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Dynamic Time-Kill Curve Characterization of Spectinamide Antibiotics 1445 and 1599 for the Treatment of Tuberculosis

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Abstract

Spectinamides are a novel class of antibiotics under development for the treatment of MDR- and XDR-tuberculosis, with 1599 and 1445 as early lead candidates within this group. In order to evaluate and differentiate the pharmacological properties of these compounds and assist in candidate selection and design of optimal dosing regimens in animal model of *Mtb* infection, time kill curve assessments were performed in a previously established *in vitro* PK/PD model system. The performed studies and subsequent pharmacometric analysis indicate that the anti-mycobacterial activity of 1599 exhibits concentration-dependent killing whereas 1445 shows time-dependent killing. These findings are supported by the fact that the PKPD index that best describes bacterial killing is T>MIC for 1445, but fC_{max}/AUC for 1599. The differential killing behavior among the lead candidates can be rationalized by the differences in post-antibiotic effect: 15.7 h for 1445 compared the 133 h for 1599. Overall, the PK/PD based analysis of the *in vitro* pharmacologic killing profile of spectinamides 1599 and 1445 on mycobacteria provided valuable insights that contributed to lead candidate selection and preclinical development of these compounds.

Graphical abstract

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Keywords

Spectinamides; Antibiotics; Tuberculosis; Time kill; Drug discovery; Mycobacteria

1. Introduction

According to the World Health Organization (WHO) annual report on tuberculosis (TB) (World Health Organization, 2017), infection with *Mycobacterium tuberculosis* (*Mtb*) remains a major burden on the health of a large fraction of the human population. Particularly multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis impose a high level of morbidity and mortality in the affected individuals (World Health Organization, 2017). Chemotherapeutic treatment options for MDR- and XDR-TB are particularly challenging with regard to efficacy, adverse drug reactions, and patient adherence: Recommended treatments include combinations of at least four potentially active anti-tuberculosis drugs, including an injectable aminoglycoside antibiotic, for MDR-TB, and six potentially active drugs for XDR-TB with treatment duration of at least 20 months (Zumla et al., 2015). Although the WHO recommended more recently shorter combination treatments for MDR-TB, these still include seven anti-mycobacterial compounds (World Health Organization, 2016). Thus, there remains an urgent need for more effective, safe, shorter and simpler treatment options of MDR- and XDR-TB.

Spectinamides are a novel class of semi-synthetic anti-tuberculosis agents derived from spectinomycin (Liu et al., 2017), that are currently being developed as antibiotics against MDR/XDR TB. Spectinamides have demonstrated potent activity against *Mtb* *in vitro* and *in vivo* in mouse models of *Mtb* infection by avoiding the mycobacterial efflux pump Rv1258c that renders their parent compound spectinomycin inactive in *Mtb* (Lee et al., 2014). Spectinamides have shown narrow spectrum activity against *Mtb*, lack of cross-resistance with existing anti-tuberculosis therapeutics, are active under hypoxic conditions, and have additive or synergistic activity with most first-line agents (Lee et al., 2014, Robertson et al., 2017). *In vitro*, they also showed activity against MDR- and XDR-TB and an excellent pharmacological profile (Lee et al., 2014, Madhura et al., 2016a, Madhura et al., 2016b).

The pharmacokinetics/pharmacodynamics (PK/PD) of anti-tuberculosis agents and other antibiotics are frequently categorized with three different PK/PD indices based exposure criteria, $fAUC/MIC$, fC_{max}/MIC and $T > MIC$, which rely on a summary measurement of *in vivo* drug exposure relative to the minimum inhibitory concentration (MIC), an *in vitro* parameter that quantifies growth inhibition to a static antibiotic concentration (Rathi et al., 2016, Trivedi et al., 2013). $fAUC/MIC$ is the ratio of area under the free plasma concentration–time curve relative to MIC, fC_{max}/MIC is the ratio of free peak plasma

concentration relative to MIC, and $T > MIC$ is the cumulative percentage of a time period that the free concentration is above MIC. Although the MIC-based approach has been widely used by clinicians for designing dosing regimens and can be useful to predict the effect of dose fractionation on antibacterial activity, it is limited by its lack of appreciating the dynamic interplay between the microorganism and fluctuating drug concentrations as experienced *in vivo* during multiple dose therapies in antimicrobial chemotherapy (Rathi et al., 2016). To overcome these limitations, especially Derendorf and coworkers (Dalla Costa et al., 1997, de la Pena et al., 2004, Nolting et al., 1996), as well as others (Firsov et al., 1997, Mattie and van der Voet, 1979), have promoted mechanism-based PK/PD modeling of dynamic time-kill curves established for multiple dose levels and dosing regimens to thoroughly capture the time-courses of bacterial growth and killing in relation to time courses of drug concentrations and to decipher drug-induced bacterial killing under appreciation of natural bacterial growth kinetics in the absence of any modulating effect by the host immune system (Trivedi et al., 2013).

In this paper, we apply the mechanism-based PK/PD modeling approach of dynamic time-kill curves to characterize the bacterial kill behavior of lead candidate compounds of the spectinamide series. In addition, we investigate the classic PK/PD indices as drivers for their anti-mycobacterial activity. This information will be useful in lead candidate selection and the design and testing of optimal dosing regimens *in vivo*.

2. Material and Methods

2.1. Culture, Media, and Antibiotics

Mycobacterium bovis BCG (ATCC, Manassas, VA) was grown in Middlebrook 7H9 broth (Becton Dickinson, Sparks, MD) supplemented with 10% albumin dextrose complex and 0.1% v/v Tween 80. The cultures were stored at -80°C in broth. For each experiment, cultures were thawed and incubated at 37°C in Middlebrook 7H9 broth until the bacteria reached the logarithmic growth phase ($\text{OD}_{600\text{nm}}$ 0.4–0.7) after approximately 4 days. Spectinamides 1599 (3'-Dihydro-3'-deoxy-3'(R)-(5-chloropyridin-2-yl) acetylamino spectinomycin dihydrochloride) and 1445 (3'-Dihydro-3'-deoxy-3'(R)-(5-fluoropyridin-2-yl) acetylamino spectinomycin dihydrochloride) (Fig. 1) were synthesized by Dr. Richard Lee's laboratory at St. Jude Children's Research Hospital, Memphis, TN as previously described (Lee et al., 2014, Liu et al., 2017). A solution of the test compounds was freshly prepared in distilled water before the start of each experiment.

2.2. *In vitro* PK/PD model

The *in vitro* dynamic time-kill curve assessment was based on a previously reported *in vitro* PK/PD model system for slow growing organisms (Vaddady et al., 2010). In brief, the system consisted of a two-armed, water jacketed chemostat (1965 series spinner flask, Bellco Glass, Vineland, NJ). A filter unit attached to a hollow steel tube representing the outflow of the model contained a prefilter (5 μm , Millipore, Billerica, MA) and filter membrane (0.22 μm , Millipore, Billerica, MA) to prevent leakage of bacteria during the dilution process. The other end of the tube was connected to plastic tubing from which the media was withdrawn continuously at a defined flow rate using a peristaltic pump

(Masterflex L/S, Cole-Parmer, Vernon Hills, IL). Any media removed from the chemostat was replaced by fresh sterile media using a second peristaltic pump. The chemostat flask was placed on a magnetic stirrer for homogeneity of the culture and preventing membrane blockage. The temperature in the flask was maintained at 37°C by circulating thermostat controlled water through the external jacket of the flask. After addition of a spectinamide dose to the chemostat, its concentration in the flask decreased exponentially due to the continuous media exchange as per the equation:

$$C = C_0 \cdot e^{-k \cdot t} \quad \text{Eq. 1}$$

where C_0 is the initial concentration of the spectinamide in the flask, C is the concentration at any time t , k is the pharmacokinetic elimination rate constant and t is the time elapsed since the addition of the drug. Previous studies on the pharmacokinetics of spectinamides, including 1445 and 1599, indicated that at therapeutically relevant concentrations the decline in plasma concentrations after intravenous dosing in rats follows mono-exponential characteristics (Lee et al., 2014). The *in vivo* half-life ($t_{1/2}$) of spectinamides 1445 and 1599 was simulated in the model by changing the flow rate according to the equation,

$$F = V \cdot \frac{\ln 2}{t_{1/2}} \quad \text{Eq. 2}$$

where the elimination rate constant k is equal to F/V , F is the flow rate of the medium and V is the volume of the medium in the flask. The apparatus was sterilized by autoclaving between experiments and was kept in a biological safety cabinet during operation.

2.3. Dynamic Time Kill Curves for Spectinamides

M. bovis BCG actively growing in the early logarithmic phase was used as an inoculum for the *in vitro* PK/PD model for a final density in the culture of 10^6 CFU/mL based on the notion that the bacillary load in tuberculosis patients varies from 6–9 log CFU/mL in cavity walls (Palaci et al., 2007). In separate experiments, different dose levels and dosing intervals of spectinamide 1445 and 1599 were tested over 4 days to simulate *in vivo* plasma concentration-time profiles, including QD, BID and TID dosing. All studies were performed in duplicate. Simulated spectinamide half-lives had previously been determined through *in vivo* PK studies in rats (Lee et al., 2014). Accuracy of nominal spectinamide concentrations and compound stability in the broth throughout the time period of the experiment were confirmed using a previously established LC-MS/MS assay for spectinamide quantification (Lee et al., 2014).

Specimens (200 μ L) were collected from the flask at time zero and at least once every day thereafter, with most experiments having 2–3 specimens per day. The specimens were centrifuged at 10,000 rpm for 10 min at 37°C, and the pellets were collected and re-suspended in an antibiotic-free medium. The number of viable bacteria in each sample was determined either by plating serial dilutions on 7H11 agar plates (Difco™, Becton

Dickinson, Sparks, MD) in duplicates, followed by incubation for 21 days at 37°C, or using the ATP-based BacTiter-Glo cell viability assay (Promega, Madison, WI). In previous investigations, we had confirmed that plated CFU counts closely correlate with the ATP-dependent luminescence signal over the range of 3 to 9 log CFU/mL, and that there is no interference of the spectinamides with the ATP measurement as a surrogate for quantifying CFU.

2.4. Model-based Time Kill Data Analysis

The established time-kill curves were analyzed with an integrated PK/PD model that combined a standard one compartment PK model with a previously described bacterial growth model (Vaddady et al., 2010), in which the antibacterial activity of the spectinamide was mediated via an E_{max} -model on the kill rate constant:

$$\frac{dN}{dt} = \left[k_0 \cdot \left(1 - \frac{N}{N_{max}} \right) - (1 - e^{-\alpha \cdot t}) \cdot \left(\frac{I_{max} \cdot C}{IC_{50} + C} \right) \right] \cdot N \quad \text{Eq. 3}$$

where N is the *M. bovis* BCG cell count in CFU/mL, k_0 is the bacterial net growth rate constant, N_{max} is the maximum number of bacteria in the system in CFU/mL, I_{max} is the maximum kill rate, C is the spectinamide concentration at time t , and IC_{50} is the concentration at half-maximal kill rate. A logistic growth function, $1 - N/N_{max}$, was used to describe the limited growth of bacteria in the absence of antibacterial agents. The delay rate constant α was incorporated to characterize the initial delay in the killing effect of spectinamides.

All time-kill data for the different dosing regimens were analyzed simultaneously by nonlinear mixed effects modeling (NONMEM v.7.2, Icon, Ellicott City, MD), using the first-order conditional estimation method. The PK parameters were fixed in the PK/PD model and set equal to their selected values ($V=55$ mL, where V is volume of the media in the system and half-life as previously reported in rats, 0.58 and 0.45 for 1599 and 1445, respectively (Lee et al., 2014)). The data and model predictions were log transformed. The between experiment variability term was incorporated only for select system parameters (N_{max} and α) to account for differences in the operation of separate experiments. Random residual variability was modeled using a proportional error term. Model performance was analyzed using goodness-of-fit plots, including observed vs. predicted, weighted residuals vs. time and weighted residuals vs. predictions plots.

For identification of the most appropriate empirical PK/PD index associated with the microbial kill, cell counts on day 4 were analyzed using an inhibitory sigmoid E_{max} model, as shown in equation 4 (Budha et al., 2009, Lee et al., 2014):

$$E = E_{max} - \frac{(E_{max} - E_0) \cdot (PKPD)^{\gamma}}{(PKPD)^{\gamma} + EC_{50}^{\gamma}} \quad \text{Eq. 4}$$

where, E is the observed *M. bovis* BCG log CFU/mL, E_0 is the bacterial cell count in the control experiment, $E_{\max}$ is the maximal antimicrobial effect in log CFU/mL, EC_{50} is the value of PK/PD index that produces half-maximal antimicrobial effect, and γ is the Hill coefficient. PKPD is one of the empirical PK/PD indices frequently used in antibiotic therapy, namely $fAUC_{0-24}/MIC$, $fC_{\max}/MIC$ or $T>MIC$. The PK/PD index that best characterized the effect of 1445 or 1599 on *M. bovis* BCG was selected based on goodness-of-fit criteria and the visual inspection following nonlinear regression analysis (Phoenix WinNonlin 64, Certara, Princeton, NJ).

3. Results

In order to evaluate and differentiate the pharmacological properties of spectinamide early lead candidates, 1599 and 1445, and assist in candidate selection and design of optimal dosing regimens in animal models of *Mtb* infection, time kill curve assessments were performed in a previously established *in vitro* PK/PD model system (Budha et al., 2009). Dynamic time-kill curve experiments were conducted for 11 dosing regimens of 1599 and 8 dosing regimens of 1445 as listed in Table 1. The corresponding time-kill effect on *M. bovis* BCG is shown for in Fig. 2. Both spectinamides exhibited a dose-dependent increase in bacterial kill, which for 1445 seemed to level off at a maximum kill rate. For 1599, no such maximum seemed to have been reached with the tested dosing regimens.

For 1445, doses of 0.4 and 2 mg/kg/day as BID regimens did not show any bactericidal effect and were comparable to untreated control. At doses of 10 and 50 mg/kg/day administered QD, 1445 displayed a stationary effect where the bacterial count remained in a state of equilibrium with the growth rate balancing out the kill rate. The same total daily dose of 10 and 50 mg/kg/day administered as BID regimens, however, showed a substantially higher killing compared to the same total daily dose administered as QD regimen. TID dosing did not further increase bacterial kill at the tested daily dose levels.

For 1599, a dose of 1 mg/kg/day given QD was not different from control, but 5 mg/kg/day given QD nearly reached a stationary effect. Further increase in the dose levels for QD doses to 10, 50 or 100 mg/kg/day increased the steepness of the CFU decline. In contrast to 1445, dosing frequency seemed to have mixed effects for 1599: At low doses of 10 mg/kg/day (3.3 mg TID vs. 5 mg BID or 10 mg QD) or 20 mg/kg/day (6.7 mg TID vs. 10 mg BID), dose fractionation seemed to decrease bacterial kill, whereas at high doses of 50 mg/kg/day (25 mg BID vs. 50 QD) and 100 mg/kg/day (50 mg BID vs. 100 mg QD), it seemed to slightly improve the killing effect, even though to a much smaller degree than that observed for 1445.

In the subsequent pharmacometric analysis, an integrated PK/PD model was fit to the experimentally obtained time-kill curves to estimate system- and compound-specific parameters for comparison of 1599 and 1445. This model combined a logistic function for growth of mycobacteria in the absence of drug and an $E_{\max}$ -model with a delay function for the bacterial kill effect. The obtained parameter estimates are listed in Table 2.

The growth rate constant (k_0) was estimated to be between 0.0995 and 0.111 h⁻¹, which is in agreement with our previous results (Budha et al., 2009) and similar to the range observed in the literature (Wayne, 1984). The compound specific parameters maximum bacterial kill rate ($I_{\max}$) and concentration of the drug required to produce half-maximum kill rate (IC_{50}) showed substantial differences among the compounds: For 1599, $I_{\max}$ was with 1.26 h⁻¹ substantially higher as the kill rate constant for 1445 (0.240 h⁻¹), but IC_{50} was approximately 10 times smaller for 1445 than 1599. That means the lower maximal kill rate for 1445 is already achieved at lower concentrations, and thus a dosing regimen maintaining concentrations above this low IC_{50} throughout the dosing interval provides the highest efficacy. For 1599, however, the maximally achievable kill rate is higher, but needs higher drug concentrations to be achieved.

The estimated between-experiment variability in $N_{\max}$, the model-estimated maximum number of bacteria in the absence of any drug, was relatively high (85.9%) for 1599, and substantially lower for 1445. This is most likely related to the earlier and more pronounced killing effect for most of the tested dosing regimens of 1599, which increased the uncertainty of the actually achievable $N_{\max}$ in each individual experiment. Thus, the relatively high value for between-experiment variability for $N_{\max}$ is likely more artefact of the modeling procedure than a true estimate for $N_{\max}$ variability. Modeling the data without between-subject variability on $N_{\max}$, however, yielded unsatisfactory model fits.

Similarly, the precision of the IC_{50} parameter estimate was much lower (i.e. higher RSE) for 1445 compared to 1599. This can likely be explained by the ‘cut-off’ like effect of IC_{50} in the scenario of time-dependent killing for 1445. Since most of the tested regimens seemed to have resulted in either very limited or substantial bacterial killing, only limited information seemed to have been in the data set to more precisely define IC_{50} in this scenario.

Since empirical PK/PD indices are a clinically widely used approach to identify the drug exposure parameters most relevant for antibacterial activity (Budha et al., 2009, Vaddady et al., 2010), the standard indices $fAUC/MIC$, $fC_{\max}/MIC$ and $T > MIC$ were calculated based on the simulated PK profiles for all dosing regimens of 1599 and 1445, and then correlated with observed CFU counts at day 4. The relationships for the different PK/PD indices are shown in Fig. 3. Based on visual inspection, goodness-of-fit criteria and correlation analysis, the PK/PD index $T > MIC$ was best able to describe the 1445-mediated microbial kill. In contrast 1599 anti-mycobacterial activity is similarly well described by $fC_{\max}/MIC$ and $fAUC/MIC$. These findings further confirm the observations made regarding the effect of dose fractionation and the PK/PD model-based analysis and suggest that 1445 shows time-dependent killing of *M. bovis* BCG *in vitro* while 1599 shows concentration-dependent killing. Time-dependent killing indicates that the antibacterial effect is at its maximum with concentrations just above the MIC and no further improvement in killing is obtained by further increasing the concentrations, whereas concentration-dependent killing requires maximum peak antibiotic concentrations for maximum efficacy (Rathi et al., 2016).

4. Discussion

In this investigation, we applied our previously developed *in vitro* PK/PD model for slow growing organisms (Budha et al., 2009) to establish dynamic time-kill curves for two spectinamide lead candidates, 1599 and 1445. The model allows to capture in depth the dynamic interplay between mycobacterial growth and changing drug concentrations as encountered during prolonged drug therapy *in vivo*, without any interference from the host immune system (Vaddady et al., 2010). As such, the derived data are useful in early drug development to compare and differentiate among competing lead candidates with regard to their interaction with the target organism. In this context dynamic time-kill curves might be seen as a useful *in vitro* tool in analogy to *in vivo* infection models in immunocompromised animals, such as the γ -interferon knockout mouse model of *Mtb* infection (Lenaerts et al., 2003). Of note, the time-kill curves only assess the effect on extracellular, actively replicating bacteria and thus cannot draw any inferences on the effect of spectinamides on intracellular and/or latent mycobacteria.

For these studies, *M. bovis* BCG was used as a surrogate for virulent *M. tuberculosis*. Both organisms are species in the *Mycobacterium tuberculosis* complex that are phylogenetically distinct from nontuberculosis mycobacteria (Frothingham et al., 1994), have close genetic similarity (Garnier et al., 2003), and similar drug sensitivity and growth rate (Hurdle et al., 2008, Lee et al., 2014, Ritz et al., 2009). They differ, however, in their ease of handling, where a biosafety level 2 environment is sufficient for *M. bovis* BCG rather than the more stringent biosafety levels 3 conditions required for *M. tuberculosis* cultures. Although this may be viewed as a limitation of the current experiments, the purpose of these time-kill curve assessment was to learn about the differential pharmacologic properties of two candidate compounds rather than characterizing anti-mycobacterial efficacy of infection in animal models or in humans. The observed differences in bacterial killing properties between the compounds in the applied model system seem to support this view.

At a first glance, the findings that 1445 exhibits time-dependent killing, but 1599 follows concentration-dependent killing may seem surprising: Both spectinamides seem to have very similar pharmacological properties. 1599 and 1445 have both potent anti-mycobacterial activity *in vitro* with MIC values of 0.8 and 1.6 $\mu\text{g/mL}$, respectively, but equal IC_{50} values in an isolated ribosomal inhibition assay (Lee et al., 2014, Liu et al., 2017). This already suggests that the structural differences of 1599 and 1445 (Fig. 1) seem to translate into different intracellular access.

The pharmacokinetic properties of 1599 and 1445 in rats are similar to other members of the spectinamide family: high metabolic stability, urinary excretion in unchanged form as major elimination pathway, and short half-life of 0.45–0.58 h at therapeutically relevant concentrations. Only clearance seems to be ~50% lower in 1445 compared to 1599, resulting in corresponding increases in systemic exposure at equivalent doses. Even though plasma protein binding is often considered highly relevant for antibiotic activity as only the free, unbound fraction is usually therapeutically active (Heuberger et al., 2013), the impact of protein binding on spectinamide activity is negligible as both compounds show only a very limited degree of binding to plasma proteins (13% for 1599, 28% for 1445).

One major difference between the two compounds, however, is their post-antibiotic effect (PAE) at $10 \times \text{MIC}$: 15.7 h (CV 22.5%) for 1445 compared the 133 h (CV 19.6%) for 1599 (Lee et al., 2014). This substantial difference may provide the basis for the observed difference in concentration- vs. time-dependent bacterial kill activity. A short PAE for 1445 would require continuous maintenance of drug concentrations above a certain threshold for antibacterial activity. On the opposite, a long PAE for 1599 would require only infrequent discrete doses with exposures high enough to trigger a long-lasting PAE effect. So far, however, all considered measures such as PAE, MIC and the PKPD indices were obtained from *in vitro* experiments, and it remains to be seen whether these observations translate for spectinamides also to *in vivo* efficacy.

At this stage, one can only speculate about the molecular basis for these differences in PAE. Since 1599 and 1445 act through the same mechanism of action, ribosomal protein synthesis inhibition, and have similar potency in isolated ribosomal assays (Lee et al., 2014), differences in intracellular access and distribution or ribosomal residency may be the cause of this divergent behavior. Investigations on the structure-activity relationship of spectinamides and molecular dynamics experiments predict enhanced ribosomal residency time of halogenated spectinamides, which 1445 and 1599 both are (Liu et al., 2017). Slight differences in physicochemical properties and structural features, however, may result in differences in interaction with bacterial membrane transporters other than Rv1258c. This hypothesis is supported by the observation that 1445 and 1599 have differential antibacterial activity (MIC) in *E.coli* K12 and its mutant, *E.coli* K12 ΔtolC , 100 vs. 200 $\mu\text{g/mL}$ for 1445 compared to 200 vs. 1.56 $\mu\text{g/mL}$ for 1599 (Lee et al., 2014). The mutant is aberrant of TolC, a membrane component of several types of bacterial efflux pumps.

Another mechanism responsible for the observed PAE differences may potentially be related to differential interaction with phospholipids in the bacterial cell membrane, a feature compounds such as spectinamides as secondary amines are known to display (Hein et al., 1990). Drug uptake experiments in primary lung derived dendritic cells and macrophage cell lines indicate at least for 1599 good intracellular uptake (Santos et al., 2018), but similar experiments in *Mycobacterium tuberculosis* complex bacteria are so far unavailable. In conclusion, we characterized the anti-mycobacterial activity of spectinamide lead candidates by establishing dynamic kill curves in an *in vitro* PK/PD model, followed by a subsequent pharmacometric analysis. Our results suggest differences in the major PK drivers for the antimicrobial activity of spectinamides 1599 and 1445 that could be explained by differences in PAE. Overall, these screening tools provided detailed insights into the antibacterial activity of spectinamide compounds that have contributed to lead candidate selection and will be further utilized in the preclinical development of spectinamides as the next treatment alternative in MDR- and XDR-tuberculosis. Since the experiments were performed in an isolated *in vitro* environment with actively replicating mycobacteria and anatomically unrestricted drug access in the absence of an immune system, a scenario that is far away from the pathophysiology of established Tb infections, the obtained results and differences between the compounds should not be viewed as indicators of *in vivo* efficacy but rather key aspects in their overall pharmacological profile.

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1599 **1445**

Figure 1.
Chemical structures of spectinamides 1599 and 1445.

Figure 2.
Mean dynamic time-kill curves for different simulated dosing regimens of spectinamides 1599 and 1445.

Figure 3.

PK/PD indices and their relationship to the log CFU on day 4. For 1445, bacterial kill is best described by the index T/MIC, whereas for 1599, the indices fC_{max}/MIC and fAUC/MIC describe the antibacterial activity equally well.

Table 1.Dosing regimens tested in the *in vitro* PK/PD model.

Compound	Dose (mg/kg)	Dosing frequency	Daily dose (mg/kg/day)
1445	-	-	-
	0.2	BID	0.4
	1	BID	2
	3.33	TID	10
	5	BID	10
	10	QD	10
	16.7	TID	50
	25	BID	50
	50	QD	50
1599	-	-	-
	1	QD	1
	5	QD	5
	5	BID	10
	3.3	TID	10
	10	QD	10
	10	BID	20
	6.7	TID	20
	25	BID	50
	50	QD	50
	50	BID	100
	100	QD	100

QD One daily; BID Twice daily; TID Three times daily

Table 2.*In vitro* PK/PD parameter estimates for spectinamides 1599 and 1445.

Parameter (unit)	1599		1445	
	Estimate (RSE)	Between experimental variability [Shrinkage]	Estimate (RSE)	Between experimental variability [Shrinkage]
K_o (h^{-1})	0.111 (35%)		0.0995 (39%)	
α (h^{-1})	0.0352 (26%)	23.2% [38.6%]	0.0404 (29%)	
N_{max} (log CFU/mL)	9.05 (6%)	85.9% [25.6%]	8.57 (3%)	11.9% [19.3%]
I_{max} (h^{-1})	1.26 (17%)		0.240 (22%)	
IC_{50} ($\mu g/mL$)	0.674 (44%)		0.0701 (74%)	
Proportional residual error	36.6%		22.1%	

RSE: Relative Standard Error

 K_o : Growth rate constant N_{max} : Maximum number of bacteria α : Delay rate constant for drug effect I_{max} : Maximum bacterial kill rate IC_{50} : Concentration required to produce the half-maximal effect