Burkholderia pseudomallei or mallei (15). It is also widely recognized as a reason for recurrence or persistence of infections caused by common human pathogens like Staphylococcus aureus (16,17) or Pseudomonas aeruginosa (18,19).

As a first attempt to determine the potential interest of RX-P873 to act upon these intracellular bacteria, the aim of the present study was to determine the intracellular activity of this molecule (as compared to its activity in broth) against S. aureus and P. aeruginosa, taken as exemplary Gram-positive and Gram-negative bacteria, respectively, in relation to its capacity to accumulate within phagocytic cells. Using multi-resistant strains and previously developed in vitro pharmacodynamic models to
assess the intracellular activities of antibiotics (18,20), we showed that the activity of RX-P873 in human THP-1 macrophages is unaffected by resistance mechanisms to other drugs commonly used to treat infections caused by these organisms. RX-P873 proved cidal in broth. In cells, RX-P873 was bacteriostatic for an extracellular concentration close to its MIC and reduced by 1 to 3 log CFU the intracellular inocula of *S. aureus* and *P. aeruginosa*, irrespective of their resistance profiles to other antibiotics. This intracellular activity of RX-P873 may be related to its ability to accumulate approx. six-fold within THP-1 monocytes.
MATERIALS AND METHODS

Cells
Studies were performed using THP-1 human monocytes (21) grown in suspension in RPMI-1640 medium supplemented with 10 % fetal bovine serum in a 5 % CO₂ atmosphere.

Cellular accumulation of RX-P873
THP-1 cells were incubated at a density of 3. x 10⁶ cells/mL during 2 h with RX-P873, collected by low speed centrifugation, washed twice in cold Phosphate Buffered Saline (PBS), pelleted, resuspended in 1 mL H₂O, and frozen at -20°C. Samples were unfrozen the day they were assayed, lysed by sonication and kept on wet ice. Standards were prepared from cell lysates spiked with known amounts of RX-P873 and treated as samples to construct calibration curves. RX-P873 was assayed by fluorimetry. Proteins from samples and standards were precipitated using acetonitrile (800 µL added to 400 µL of cell lysate; incubation for 1 h at -20°C). Samples were then centrifuged 10 min at 14.000 g; supernates were collected, evaporated to dryness and reconstituted in 100 µL acetonitrile. Fifty µL of standards or of samples were then transferred in 96-well plates and fluorescence was measured (λ<sub>exc</sub> = 280 nm; λ<sub>em</sub> = 450 nm) using a Spectramax reader (Molecular Devices, Sunnyvale, CA). The assay was linear in the 0.2 to 10 mg/L concentration range (R² 0.9959; lower limit of quantification: 0.2 mg/L).
RX-P873 concentrations in samples were expressed by reference to the total protein content, as determined using the method of by the Folin-Ciocalteu/biuret method (Biorad kit 500-0113 & 500-0114; Hercules, CA).
Bacterial strains

The strains used in this study are shown in Table 1. They include reference strains and clinical isolates obtained via collaborating clinical microbiology laboratories. Bacteria were routinely grown in Mueller-Hinton broth and CFU counting was performed by plating on Tryptic Soy Agar.

Determination of Minimal Inhibitory Concentrations (MICs) and concentration-kill curves studies in extracellular medium.

MICs were measured by serial two-fold microdilution according to CLSI guidelines in cation-adjusted Mueller-Hinton broth (22). Extracellular activity was assessed with a starting inoculum of 10^6 CFU/mL in Mueller-Hinton broth, as previously described (18,20).

Assessment of cell viability.

Viability of THP-1 cells was evaluated by measuring the release of the cytosolic enzyme lactate dehydrogenase in the culture medium (Cytotoxicity Detection KitPLUS [LDH], Roche Diagnostics GmbH, Manheim, Germany) after 24 h of incubation in the presence of increasing concentrations of RX-P873.

Intracellular infection and assessment of antibiotic intracellular activity

These experiments were performed according to the procedures described in details for Staphylococcus aureus (20) and Pseudomonas aeruginosa (18), respectively. In brief, bacteria were opsonized by a 30 min (S. aureus) or 60 min (P. aeruginosa) incubation at 37°C with 10% human serum in RPMI-1640. Phagocytosis was then allowed for 1 h with an inoculum of 4 bacterial per cell for S. aureus and during 2 h with an inoculum of 10 bacteria per cell for P. aeruginosa. Medium was removed, cells were washed once with PBS and incubated during 45 min with gentamicin at 100 X its MIC to eliminate extracellular bacteria, washed 3 x with PBS to eliminate
gentamicin, and reincubated during 24 h with increasing concentrations of antibiotics. At the end of the incubation period, cells were washed with PBS and collected in H₂O. CFU were determined by plating and proteins were assayed by the method of Lowry. Data are expressed as changed from the initial post-phagocytosis inoculum (typically ~ 10⁶ CFU/mg cell protein).

Materials
RX-P873 was provided by Melinta Therapeutics (New Haven, CT). The other antibiotics were obtained as microbiological standards from their corresponding manufacturers (ciprofloxacin [chlorhydrate; potency, 85%] and moxifloxacin [chlorhydrate; potency, 91%] from Bayer AG; Wuppertal, Germany) or as commercial products registered in Belgium for parenteral use from their respective marketing authorization holders or resellers as Gentalline® (Gentamicin; Schering-Plough; Brussels, Belgium), vancomycin (Mylan, Hoeilaart, Belgium), Zyvoxid® (linezolid, Pfizer Inc, Brussels, Belgium), Cubicin® (Cubist; Paris, France), and Glazidim® (ceftazidime; Glaxo-SmithKline, Genval, Belgium). Colistin (sulphate salt; potency 67.50%) and oxacillin (potency, 81.5 %) were purchased from Sigma-Aldrich (St. Louis, MO). Unless stated otherwise, all other reagents were of analytical grade and were purchased from Sigma-Aldrich-Fluka. Cell culture or microbiology media were from Invitrogen (Paisley, Scotland) and BD Diagnostics (Sparks, MD).

Statistical analyses, curve fittings, and software
Statistical analyses, curve fittings and calculations of the corresponding regression parameters were performed using GraphPad Prism (version 6.05) software for Windows (GraphPad Prism Software, San Diego, CA). More specifically, the Hill equations of the concentration-response curves were used to calculate the maximal efficacies (Eₘₐₓ; maximal decrease in CFU counts [in log₁₀ units] from the corresponding initial inoculum as extrapolated from infinitely large antibiotic
concentration), and the static concentrations (\(C_s\); extracellular concentration resulting in no apparent bacterial growth [number of CFU identical to the initial inoculum]) of each drug for each strain). Physicochemical properties of antibiotics were calculated using Reaxys® software version 2014, Elsevier.
RESULTS

Accumulation of RX-P873 in THP-1 human monocytes

Figure 2 shows the accumulation of RX-P873 in THP-1 cells incubated with different extracellular concentrations during 2 h. The cellular concentration of the antibiotic increased linearly as a function of the extracellular one in the range investigated, leading to an apparent accumulation factor similar for the three extracellular concentrations tested (mean value: 6.3 ± 0.7).

MICs of RX-P873 and comparator antibiotics

Table 1 shows the MICs of RX-P873 in comparison with antibiotics representative of the main classes currently used in clinics (moxifloxacin, oxacillin, linezolid, daptomycin, vancomycin for *S. aureus*; gentamicin, ciprofloxacin, ceftazidime and colistin for *P. aeruginosa*) against a series of laboratory and clinical strains of *S. aureus* and *P. aeruginosa*. Considering first activity on *S. aureus*, MICs of RX-P873 ranged from 0.125 to 0.5 mg/L, irrespective of the phenotype of resistance of the strains to other antibiotics. Against *P. aeruginosa*, MICs were higher (0.5-4 mg/L), but again not affected by resistance to other drugs. Of note, the lowest MIC was observed for the strain PAO509 that does not express active efflux systems.

Intracellular activity of RX-P873 against *S. aureus* and *P. aeruginosa*

The intracellular activity of RX-P873 was then evaluated against reference strains (*S. aureus* ATCC25923 and *P. aeruginosa* PAO1), selected clinical isolates showing multidrug resistance (VISA SA618bis also resistant to moxifloxacin and daptomycin; MRSA NRS119 also resistant to moxifloxacin and linezolid; *P. aeruginosa* PA256 resistant to ceftazidime and ciprofloxacin) or hypersusceptibility due to the absence of efflux (*P. aeruginosa* PAO509). To this effect, infected THP-1 cells were exposed during 24 h to a broad range of antibiotic concentrations in order obtain full dose-
response effects and to calculate the corresponding pertinent pharmacodynamics parameters for each antibiotic – strain combination (18,20). The concentration of RX-P873 in the culture medium of THP-1 cells was limited to 50 mg/L to avoid undue cellular toxicity (less than 15 % LDH release, i.e. twice the value measured for control cells). The results are shown graphically in Figure 3 and the values of the corresponding pharmacodynamic parameters in Figure 4. As previously described, concentration-effect relationships followed sigmoidal responses for all antibiotics and against both bacteria.

Against S. aureus (Figure 3, left panels), moxifloxacin was the most effective antibiotic (Emax of 1.7, 1.2, and 2.1 log10 against ATCC25923, SA616bis, and NRS 119, respectively). The other drugs, including RX-P873, caused globally 0.5 to 1-log10 decrease in the intracellular inoculum for all strains. While moxifloxacin was the most potent (lowest Cs) against the susceptible strain ATCC25923, RX-P873 was the most potent against the two resistant strains.

Against P. aeruginosa (Figure 3, right panels), ciprofloxacin reduced the intracellular inoculum by 3-log10 for the wild-type strain PAO1 as previously described (18) and by about 4-log10 for the hypersusceptible strain PAO509. For the resistant strain PA256, a 2-log10 decrease at the highest concentration tested was observed (no plateau could be reached). Ceftazidime caused only a 2-log10 reduction in intracellular inoculum for all strains. In contrast, RX-P873 was bactericidal against both the susceptible strain PAO1 and the hypersusceptible strains PAO509, and decreased the intracellular inoculum of 2.5 log10 against the multi-resistant strain PA256 at the highest concentration tested. It was much more potent (lowest CFU50) than both ciprofloxacin and ceftazidime against this resistant strain. Focusing on relative potencies, Figure 4 (upper panels) shows that static concentrations of each antibiotic were always close to their respective MIC values, and therefore significantly higher for antibiotics to which strains were resistant (except for vancomycin against SA618bis, probably due to the small difference in MIC (2 dilutions only compared to
ATCC25923). RX-P873 static concentrations were similar among strains from the same species (in accordance with its almost unchanged MIC) and slightly higher against *P. aeruginosa* than against *S. aureus* (again in accordance with its higher MIC against the first species). When moving to maximal relative efficacies (Figure 4, lower panels), we did not observe systematically a reduction in this parameter in resistant strains. Fluoroquinolones were more effective against both types of intracellular bacteria than other antibiotics used as comparators. Interestingly enough, RX-P873 was as effective as ciprofloxacin against *P. aeruginosa* but less effective than moxifloxacin against *S. aureus*.

**Comparison of the extracellular and intracellular activities of RX-P873 against *S. aureus* and *P. aeruginosa***

Figure 5 compares the activity of RX-P873 against the extracellular and intracellular forms of the same bacterial strains. When drug concentrations were expressed in multiples of the respective MICs, RX-P873 activity was undistinguishable among strains of the same species. Static concentrations were close to the MICs for both the extracellular and intracellular bacteria of both species, with a trend (not statistically significant) to higher values against intracellular forms. As observed for all antibiotic classes in these models, maximal relative efficacies were always lower against intracellular than extracellular bacteria. Noteworthy also, the maximal relative efficacy of RX-P873 was significantly larger (more negative E\text{max}) not only against the intracellular forms, but also against the extracellular forms of *P. aeruginosa* compared to *S. aureus*. More specifically, the limit of detection (5 log\text{10} decrease) was reached at 10 times the MIC against extracellular *P. aeruginosa* while the equivalent concentration reduced the extracellular *S. aureus* inoculum of only 4 log\text{10} units.
In a last set of experiments, we compared the kinetics of bacterial killing by RX-P873 against reference strains of *S. aureus* and *P. aeruginosa*. Figure 6 shows that RX-P873 was bactericidal against both pathogens as soon as its concentrations were higher than the MIC, but this effect developed slower against *S. aureus* than against *P. aeruginosa*, the limit of detection being reached after more than 8 h and only 2 h, respectively.
DISCUSSION

In this paper, we document that a representative of the pyrrolocytosines, a novel class of inhibitors of bacterial protein synthesis, is active against the intracellular forms of both Gram-positive and Gram-negative bacteria, with a potency unaffected by their resistance phenotype to currently used antibiotics. The present work, therefore, brings new information about this class of antibiotics, which we critically examine here.

First, we show that the intrinsic activity of RX-P873, as determined in broth, is similar against wild-type or multi-resistant strains of both S. aureus and P. aeruginosa, which is to be expected for a drug displaying a novel mode of action. Thus, our data are in accordance and expand the observations made with other collections that included different bacterial species but were only reported as posters so far (8,23). We also observed that RX-P873 MICs are approximately 2 dilutions higher against P. aeruginosa than against S. aureus, as was also reported in the above-mentioned studies. It is tempting to speculate that this could be due to a modest but significant outward efflux by the broad-spectrum transporters constitutively expressed in P. aeruginosa because this difference vanished when considering strain PAO509, which does not express multidrug efflux systems. Notably, however, RX-P873 seems less affected by efflux than ciprofloxacin, for which the MIC was 4 dilutions lower in PA0509 than in PAO1.

Second, we show that the intracellular activity of RX-P873 is at a level that makes it as potent and as effective against the tested bacteria whatever their resistance phenotype to other antibiotics. Thus, while fluoroquinolones remain globally more active intracellularly against susceptible strains, our data clearly highlight that RX-P873 may offer a clear advantage when dealing with resistant
strains. However, and as previously described for many other classes of antibiotics, 
even those accumulating within the cells (18,20,24,25), the intracellular static 
concentration of RX-P873 remains close to its MIC as measured in broth. While it is 
premature to ascribe this to insufficient bioavailability (as was suggested for 
fluoroquinolones [26]), it nevertheless points again to the fact that intracellular 
potency and accumulation are not necessarily linked. Yet, it must remain clear that 
intracellular penetration and reaching a critical concentration is a first and necessary 
property for an antibiotic to express intracellular activity.

This brings us to a third observation made in this study, namely that RX-P873
is accumulating in eukaryotic cells, with an apparent cellular concentration 6-fold 
higher than the extracellular one. Of interest, recent pharmacokinetic data in mice 
found concentrations of RX-P873 sustainably higher in the thigh than in the serum, 
with a mean tissular penetration ratio of 6 (27). Although we do not have at this stage 
any clue about the mechanism of the cellular accumulation of RX-P8763 or about its 
subcellular distribution, we can state that the behavior of RX-P873 differs from that of 
other positively-charged antibiotics such as aminoglycosides on the one hand and 
macrolides on the other hand. Aminoglycosides enter only very slowly inside 
eukaryotic cells (28) due to their highly hydrophilic character at the extracellular pH 
(log D ~ -10 to -15 at pH 7.4). In contrast, macrolides or ketolides that are also 
cationic but much more lipophilic at pH 7.4 (log D close to 0 at pH 7.4) easily diffuse 
into cells (29,30) where they eventually are retained by proton trapping in acidic 
compartments (31). The cellular accumulation of a hydrophilic drug such as RX-
P873 (which is mainly under its cationic form at pH 7.4) is thus unexpected.

However, the investigational hydrophilic fluoroquinolone finafloxacin accumulates 
about 8-fold in THP-1 cells in conditions where it is positively-charged (medium at pH 
5.5; calculated log D of -1.1 at this pH value) (32). Thus, whatever the underlying 
mechanism of this accumulation, and pending for further investigations, these data at
least show that the quite hydrophilic character of RX-P873 is not incompatible with cell penetration and subsequent expression of intracellular activity.

The last, and probably more intriguing observation in this study, is that RX-P873 has a much lower relative efficacy (lesser negative $E_{\text{max}}$) against the intracellular forms of *S. aureus* than against the intracellular forms of *P. aeruginosa* although its MICs are lower against *S. aureus* compared to *P. aeruginosa*. In previous work comparing the intracellular activity of different classes of drugs against a same bacterial strain, we documented that relative efficacies are not related to intrinsic activities of antibiotics in broth (as determined by the measurement of their MICs), which is actually predictive of the relative potency, but rather related to the mode of action of the drugs. Thus, highly bactericidal antibiotics tend to bring intracellular CFUs to lower levels than slowly cidal or bacteriostatic ones, regardless of their respective MICs (18,20,33,34). The same reasoning could possibly apply here to RX-P873, as we show a slower bactericidal effect against *S. aureus* than *P. aeruginosa* when grown in broth (as confirmed by independent studies reported as posters so far [10,11]). Yet, in the present study, fluoroquinolones also show a lower maximal relative efficacy against *S. aureus* compared to *P. aeruginosa*. This may denote a difference in response related to the type of bacteria studied. Thus, we previously showed that the intracellular maximal relative efficacy of ciprofloxacin is high (more negative $E_{\text{max}}$) for *Listeria monocytogenes*, intermediate against *P. aeruginosa*, and lower (less negative $E_{\text{max}}$) against *Legionella pneumophila* and *S. aureus* (18,32).

Thus, taken as a whole, this study suggests that RX-P873 may constitute a useful weapon in our future armamentarium, especially for bacteria displaying multi-resistance to currently available antibiotics and capable of surviving intracellularly. More specifically, our work underlines the interest in this molecule against both
against a Gram-positive organism (based on its low MIC), but more strikingly, against a Gram-negative organism, based on its higher intracellular efficacy. This work therefore opens the door to further investigations focusing on other challenging bacterial species.
ACKNOWLEDGMENTS

We are grateful to M.C. Cambier, V. Mohymont, K. Santos, and V. Yfantis for excellent technical assistance. J.M.B. was a postdoctoral fellow on research programs sponsored by the Région Wallonne, Belgium and F.V.B. is Maître de Recherche of the Belgian Fonds National de la Recherche Scientifique. This work was supported by the Fonds de la Recherche Scientifique Médicale (grant 3.4530.12), the Interuniversity Attraction Poles Program initiated by the Belgian Science Policy Office (program IAP P7/28), and a grant from Melinta Therapeutics, New Haven, CT.
REFERENCES


Table 1: susceptibility of reference strains and clinical isolates to RX-P873 and comparators

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<th>Strain</th>
<th>Description and reference</th>
<th>MIC (mg/L)</th>
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<tr>
<td></td>
<td>RX-P873 OXA MXF VAN LZD DAP</td>
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<tr>
<td><strong>S. aureus</strong></td>
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*Abbreviations: CAZ, ceftazidime; CIP, ciprofloxacin; CLR, clarithromycin; CST, colistin; DAP, daptomycin; GEN, gentamicin; LZD, linezolid; MOX, moxifloxacin; OXA, oxacillin; VAN, vancomycin.

*Values in bold are higher than the EUCAST susceptibility breakpoint for registered antibiotics (resistant strain)*
Figure 1

Caption to Figure 1: Chemical structure and ionization status of RX-P873. The graph shows the evolution of the proportions of the two majority microspecies of the molecule in the range of pH it could face in biological environments, as well as the LogD values calculated at pH 5.5 and 7.4 (using Reaxys® software).
Caption to Figure 2: Apparent cellular accumulation of RX-P873 in THP-1 cells incubated during 2 h with increasing extracellular concentrations. The left axis shows the cellular concentration of the drug expressed in ng/mg cell protein. Data were used to fit a linear regression with a slope of 30.0 ± 1.9 ng.mL⁻¹/mg.L⁻¹ and a R² of 0.9923. The right axis shows the apparent cellular accumulation factor calculated using a conversion factor of 5 µL/mg cell prot. All values are means ± standard deviations (SD) of three independent determinations.
Figure 3

Caption to Figure 2: Intracellular activity of RX-P873 and selected comparators (linezolid [LZD], vancomycin [VAN], daptomycin [DAP], moxifloxacin [MXF] or ciprofloxacin [CIP] and ceftazidime [CAZ]) against different strains of *S. aureus* (left), or *P. aeruginosa* (right), as determined after 24 h of incubation with increasing concentrations of each drug. The ordinate shows the change in the number of CFU (log scale) per mg of cell protein as compared to the
initial inoculum. The plain horizontal line corresponds to an apparent static effect; the dotted horizontal line, to the limit of detection. All values are means ± standard deviations (SEM) of 2-3 experiments performed in triplicate (when not visible, the SEM are smaller than the size of the symbols)
Figure 4

Caption to Figure 4: Comparison of intracellular static concentrations (Cs; top) and maximal efficacy (Emax; bottom) for RX-P873 and its comparators as calculated from the sigmoidal regressions of concentration-effects studies shown in Figure 3 (Hill slope = 1 except for ciprofloxacin against PA256, because E_{max} could not be calculated using a Hill slope of 1; highest concentration tested too far from that allowing to reach the plateau value). The horizontal lines with central squares superimposed to Cs values points to MICs values. Statistical analyses: 2-ways ANOVA with Tukey multiple comparison test considering the different strains for each antibiotic: data with different letters are significantly different from one another (p < 0.05). nd: not determined
Figure 5

Caption to Figure 5: Left and middle panels: Extracellular (dotted line) and intracellular (plain line) activity of RX-P873 against different strains of *S. aureus* (left), or *P. aeruginosa* (right), as determined after 24 h of incubation with increasing concentrations of each drug. The ordinate shows the change in the number of CFU (log scale) per mg of cell protein as compared to the initial inoculum; concentrations are expressed in multiple of the MIC for each strain. A single sigmoidal regression was fit to the whole set of data obtained for the three independent strains of each bacterial species. The plain horizontal line corresponds to an apparent static effect; the dotted horizontal line, to the limit of detection, and the dotted vertical line, to the MIC. All values are means ± standard deviations (SEM) of 2-3 experiments performed in triplicate (when not visible, the SEM are smaller than the size of the symbols). Right panel: comparison of static concentration (top) and maximal efficacy (bottom) of RX-P873 against intracellular (plain bars) or extracellular (open bars) bacteria. Statistical analyses: 2-ways ANOVA with Tukey multiple comparison test: data with different letters are significantly different from one another (p < 0.05).
Caption to Figure 6: Influence of time on the rate and extent of activity of RX-P873 against *S. aureus* ATCC25923 (left) or *P. aeruginosa* PAO1 (right), as determined over 24 h of incubation. The ordinate shows the change in the number of CFU (log scale) per mL as compared to the initial inoculum; concentrations are expressed in multiple of the MIC for each strain. The plain horizontal line corresponds to an apparent static effect and the dotted horizontal line, to the limit of detection. All values are means ± standard deviations (SD) of 3 independent determinations (when not visible, error bars are smaller than the size of the symbols).