Figure S1

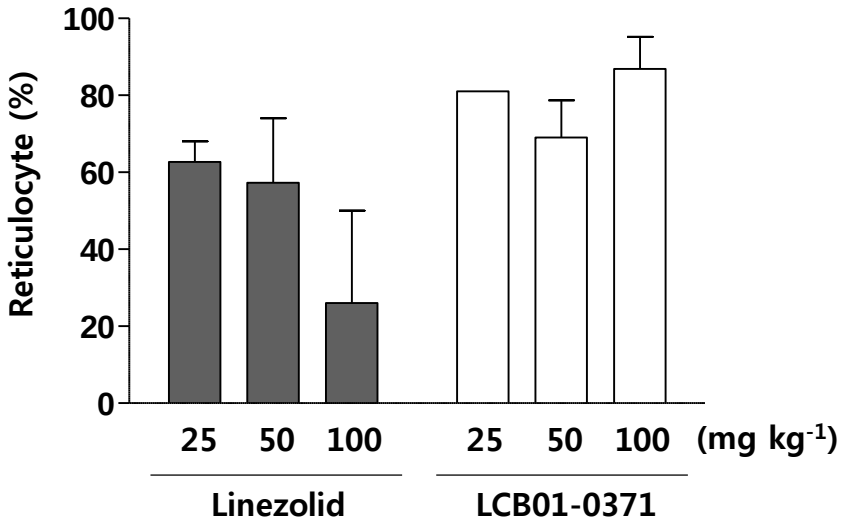

Figure S1 Reticulocyte count of treated groups following 7 days of LCB01-0371 therapy.

Linezolid and LCB01-0371 at 25, 50, and 100 mg/kg were orally administered for 7 days in SD rat twice-daily (total daily doses are: 50, 100, 200 mg/kg) and blood samples were analyzed at day 8. Reticulocyte count on 7 day was significantly higher when LCB01-0371 was administered at all different conditions.

Figure S2

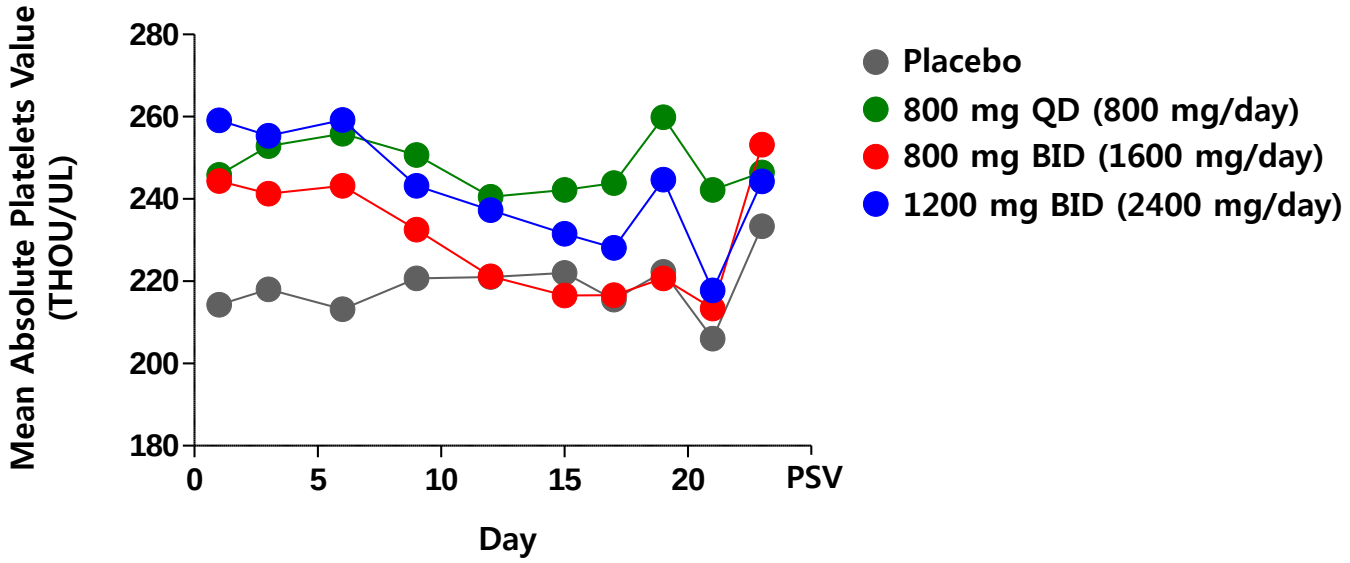

Figure S2 Mean platelet counts after treatment with LCB01-0371 over time (Multiple Ascending Dose) in phase 1b trial.

The patients receiving LCB01-0371 (800, 1600, and 2400 mg/day) are shown.

Figure S3

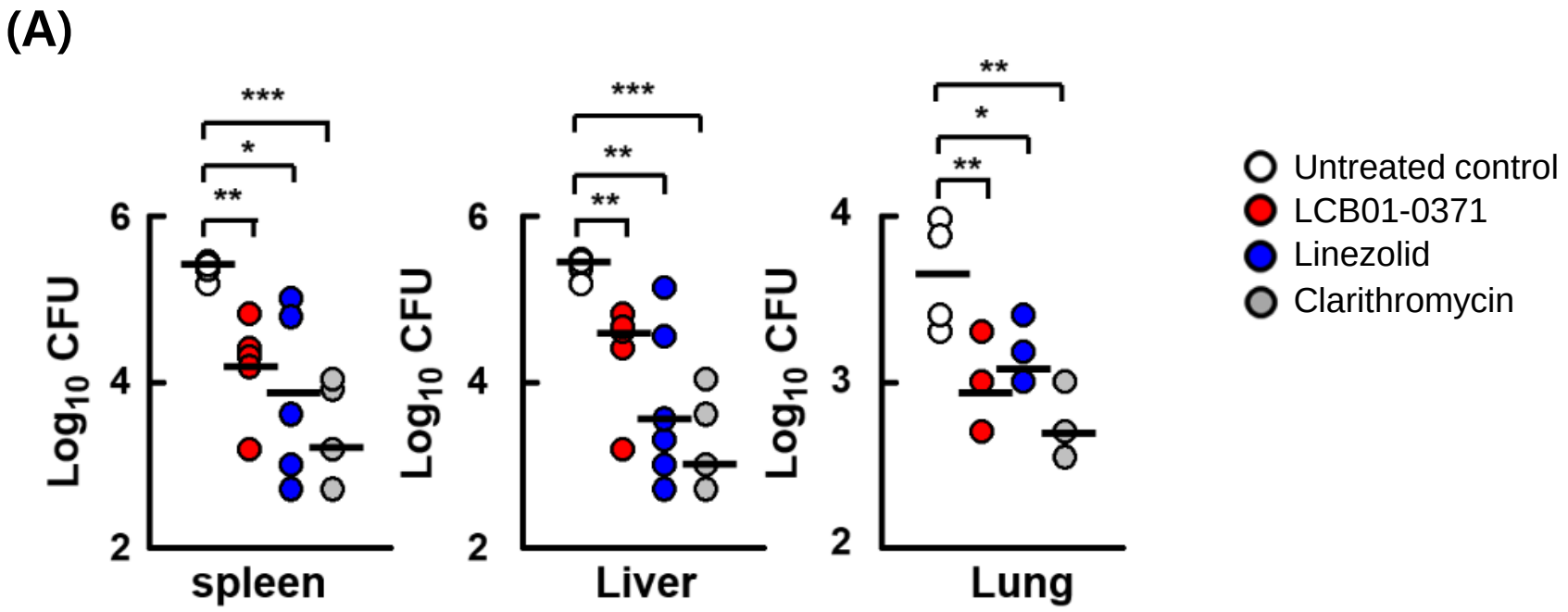

Figure S3 *In vivo* efficacy of LCB01-0371, linezolid, and clarithromycin against *Mycobacterium abscessus* infection in two different media.

C57BL/6 WT female mice (n=6) were infected intravenously with 1×10^7 CFU *M. abscessus*. After 2 days of infection, mice were treated for 4 consecutive days with 1000 mg/kg LCB01-0371 and linezolid. Clarithromycin (200 mg/kg) and distilled water were used as positive and negative controls, respectively. The spleen, liver, and lung were harvested, and bacterial load was determined using 7H11 agar enriched with 10% OADC (A) and 5% BSA (B). Error bars represent ±SEM from two independent experiments (*P < 0.05, **P < 0.01, ***P < 0.001).

Figure S3

(B)

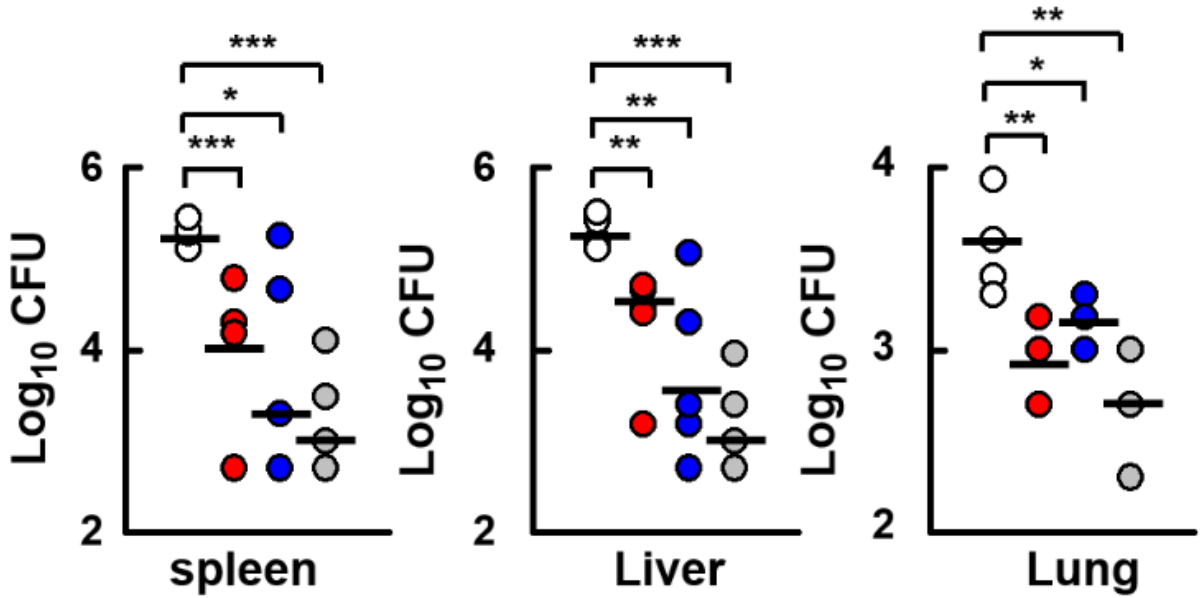

Figure S4

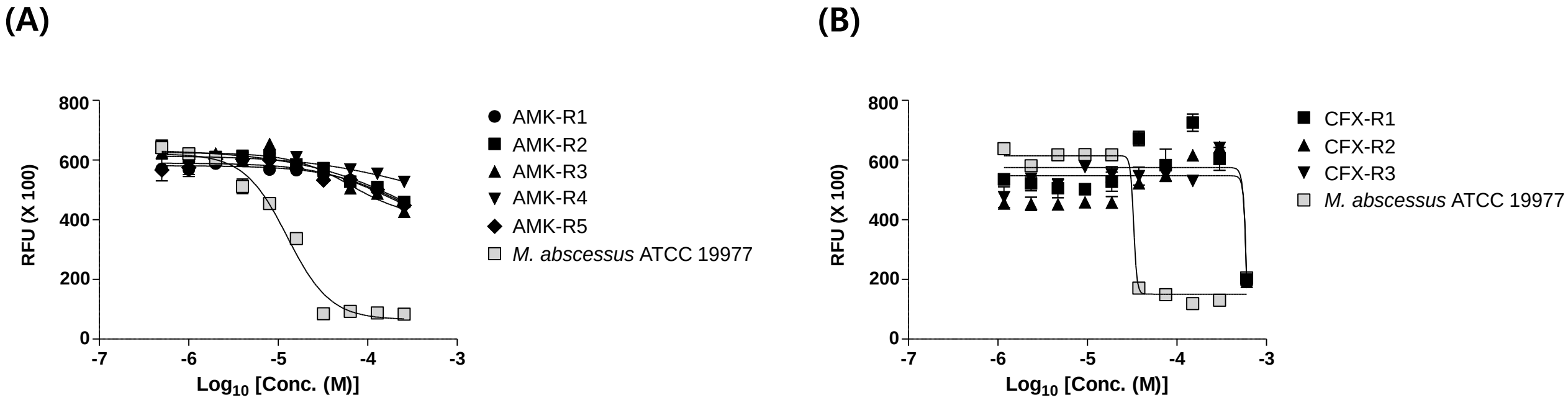

Figure S4 Activities of amikacin, cefoxitin, and clarithromycin against laboratory-generated amikacin, cefoxitin, and clarithromycin-resistant clones.

Dose-response curves of amikacin, cefoxitin, and clarithromycin against *Mycobacterium abscessus* AMK-R clones #1-5 (A), *M. abscessus* CFX-R clones #1-3 (B), and *M. abscessus* CLA-R clones #1-5 (C), using the resazurin-based assay. Each concentration was tested in triplicate.

Figure S4

(C)

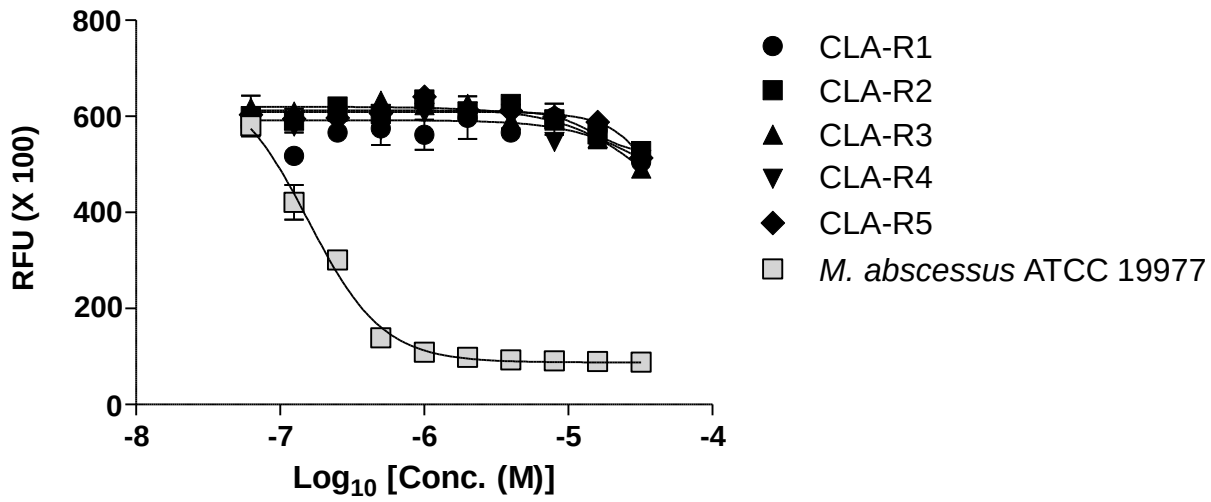
800
600
400
200
0
RFU (X 100)
-8
-7
-6
-5
-4
Log10 [Conc. (M)]
CLA-R1
CLA-R2
CLA-R3
CLA-R4
CLA-R5
M. abscessus ATCC 19977

Figure S5

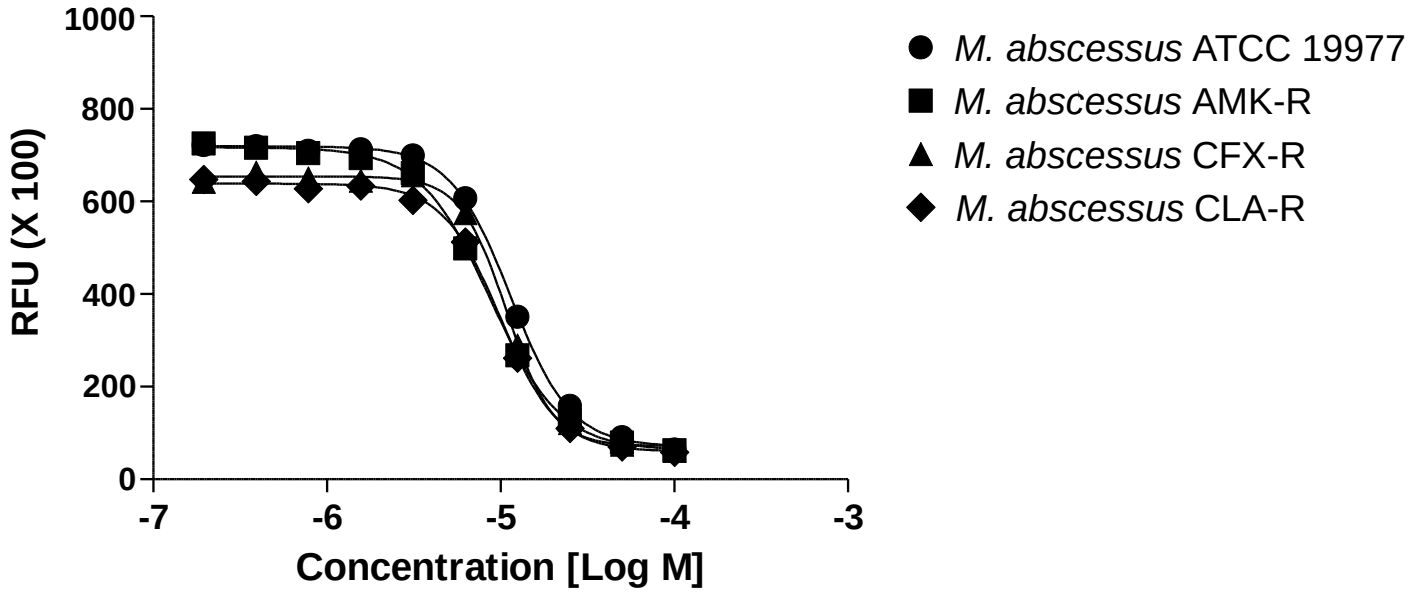

Figure S5 Activity of LCB01-0371 against laboratory-generated amikacin, cefoxitin, and clarithromycin-resistant clones.

Dose-response curve was generated by treatment with LCB01-0371 against *Mycobacterium abscessus* AMK-R, *M. abscessus* CFX-R, and *M. abscessus* CLA-R in comparison with *M. abscessus* ATCC 19977. Each concentration was tested in triplicate.

TABLE S1 In vivo pharmacokinetic parameters of linezolid and LCB01-0371.

	Mouse		Rat	
	Linezolid	**LCB01-0371**	**Linezolid**	**LCB01-0371**
PO (mg/kg)	30	30	10	10
AUC (mg.h/L)	73.39	48.28	23.91	16.24
AUC_{norm} (kg.h/L)	2.45	1.61	2.39	1.62
C_{max} (mg/L)	29.73	24.45	6.16	4.03
$C_{max\ norm}$ (kg/L)	0.99	0.82	0.62	0.40
t_{max} (h)	0.25	0.5	0.5	1.17
$t_{1/2}$ (h)	1.42	1.11	1.00	1.13
F (%)	ND	ND	155.6	68.6

TABLE S2 Pharmacokinetics of LCB01-0371 from phase 1b multiple ascending dose (MAD) study.

	400 mg		800 mg		1200 mg	
	1 day	**7 day**	**1 day**	**7 day**	**1 day**	**7 day**
C_{max} (μg/mL)	5.62 ±2.39	5.11 ±1.98	11.15 ±5.88	14.17 ±4.69	13.83 ± 2.07	20.25 ±7.15
T_{max} (hr)	0.60 ±0.14	0.92 ±0.56	1.04 ±0.75	0.71 ±0.25	1.13 ±0.63	1.04 ±0.60
$T_{1/2}$ (hr)	1.56 ±0.07	2.36 ±0.94	1.58 ±0.17	1.97 ±0.39	1.69 ±0.17	2.23 ±0.26
AUC_{tau} (μg.hr/mL)	7.79 ±2.96	8.38 ±2.75	19.46 ±4.38	28.10 ±7.75	38.15 ± 14.22	41.60 ±12.99
$AUC_{0\text{-}inf}$ (μg.hr/mL)	7.83 ±2.99	8.36 ±2.75	19.56 ±4.44	28.36 ±7.91	38.56 ±14.52	41.62 ±13.00
Accumulation ratio (D7AUC/D1AUC)	1.13 ± 0.12		1.47 ± 0.44		1.11 ± 0.20	
Vz/F (L/kg)	2.02 ±0.93	2.89 ± 2.13	1.49 ± 0.31	1.37 ±0.71	1.28 ± 0.37	1.62 ±0.81
CL/F (L/hr/kg)	0.91 ±0.42	0.80 ± 0.25	0.66 ±0.17	0.47 ±0.18	0.54 ±0.19	0.50 ±0.23
MRTlast (hr)	2.23 ±0.20	2.55 ± 0.61	2.37 ±0.29	2.43 ±0.49	2.90 ±0.43	2.81 ±0.40
C_{max_norm}(kg/L)	0.91 ±0.39	0.83 ±0.32	0.91 ±0.48	1.15 ±0.38	0.75 ±0.11	1.10 ±0.39
$AUC_{0\text{-}inf\_norm}$(kg.hr/L)	1.27 ±0.49	1.36 ± 0.45	1.59 ±0.36	2.30 ±0.64	2.09 ±0.79	2.25 ±0.70

Double blind, randomized, placebo control
MAD dose : 400 mg, 800 mg, 1200 mg or 1600 mg LCB01-0371 twice daily (BID) for 7 days
N = 8 (6 active+2 placebo)

TABLE S3 GLP-Tox study.

Study	Comments	
General Toxicity	PO (mg/kg)	IV (1 hr infusion) (mg/kg)
Single dose acute toxicity study in rats	MTD = 2000	MTD = 1000
14-day dose range-finding study in rats with TK	NOAEL = 100	
4-week toxicity study in rats with 4-week recovery	NOAEL = 60	NOAEL = 120
Single dose acute toxicity study in dogs	MTD = 1000	MTD = 500
14-day dose range-finding study in dogs with TK	NOAEL < 40	NOAEL < 40
4-week toxicity study in dogs with 4-week recovery	NOAEL (male = 20 and female=10)	NOAEL = 15
Genetic Toxicity		
Ames test	Negative	
In vitro chromosomal aberration test	Negative	
Rat micronucleus test	Negative	
Safety Pharmacology		
Assessment of blockage of hERG potassium channels	Negative (IC50 > 100 μM)	
Cardiovascular telemetry study in beagle dogs	Negative	
Respiratory (Pulmonary) study in rats	Negative	
Neurobehavioral safety evaluation in rats	Negative	