

Supplementary Figures S1- S3 for

**Cadazolid, a New Antibiotic for the Treatment of *Clostridium difficile*
Infections: *In Vitro* and *in Vivo* Antibacterial Evaluation**

Authors: Hans H. Locher^{1#}, Peter Seiler¹, Xinhua Chen², Susanne Schroeder¹,
Philippe Pfaff¹, Michel Enderlin¹, Axel Klenk¹, Elvire Fournier¹, Christian
Hubschwerlen¹, Daniel Ritz¹, Ciaran P. Kelly², Wolfgang Keck¹

Affiliations: ¹ Actelion Pharmaceuticals Ltd, Allschwil, Switzerland. ²Department of
Medicine, Division of Gastroenterology, Beth Israel Deaconess Medical Center,
Harvard Medical School, Boston, MA, USA

#Correspondence: Hans H. Locher

Actelion Pharmaceuticals Ltd

Gewerbestrasse 16

CH-4123 Allschwil

Switzerland

FAX : +41 61 565 80 90

E-mail : hans.locher@actelion.com

FIG. S1

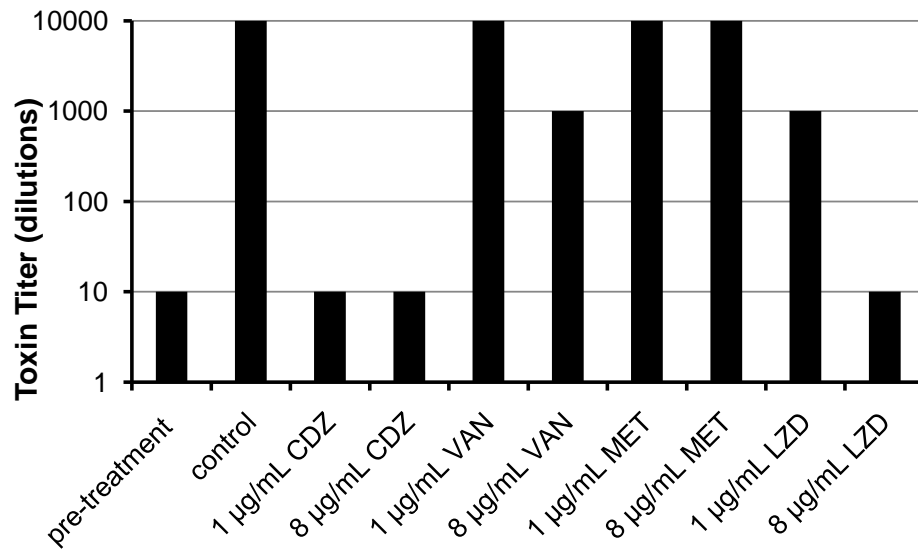
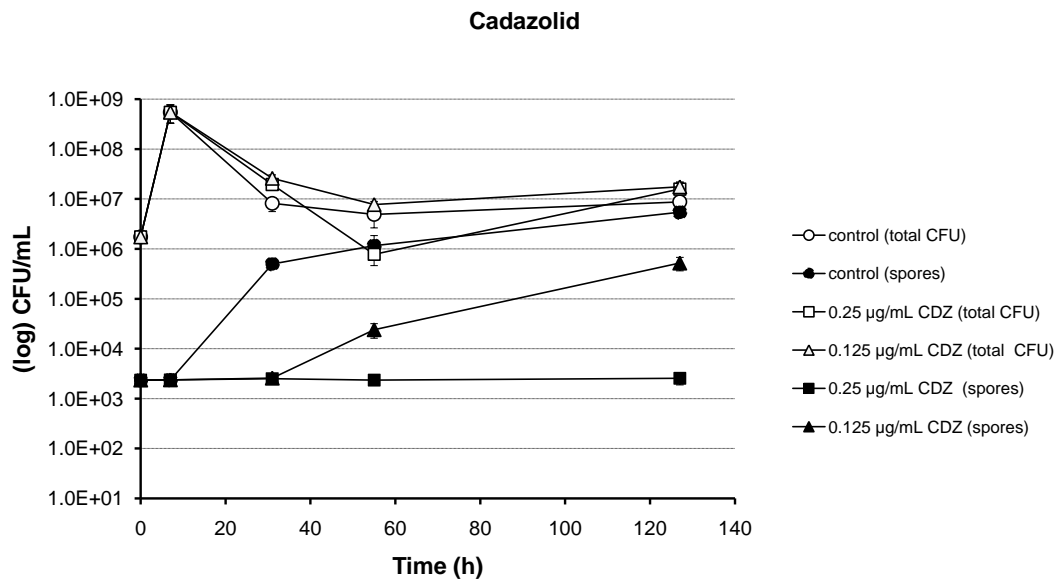


FIG. S1. Effect of antibacterial treatment on cytotoxin formation in stationary phase cultures of hyper virulent *C. difficile* NCTC 13366. High-density stationary phase cultures were treated with supra-MIC concentrations for 5 days and cytotoxin in culture supernatants was measured by a cell-rounding assay using CHO cells. Toxin content was quantified by titration (10-fold dilutions) and toxin titer was defined as the highest dilution resulting in > 90% cell rounding (Barc, MC, Depitre G, Corthier A, Collignon W, Su J, and Bourlioux P. Effects of antibiotics and other drugs on toxin production in *Clostridium difficile* *in vitro* and *in vivo*. Antimicrob Agents Chemother, 1992; 36, 1332-5.). Assays were done in duplicates with identical results. Abbreviations: CDZ, cadazolid; VAN, vancomycin; MET, metronidazole; LZD, linezolid.

FIG. S2

A.



B.

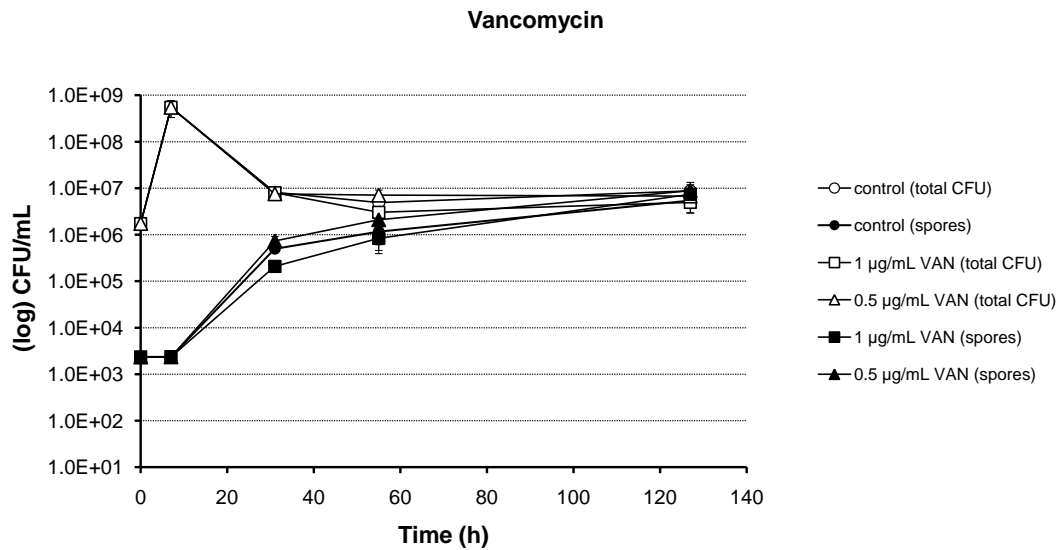
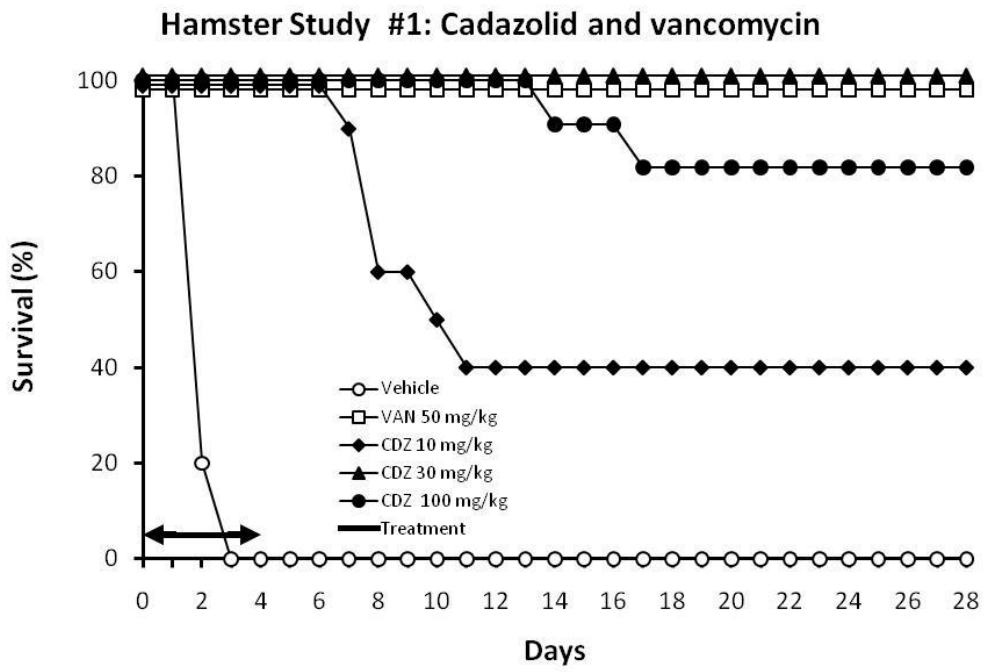


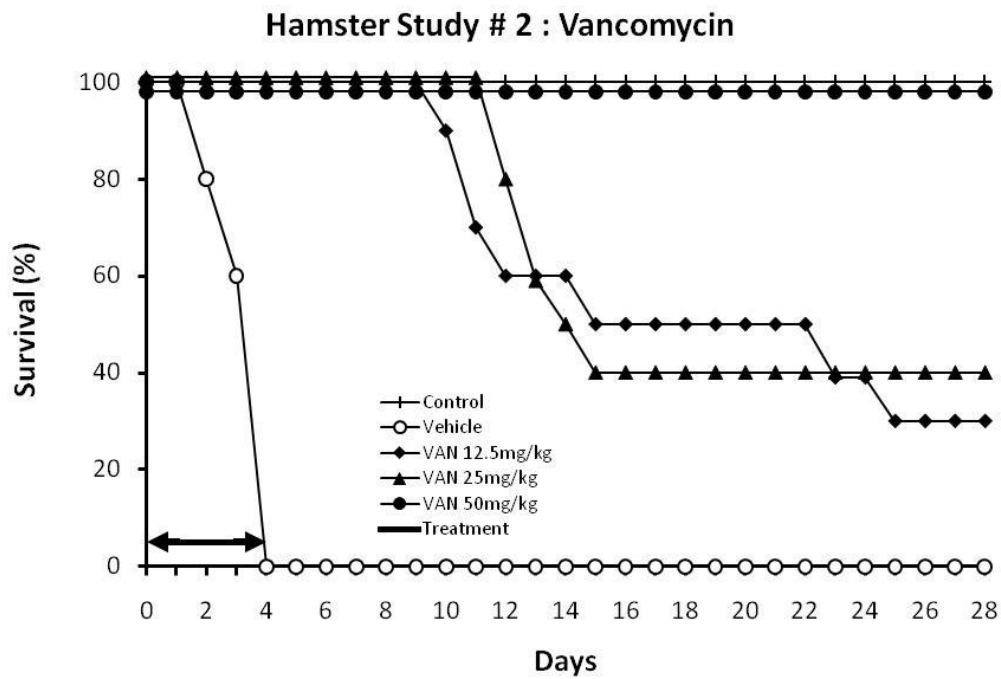
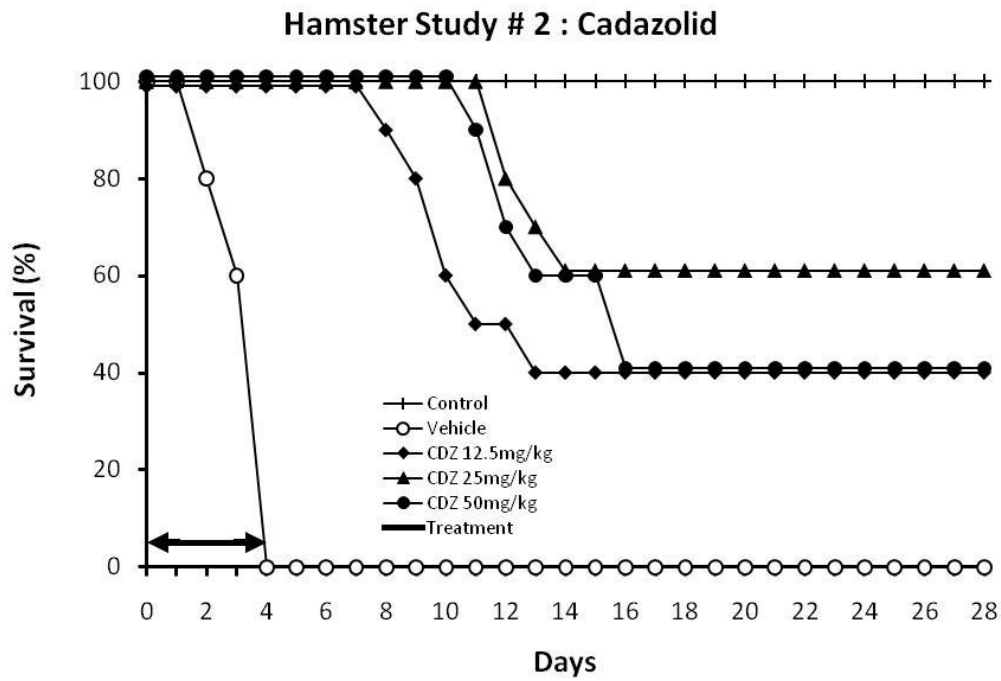
FIG. S2. Effect of sub-growth inhibitory concentrations of cadazolid (A) and vancomycin (B) on spore formation in *C. difficile* NCTC 13366. Antibiotics were added to late-exponential cultures ($t=6$ hours) at concentrations representing 0.5 and 1x the agar dilution MIC. Controls received no antibiotics. Total viable cells and spores (after ethanol treatment) were enumerated by colony counting. Abbreviations: CDZ, cadazolid; VAN, Vancomycin

Fig. S3

A



B



C

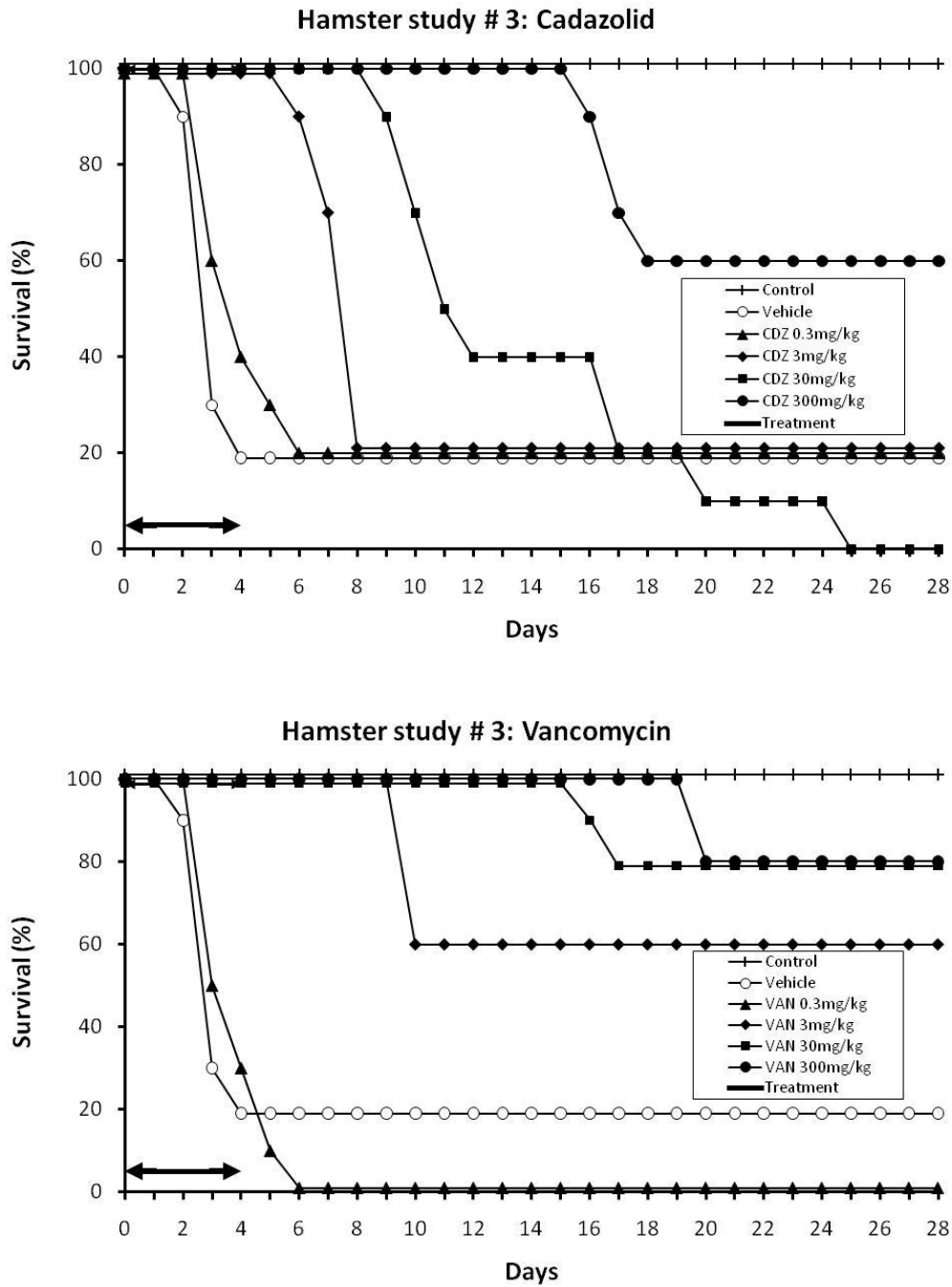


Fig S3. Efficacy in the hamster model of CDAD (A, study #1; B, study #2; C, study #3). Hamsters were infected with *C. difficile* strain VPI 10463 p.o. on day 0. Cadazolid (CDZ) or vancomycin (VAN) was administered p.o. once daily for 4 days (10 hamster per dose group), starting 2 h post infection. Plotted is survival (% of starting group size) over time. The treatment period is marked with a black arrow.