In Vivo Pharmacodynamic Characterization of a Novel Antibiotic Odilorhadbins, NOSO-502 against *Escherichia coli* and *Klebsiella pneumoniae* in a Murine Thigh Infection Model

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Running Title: In vivo PK/PD of NOSO-502 against *Escherichia coli* and *Klebsiella pneumoniae*

Key Words: NOSO-502, Pharmacodynamic, *Escherichia coli*, *Klebsiella pneumoniae*
ABSTRACT

NOSO-502 is a novel odororhabdin antibiotic with potent activity against Enterobacteriaceae. The goal of these studies was to determine which pharmacokinetic/pharmacodynamic (PK/PD) indice and magnitude best correlated with efficacy in the murine thigh infection model. Six E. coli and 6 K. pneumoniae were utilized. MICs were determined using CLSI methods and ranged from 1 to 4 mg/L. A neutropenic murine thigh infection model was utilized for all treatment studies. Single-dose plasma pharmacokinetics were determined in mice after subcutaneous administration of 7.81, 31.25, 125 and 500 mg/kg. Pharmacokinetic studies exhibited peak concentration (Cmax) values of 1.49 to 84.6 mg/L, area under the concentration-time curve (AUC0–τ) values of 1.94 to 352 mg*h/L, and beta-elimination half-lives of 0.41 to 1.1 h. Dose fractionation studies were performed using total drug doses 7.81 mg/kg to 2000 mg/kg fractionated into q3-, q6-, q12-, or q24-hourly regimens. Nonlinear regression analysis demonstrated AUC/MIC was the PK/PD parameter that best correlated with efficacy (R² 0.86). In subsequent studies, we used the neutropenic murine thigh infection model to determine the magnitude of NOSO-502 AUC/MIC needed for the efficacy against a diverse group of Enterobacteriaceae. Mice were treated with four-fold increasing doses (range 3.91 to 1000 mg/kg) of NOSO-502 every 6 hours. The mean fAUC/MIC magnitudes associated with net stasis and 1-log kill endpoint for K. pneumoniae were 4.22 and 17.7, respectively. The mean fAUC/MIC magnitude associated with net stasis endpoint for E. coli was 10.4. NOSO-502 represents a promising novel, first-in-class
odilorhabdin antibiotic with in vivo potency against Enterobacteriaceae.

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INTRODUCTION

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The epidemic of antimicrobial resistance is a growing public health threat that warrants the discovery and evaluation of new antimicrobial classes. NOSO-502 belongs to a novel class of peptide antibiotics, odilorhabdins (ODLs), produced by an NRPS gene cluster within the genome of Xenorhabdus nematophila (1). ODLs exhibit broad-spectrum activity against gram-positive and gram-negative pathogens, including carbapenem-resistant Enterobacteriaceae (CRE), by binding to the decoding center of the 16S subunit of the bacterial ribosome at a site not exploited by any known ribosome-targeting antibiotic (1). Preclinical data has shown NOSO-502 is safe and efficacious in animal models (1). The goals of our experiments were to determine the NOSO-502 pharmacokinetic/pharmacodynamic (PK/PD) index predictive of therapeutic success against E. coli and K. pneumoniae and to determine the PK/PD index magnitude associated with stasis and cidal outcomes in the murine neutropenic thigh model.

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RESULTS

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In vitro susceptibility testing. The median MIC results for NOSO-502 are shown in Table 1. The MIC range for NOSO-502 was narrow for the pathogen group, varying only 4-fold despite using a broad subset of clinical strains with demonstrated resistance phenotypes and genotypes to other agents. Consistent with a novel
mechanism, the NOSO-502 MIC results were not affected by beta-lactam or
tetracycline resistance mechanisms.

**Drug pharmacokinetics.** Single-dose plasma pharmacokinetics of NOSO-502
following subcutaneous administration are shown in Figure 1. NOSO-502 drug
concentrations increased in a dose-dependent manner across the dose range. \( C_{\text{max}} \)
concentrations ranged from 1.49 to 84.6 mg/liter. AUC\(_{0-\infty}\) values ranged from 1.94 to
352 mg*h/liter and were linear across the 7.81- to 500-mg/kg dosing range (\( R^2 \) of
0.99). The elimination half-life ranged from 0.41 to 1.1 h.

**PK/PD parameter determination.** The dose-response relationships of NOSO-502
over a 256-fold range of doses, fractionated into one of four dosing intervals, against
*E. coli* ATCC 25922 are shown in Figure 2. At the start of therapy, mice had 6.70 ±
0.09 log\(_{10}\) CFU/thigh of *E. coli* ATCC 25922, and the organism burden increased 3.28
± 0.11 log\(_{10}\) CFU/thigh from 0 to 24 h in untreated control mice. Each fractionation arm
demonstrated relatively similar concentration-dependent activity as the total daily
dose was escalated with the highest doses studied resulting in net cidal activity. The
similarity in the dose-response curves for each of the fractionated regimens usually
indicates AUC/MIC is the predictive PK/PD index as this PK/PD parameter is held
relatively constant in each fractionated regimen. In contrast, \( C_{\text{max}} \) and time above MIC
change proportionally (and inversely to each other) based on dose in each
fractionated regimen.
The relationships between microbiologic effect and each of the pharmacodynamic indices, 24 h free-drug AUC/MIC (fAUC/MIC), 24 h free-drug C\text{max}/MIC (fC\text{max}/MIC), and the percent of time free drug concentrations exceed the MIC (%T/MIC) over 24 h against *E. coli* ATCC 25922 is shown in Figure 3. The strongest relationship was observed when results were regressed using the 24 h fAUC/MIC index with an \( R^2 \) value of 0.86. Regression with both the %T>MIC and fC\text{max}/MIC resulted in weaker relationships based upon both visual inspection and \( R^2 \) values. Consideration of total or free drug levels did not appreciably impact the relationships between efficacy and PK/PD parameters (free-drug data is shown).

**PK/PD magnitude determination.** The dose-response relationships for treatment against each of the 6 *E. coli* and 6 *K. pneumoniae* strains in the neutropenic murine thigh model are shown in Figure 4A and 4B. The burden at the start of therapy, growth in untreated controls (i.e. fitness), and drug effect were relatively similar for each isolate (Table 2). At the start of therapy, mice had 6.62 ± 0.23 log\text{10} CFU/thigh of *E. coli*. The organisms grew 3.37 ± 0.28 log\text{10} CFU/thigh in untreated control mice. The average maximal reduction in 6 *E. coli* strains with NOSO-502 treated mice compared to untreated controls was -4.17 ± 0.49 log\text{10} CFU/thigh and average maximum kill from zero hour was -0.77 ± 0.58 log\text{10} CFU/thigh. Net stasis was achieved against all strains and a more than 1 log\text{10} kill against two of six strains.

The initial burden and burden increase from 0 to 24 h in untreated controls for *K. pneumoniae* were 7.12 ± 0.41 log\text{10} CFU/thigh and 2.81 ± 0.40 log\text{10} CFU/thigh,
respectively. NOSO-502 showed slightly more potent activity against *K. pneumoniae*, with average maximal reduction compared to untreated controls at -4.43 ± 0.54 log_{10} CFU/thigh and average maximum kill from zero hour at -1.62 ± 0.64 log_{10} CFU/thigh. One log_{10} kill was achieved against all strains and a more than 2 log_{10} kill against two of six strains.

The relationship between organism burden in the thigh and the plasma 24h fAUC/MIC ratio is shown in Figure 5A and 5B. Calculation of the doses necessary to achieve a static and 1 log_{10} kill effect against multiple organisms is shown in Table 3. Also shown are the associated total and free-drug 24 h AUC/MIC target ratios necessary to achieve these outcomes. The mean 24h fAUC/MIC values associated with net stasis endpoint for *E. coli* was 10.4. The mean 24h fAUC/MIC values associated with net stasis and 1 log_{10} kill endpoints for *K. pneumoniae* were 4.22 and 17.7, respectively. The presence of antimicrobial resistance in both bacterial species did not alter the 24-h AUC/MIC required to produce efficacy. Additionally, while numerically lower stasis targets were observed for *K. pneumoniae* compared to *E. coli*, this difference was not statistically significant (*t*-test p=0.08).

**DISCUSSION**

The rapid spread of antibiotic resistance poses a serious threat to global public health. In recent years, carbapenem-resistant *Enterobacteriaceae* (CRE) has dramatically increased and represents an important threat to global health (2-5). The U.S. Centers for Disease Control and Prevention (CDC) has classified CRE as urgent threats (6). In
response to these concerns, the development of effective antimicrobial agents, in
particular novel classes, to treat these infections has been an area of intense
research.

In antibiotic discovery and development, PK/PD evaluation in animal infection
models play an essential role in designing the optimal dosing regimen and planning
clinical trials (7, 8). Although a drug may fail in clinical trials or for an individual patient
for many reasons, one obvious potential explanatory factor is suboptimal drug
exposure, which can be mitigated by optimal dosing design based on PK/PD study. In
pre-clinical stages, PK/PD studies help define whether a drug’s activity is linked
optimally to concentration- or time-dependence. Practically, this translates into
determining whether dosing regimen optimization is achieved by administering very
large doses to achieve maximal concentrations in excess of the MIC, or whether small,
frequent doses are necessary to maintain drug concentrations above a threshold
(MIC) and then maintain those concentrations for a prolonged period of time.
Preclinical studies additionally serve to provide target drug exposures indexed to the
MIC to achieve various microbiological outcomes such as net stasis or 1-log10 kill.
These target exposures are then utilized in the context of human pharmacokinetics
and MIC distribution in Monte Carlo simulations to estimate target achievement to
enable rational go or no-go decision making in a relatively early stage of new
antibiotic development (9, 10). Herein, we examined the PK/PD relationships and
determined the magnitude associated with efficacy endpoints for a novel antibiotic
NOSO-502 in an established neutropenic murine thigh infection model. NOSO-502
belongs to a novel peptide antibiotic class, the odilorhabdins (ODLs), which exhibit a
number of potential advantages. Mechanistic studies have demonstrated ODLs
interfere with protein synthesis by binding to the bacterial 16S ribosomal subunit
leading to errors in translation and cell death (1). While other antibiotic classes also
bind to the small ribosomal subunit (e.g. tetracyclines and aminoglycosides), the site
of binding for ODLs is distinct such that there has been no evidence in pre-clinical
studies of cross-resistance to other antibiotics that affect protein translation (1).
Importantly, the activity of NOSO-502 has been demonstrated against gram-positive
and gram-negative bacterial pathogens, including CRE (1).

Prior in vivo study of ODLs showed dose-dependent response in mouse models
of *E. coli* and *K. pneumoniae* sepsisemia, UTI and lung infection, but only single dose
levels were studied which precludes adequate PK/PD assessment (1). The current
study represents the first traditional PK/PD evaluation of a novel ODL antibiotic,
NOSO-502, using the murine thigh infection model. Using a dose-fractionation study
design, we found AUC/MIC was the PK/PD index predictive of efficacy for NOSO-502.
This is perhaps not unexpected given other agents that affect protein translation have
also been predominantly associated with the PK/PD index AUC/MIC. For example the
PK/PD indices for tetracycline class, such as tetracycline (11), minocycline (12),
tigecycline (13), eravacycline (14), and omadacycline (15), have been shown to be
AUC/MIC.

Our multi-organism PK/PD target studies performed with NOSO-502
demonstrated very similar dose-dependent activity with net static activity against all
strains. Given the relatively narrow MIC distribution this similarity was expected. This also demonstrated that similar to the previous in vitro evaluations, resistance mechanisms to beta-lactams and tetracyclines did not appreciably affect the in vivo pharmacodynamic activity. A limitation to the current study is that we did not use any strains with known aminoglycoside resistance mechanisms.

We did observe enhanced activity of NOSO-502 against K. pneumoniae, as approximately 4-fold less drug was needed (i.e. dose-response curves shifted to the left) for stasis and cidal endpoints compared to those for E.coli. We also observed, similar to previous studies (data provided by sponsor and not shown), enhanced in vitro effect against K. pneumoniae with numerically lower MICs for most K. pneumoniae strains. However, the difference in MIC was only 2-fold between K. pneumoniae and E. coli. This resulted in free 24-h AUC/MIC targets being roughly 2-fold lower for K. pneumoniae than for E. coli, but this difference was not statistically significant. A killing endpoint (1-log kill) was achieved for K. pneumonia at approximately 4-fold the stasis target; however, it should be noted that we did not achieve a large number of 1-log kill endpoints against E. coli in order to make similar comparisons for this endpoint.

In conclusion, these studies demonstrated that NOSO-502 exhibits dose-dependent in vivo activity against K. pneumoniae and E. coli strains, including those with resistance mechanisms to beta-lactams and tetracyclines. It is a promising novel antibiotic from the newly discovered odilorhabin class. AUC/MIC was the PK/PD index that best predicted efficacy, and we were able to demonstrate both net stasis
and cidal endpoints in a clinically relevant animal infection model. The PD index and targets identified in this study will be useful in guiding appropriate dosing regimen design for future clinical studies in the context of human pharmacokinetic exposures and MIC distribution. Further development and studies are warranted, especially in light of the urgent need for novel drugs to address the rise of drug resistant infections.

MATERIALS AND METHODS

Organisms, media, and antibiotic. Six *E. coli* and 6 *K. pneumoniae* strains were used for these studies (Table 1). The strains were chosen to include common tetracycline and beta-lactam resistance phenotypes. They were grown, sub-cultured, and quantified using Mueller-Hinton broth (MHB) and agar (Difco Laboratories, Detroit, MI). NOSO-502 for *in vitro* and *in vivo* studies was supplied by the study sponsor (Nosophysm SAS, Nîmes, France). Compound was prepared by reconstitution in sterile water to pH 5.5-6.5 and subsequent dilution in sterile 5% D-mannitol solution.

In vitro susceptibility testing. The MICs of NOSO-502 for the various isolates were determined using Clinical and Laboratory Standards Institute (CLSI) microdilution methods (16, 17). All MIC assays were performed in duplicate on three separate occasions. The median MIC of replicate assays is reported and utilized in PK/PD analyses.

Murine thigh infection model. Animals for the present studies were maintained in...
accordance with criteria of the Association for Assessment and Accreditation of
Laboratory Animal Care International (AAALAC). All animal studies were approved by
the Animal Research Committee of the William S. Middleton Memorial Veterans
Hospital. Six-week-old, specific-pathogen-free, female ICR/Swiss mice weighing 23 to
27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). Mice were
rendered neutropenic (neutrophils, <100/mm³) by injecting them with
cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) subcutaneously
4 days (150 mg/kg) and 1 day (100 mg/kg) before thigh infection. Previous studies
have shown that this regimen produces neutropenia in this model for 5 days (18).
Broth cultures of freshly plated bacteria were grown to logarithmic phase overnight to
an absorbance of 0.3 at 580 nm (Spectronic 88; Bausch and Lomb, Rochester, NY).
After a 1:10 dilution into fresh Mueller-Hinton broth, bacterial counts of the inoculum
ranged from 10^7.1 to 10^7.4 CFU/mL. Thigh infections with each of the isolates were
produced by injection of 0.1 ml of inoculum into the thighs of isofoirane-anesthetized
mice. NOSO-502 therapy was initiated 2 h after the infection procedure. After 24 h,
the animals were euthanized and thighs aseptically removed, homogenized, and
plated for CFU determination. No treatment and zero-hour controls were included in
all experiments. Four thigh replicates were included for all treatment and control
groups.

**Drug pharmacokinetics.** Single-dose plasma pharmacokinetics of NOSO-502
were performed in mice. Animals were administered single subcutaneous doses (0.2
mL/dose) of NOSO-502 at dose levels of 7.81, 31.25, 125 and 500 mg/kg. Groups of
three mice were sampled at each time point (seven time points, consisting of 0.5, 1, 2,
3, 4, 8, and 12 h) and dose level. Samples were then centrifuged for 5 min at 4,000
rpm, and plasma was removed and frozen at -20 °C until assay. Plasma
concentrations were determined using liquid chromatography-tandem mass
spectrometry (LC-MS/MS) by the sponsor. The lower limit of detection of the
LC-MS/MS was 7.2 ng/mL. Pharmacokinetic parameters, including elimination
half-life (T₁/₂), area under the concentration-time curve (AUC₀₋₋), and peak
concentrations (Cₘₐₓ) were calculated using a non-compartmental model. The
beta-elimination half-life (T₁/₂) was determined by linear least-squares regression. The
AUC₀₋₋ was calculated from the mean concentrations using the trapezoidal rule.
Pharmacokinetic estimates for dose levels that were not directly measured were
calculated using linear interpolation for dose levels between those with measured
kinetics and linear extrapolation for dose levels above or below the highest and lowest
dose levels with kinetic measurements. A plasma protein binding of 80.2% was used
in the current study to calculate free drug concentrations for analysis based upon prior
unpublished data from the sponsor.

PK/PD parameter determination. A dose fractionation study was undertaken to
determine the PK/PD index (AUC/MIC, Cₘₐₓ/MIC or %Time above MIC) that was
predictive of efficacy for NOSO-502. Four-fold increasing doses (range 7.81 mg/kg to
2000 mg/kg) of NOSO-502 were fractionated into q3, q6, q12, and q24 h dosing
regimens. Mice were infected with isolate ATCC 25922 as described above and administered NOSO-502 by subcutaneous injection according to the dosing regimen prescribed in the fractionation design. After 24 h, the mice were euthanized and CFU count determined in the thighs. To determine which PK/PD index was most closely linked with efficacy, the number of bacteria in the thigh at the end of 24 h of therapy was correlated with (i) the free $C_{\text{max}}$/MIC ratio ($fC_{\text{max}}$/MIC), (ii) the 24-hour free AUC/MIC ratio ($f$AUC/MIC), and (iii) the percentage of the dosing interval during which plasma free drug levels exceeded the MIC for each of the dosage regimens studied ($%T$/MIC). The correlation between efficacy and each of the three PK/PD indices was determined by nonlinear least-squares multivariate regression (SigmaPlot version 13.0; Systat Software, San Jose, CA). The model is derived from the Hill equation. The coefficient of determination ($R^2$) was used to estimate the variance that might be due to regression with each of the PK/PD indices.

**PK/PD parameter magnitude studies** Dose-response experiments using the thigh model were performed for six *E. coli* isolates as described in methods above. The dose range consisted of four-fold increases (range 3.91 to 1000 mg/kg/6 h) in drug concentration with administration by subcutaneous route. The dose-response relationships were quantified and the relationship between the PK/PD parameter AUC/MIC and treatment efficacy using the sigmoid $E_{\text{max}}(hll)$ model was performed using Sigma Plot version 13.0, Systat Software, San Jose, CA. These PK/PD relationships were examined utilizing the plasma total and free drug concentrations.

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from pharmacokinetic studies. The coefficient of determination ($R^2$) from this model was used to numerically quantify the strength of this relationship. This coefficient represents the percentage of the variance in bacterial numbers that can be attributed to the PK/PD parameter. The doses required for a static effect (static dose) and 1-log kill (1 log-kill dose) compared to the start of therapy for multiple *E. coli* and *K. pneumoniae* pathogens in the thigh model were determined utilizing the plasma total and free drug concentrations using the following equation:

$$\log_{10} D = \log_{10} \left( \frac{E/(E_{\text{max}} - E)}{N} \right) + \log ED_{50}$$

Where $E$ is the growth from zero hour, $E_{\text{max}}$ is the maximum effect, $ED_{50}$ is the dose required to achieve 50% of the $E_{\text{max}}$, and $N$ is the slope of the dose-effect curve, and $D$ is the dose required to achieve net stasis. For one log kill, the $E$ was set to growth from zero hour plus 1 in order to calculate dose ($D$) for 1 log kill. The associated 24 h total and free drug AUC/MIC targets for each organism were calculated.

**ACKNOWLEDGMENTS**

This study was funded by Nosopharm SAS, Nîmes, France.

**REFERENCES**


Table 1. *In vitro* susceptibility of NOSO-502 and strain fitness in the neutropenic thigh infection model.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Median MIC (mg/L)</th>
<th>Increase in burden (Mean ± Std dev CFU/thigh) from 0 to 24 h</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC 25922</td>
<td>4</td>
<td>3.28 ± 0.1</td>
<td>ATCC</td>
</tr>
<tr>
<td>EC 6042</td>
<td>4</td>
<td>3.05 ± 0.3</td>
<td>TEM 10</td>
</tr>
<tr>
<td>EC 1135</td>
<td>4</td>
<td>3.62 ± 0.3</td>
<td>Tet(M), ESBL</td>
</tr>
<tr>
<td>EC 681</td>
<td>2</td>
<td>3.62 ± 0.1</td>
<td>ESBL</td>
</tr>
<tr>
<td>EC 1-894-1</td>
<td>4</td>
<td>3.47 ± 0.1</td>
<td>Tetracycline Resistant</td>
</tr>
<tr>
<td>EC 1-741-1</td>
<td>4</td>
<td>3.13 ± 0.1</td>
<td>Tetracycline Resistant</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP 43816</td>
<td>2</td>
<td>2.65 ± 0.1</td>
<td>ATCC</td>
</tr>
<tr>
<td>KP BAA 2146</td>
<td>1</td>
<td>2.64 ± 0.05</td>
<td>NDM-1</td>
</tr>
<tr>
<td>KP 216</td>
<td>2</td>
<td>2.67 ± 0.1</td>
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</tr>
<tr>
<td>KP 4105</td>
<td>2</td>
<td>2.37 ± 0.1</td>
<td>TEM 26, SHV-1</td>
</tr>
<tr>
<td>KP 4110</td>
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<td>2.98 ± 0.3</td>
<td>TEM 10, SHV-1</td>
</tr>
<tr>
<td>KP 81-1260A</td>
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<td>3.54 ± 0.1</td>
<td>CTX-M3, AmpC</td>
</tr>
</tbody>
</table>
Table 2. Initial burden, growth in untreated controls, and maximum kill for each organism in the in vivo murine thigh model (Mean ± Std dev).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Initial burden (0 hour) CFU/thigh</th>
<th>24 h CFU/thigh in Untreated Controls</th>
<th>Maximum kill CFU/thigh (from 0 hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC 25922</td>
<td>6.70 ± 0.09</td>
<td>9.98 ± 0.06</td>
<td>-0.395 ± 0.09</td>
</tr>
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<td>EC 6042</td>
<td>6.78 ± 0.28</td>
<td>9.83 ± 0.11</td>
<td>-1.33 ± 0.15</td>
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<td>EC 1135</td>
<td>6.52 ± 0.06</td>
<td>10.1 ± 0.23</td>
<td>-0.32 ± 0.51</td>
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<td>EC 681</td>
<td>6.39 ± 0.04</td>
<td>10.0 ± 0.06</td>
<td>-0.83 ± 0.10</td>
</tr>
<tr>
<td>EC 1-894-1</td>
<td>6.48 ± 0.10</td>
<td>9.95 ± 0.08</td>
<td>-0.16 ± 0.04</td>
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<td>EC 1-741-1</td>
<td>6.95 ± 0.09</td>
<td>10.1 ± 0.05</td>
<td>-1.60 ± 0.12</td>
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<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KP 43816</td>
<td>7.37 ± 0.09</td>
<td>10.0 ± 0.03</td>
<td>-2.65 ± 0.09</td>
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<td>9.96 ± 0.04</td>
<td>-2.05 ± 0.04</td>
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<tr>
<td>KP 216</td>
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<td>10.1 ± 0.02</td>
<td>-1.07 ± 0.05</td>
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<tr>
<td>KP 4105</td>
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<td>9.71 ± 0.04</td>
<td>-1.80 ± 0.22</td>
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<tr>
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<td>9.80 ± 0.08</td>
<td>-1.14 ± 0.47</td>
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<tr>
<td>KP 81-1260A</td>
<td>6.43 ± 0.08</td>
<td>9.98 ± 0.08</td>
<td>-1.05 ± 0.10</td>
</tr>
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</table>
Table 3. Pharmacodynamic targets associated with net stasis and 1-log kill in the neutropenic murine thigh model for *E. coli* and *K. pneumoniae*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>MIC (mg/L)</th>
<th>Stasis</th>
<th>1 log Kill</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h dose</td>
<td>24 h fAUC/MIC</td>
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<tr>
<td><em>E. coli</em></td>
<td>25922</td>
<td>4</td>
<td>453.10</td>
<td>57.17</td>
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<td></td>
<td>6042</td>
<td>4</td>
<td>214.67</td>
<td>19.45</td>
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<td></td>
<td>1135</td>
<td>4</td>
<td>474.76</td>
<td>60.81</td>
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<tr>
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<td>681</td>
<td>2</td>
<td>364.98</td>
<td>84.78</td>
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<td>1-894-1</td>
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<td>615.97</td>
<td>84.49</td>
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<td>1-741-1</td>
<td>4</td>
<td>119.75</td>
<td>9.63</td>
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<tr>
<td><strong>Mean</strong></td>
<td></td>
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<td>373.87</td>
<td>52.72</td>
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<tr>
<td><strong>Median</strong></td>
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<td>409.04</td>
<td>58.99</td>
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<td><strong>Std Dev</strong></td>
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<td>181.70</td>
<td>31.89</td>
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<tr>
<td><em>K. pneumoniae</em></td>
<td>43816</td>
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<td>55.78</td>
<td>6.63</td>
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<td>2.30</td>
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* †AUC/MIC, total drug AUC/MIC; fAUC/MIC, free drug AUC/MIC
†NA, not achieved
LEGENDS

FIGURE 1. Single dose plasma pharmacokinetics of NOSO-502. Four different doses that varied 4-fold on a mg/kg basis were administered to mice by subcutaneous route. Groups of three mice were sampled for each time point. Each symbol represents the mean ± SD from three animals. Shown in the legend is the maximum plasma concentration (C_max), the area under the concentration curve from 0–∞ (AUC), and the beta-elimination half-life (T_1/2).

FIGURE 2. In vivo dose fractionation with NOSO-502 using a neutropenic mouse thigh model. Each symbol represents the mean and standard deviation from four thighs infected with E. coli ATCC 25922. The error bars represent the standard deviation. The burden of organisms was measured at the start and end of therapy. Five total drug (mg/kg/24 h) dose levels were fractionated into one of four dosing regimens and is shown on the x-axis. The y-axis represents the change in organism burden from the start of therapy. The dashed horizontal line represents net stasis over the treatment period. Points above the line represent net growth and points below represent net killing (cidal activity).

FIGURE 3. Impact of pharmacodynamic regression of the in vivo dose fractionation study with NOSO-502 against E. coli ATCC 25922. Each symbol represents the mean and standard deviation from four thighs. The dose data is expressed as fAUC/MIC (free drug AUC/MIC), fC_max/MIC (free drug C_max/MIC), and the percentage of time plasma free drug concentrations exceed the MIC (%T/MIC). The R^2 is the coefficient of determination. Also shown for each PD index is the maximal effect (E_max), the PD index value associated with 50% of the maximal effect (ED_50), and the slope of the relationship or the Hill coefficient (N). The line drawn through the data points is the best-fit line based upon the sigmoid E_max formula.

Figure 4. In vivo dose effect of NOSO-502 against six E. coli and six K. pneumoniae strains using a neutropenic mouse thigh model. Each symbol represents the mean and standard deviation from four thighs. Five total drug dose levels were fractionated into an every 6 h regimen. The burden of organisms was measured at the start and end of therapy. The study period was 24 hours. The horizontal dashed line at 0 represents the burden of organisms in the thighs of mice at the start of therapy. Data points below the line represent killing and points above the line represent growth.

Figure 5. In vivo dose effect of NOSO-502 against six E. coli strains (A) and six K. pneumoniae strains using a neutropenic mouse thigh model. NOSO-502 exposure is expressed as the free drug 24 h AUC/MIC (fAUC/MIC). The R^2 represents the coefficient of determination. The ED_50 represents the AUC/MIC associated with 50% of the maximal effect (E_max) and N is the slope of the relationship or the Hill coefficient. The line drawn through the data points is the best-fit line based upon the sigmoid E_max.
formula. The dashed line represents the burden at the start of therapy. Points above the line represent net growth and those below the line represent killing.