Supplemental Information

# Pharmacokinetic and pharmacodynamic evaluation of the atypical tetracyclines chelocardin and amidochelocardin in murine infection models

Running title: PK/PD profiling of chelocardin and amidochelocardin

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# Supplemental figures

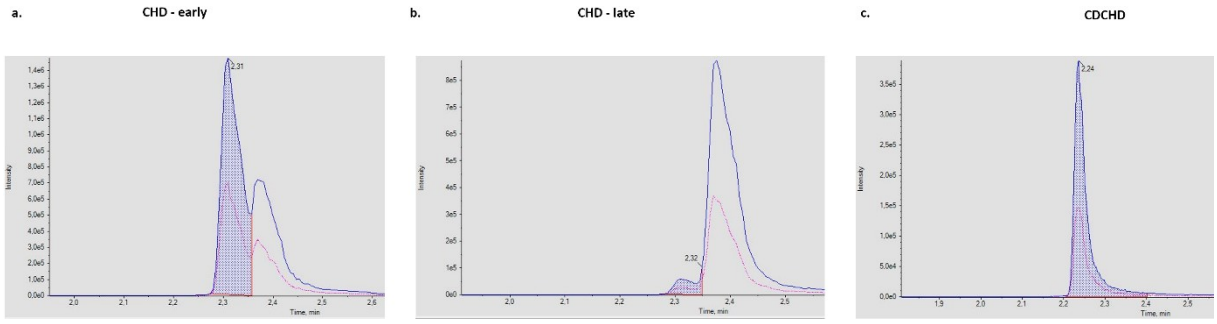


**Figure S1.** LC-MS/MS traces of CHD, epi-CHD and CDCHD in plasma after IV administration.

LC-MS/MS traces of CHD (a,b) and CDCHD (c) are displayed. CHD and epi-CHD in plasma are displayed 15 min (a) and 24 hours (b) post IV administration of 15 mg/kg. CDCHD in plasma is displayed 15 min after administration (c). The active epimer is marked in blue. Whereas the active epimer decreases for CHD (a,b), no epi-CDCHD can be found (c).

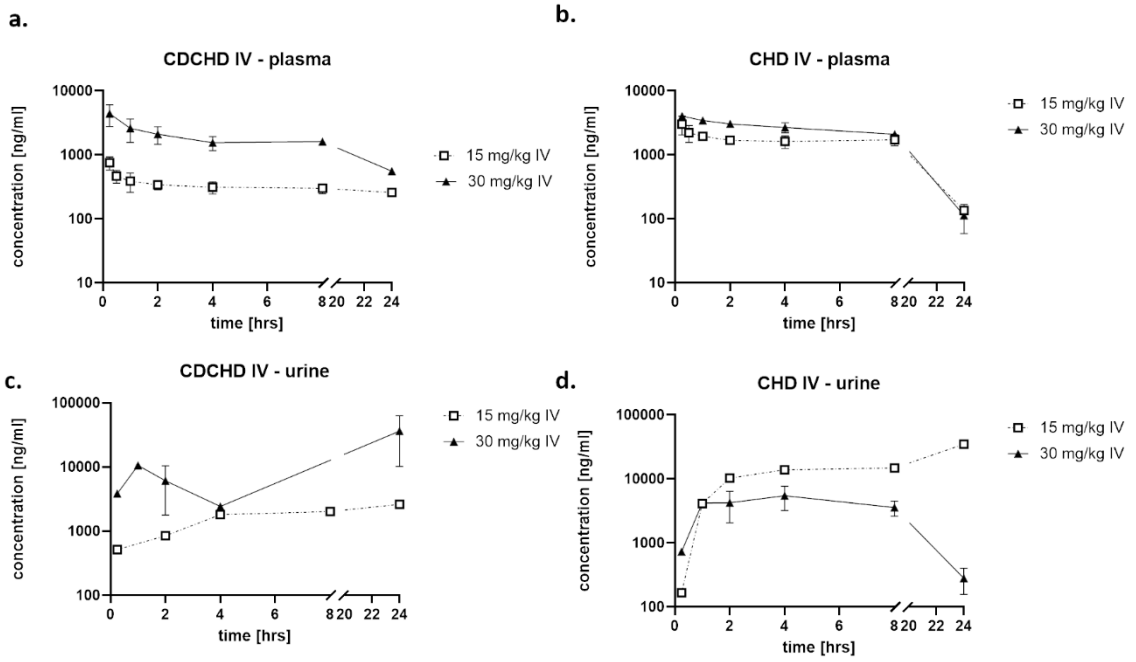


**Figure S2.** Pharmacokinetic profiles of CHD and CDCHD at 15 and 30 mg/kg IV.

CDCHD (a,c) and CHD (b,d) were administered at 15 mg/kg IV and 30 mg/kg IV, respectively. Plasma concentrations of CDCHD (a) and CHD (c) at 15 mg/kg IV (white) and 30 mg/kg IV (black) are displayed. Moreover, urine concentrations of CDCHD (b) and CHD (d) at 15 mg/kg IV (white) and 30 mg/kg IV (black) are displayed. N=3 mice were used per time point.

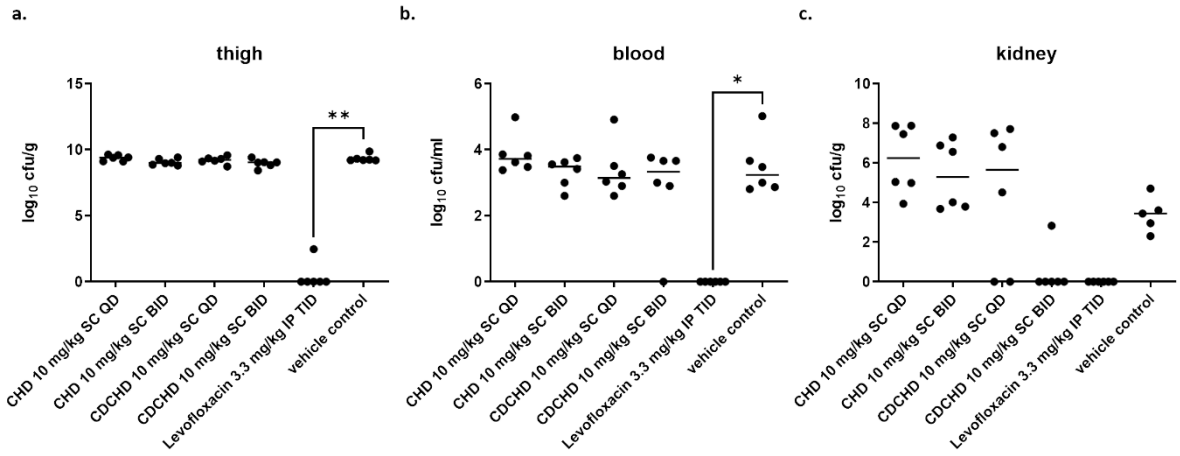


**Figure S3.** Assessment of low doses of CHD and CDCHD in the neutropenic thigh infection model with *K. pneumoniae*.

CHD and CDCHD were tested in the neutropenic thigh infection model with *K. pneumoniae* at 10 mg/kg SC QD and 10 mg/kg SC BID, respectively, against levofloxacin 3.3 mg/kg IP TID. Bacterial loads were determined in thigh (a), blood (b) and kidney (c). Bacterial loads were expressed as $\log_{10}$ cfu/g in thigh and kidney and as $\log_{10}$ cfu/ml in blood. Per group n=6 animals were used. Two thighs from one animal were pooled. *: $p < 0.05$, ** $p < 0.01$.

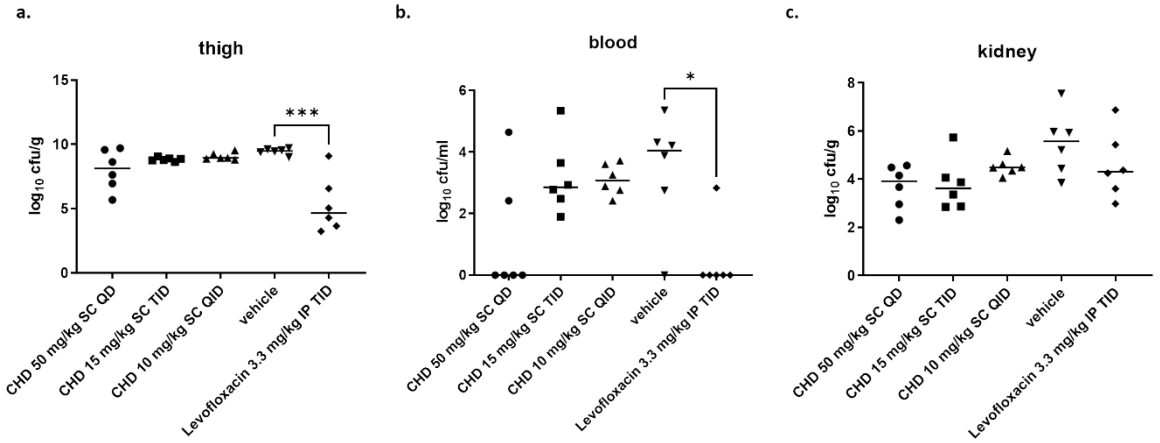


**Figure S4.** Assessment of higher doses of CHD in the neutropenic thigh infection model with *K. pneumoniae*.

CHD was tested in the neutropenic thigh infection model with *K. pneumoniae* at 50 mg/kg SC QD, 15 mg/kg SC TID and 10 mg/kg SC QID against levofloxacin 3.3 mg/kg IP TID. Bacterial loads were determined in thigh (a), blood (b) and kidney (c). Bacterial loads were expressed as $\log_{10}$ cfu/g in thigh and kidney and as $\log_{10}$ cfu/ml in blood. Per group n=6 animals were used. Two thighs from one animal were pooled. *: $p < 0.05$, ***: $p < 0.001$.

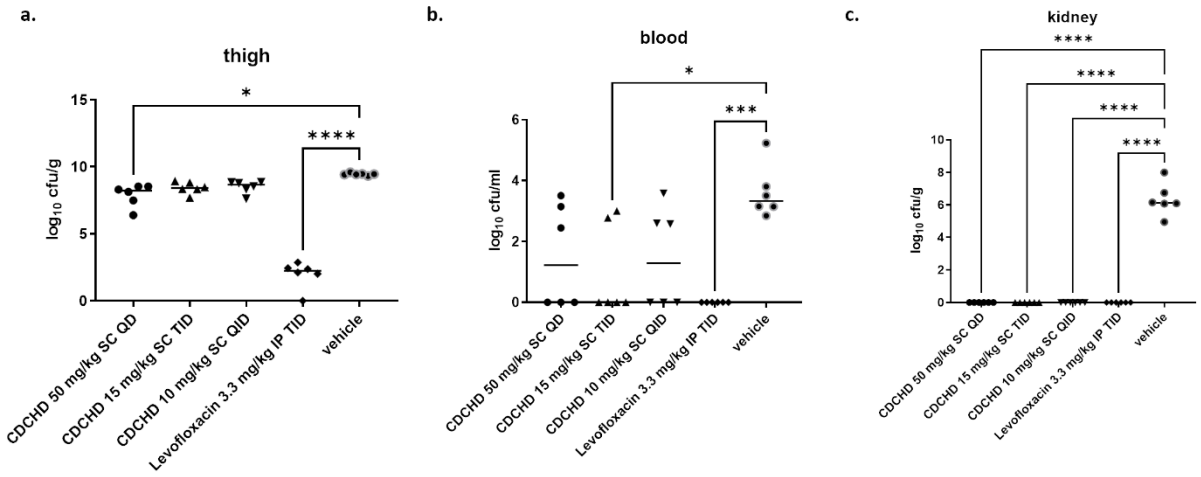


**Figure S5.** Assessment of higher doses of CDCHD in the neutropenic thigh infection model with *K. pneumoniae*.

CDCHD was tested in the neutropenic thigh infection model with *K. pneumoniae* at 50 mg/kg SC QD, 15 mg/kg SC TID and 10 mg/kg SC QID against levofloxacin 3.3 mg/kg IP TID. Bacterial loads were determined in thigh (a), blood (b) and kidney (c). Bacterial loads were expressed as $\log_{10}$ cfu/g in thigh and kidney and as $\log_{10}$ cfu/ml in blood. Per group n=6 animals were used. Two thighs from one animal were pooled. *: $p < 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$.

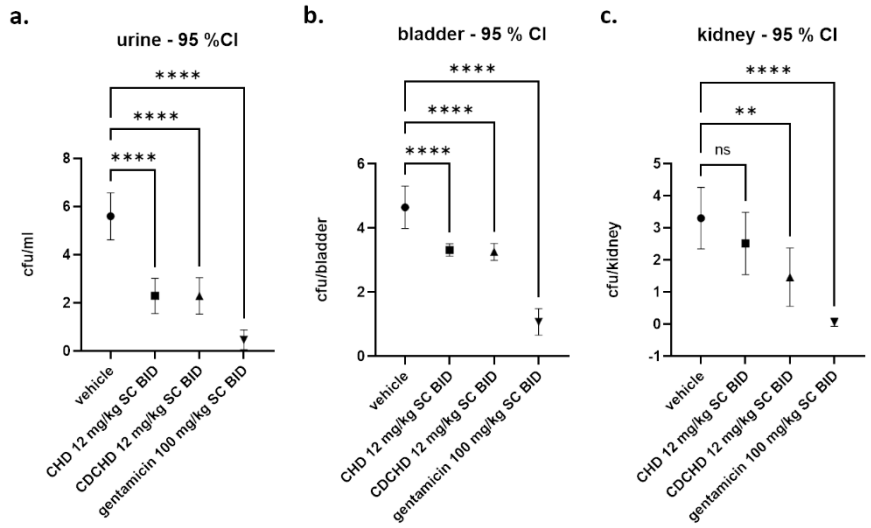


**Figure S6.** Assessment of CHD and CDCHD in an ascending urinary tract infection model with display of the 95 % confidence interval.

CHD and CDCHD were tested in an ascending urinary tract infection model with *E. coli* at 10 mg/kg SC BID against gentamicin 100 mg/kg SC BID. Bacterial loads were determined in urine (a), bladder (b) and kidney (c). Bacterial loads were expressed as $\log_{10}$ cfu/ml in urine, $\log_{10}$ cfu/bladder for bladder and as and kidney and as $\log_{10}$ cfu/kidney for kidney. Per group n=21 animals were used. Here, the mean with the 95% confidence interval (CI) is plotted. ns : not significant, **: $p < 0.01$, **** : $p < 0.0001$.

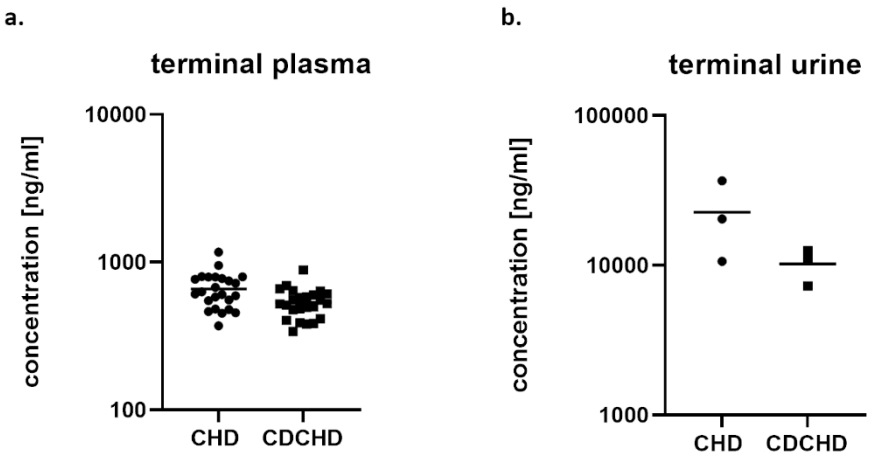


**Figure S7.** Bioanalysis of terminal plasma and urine samples from the ascending urinary tract infection model.

CDCHD and CHD were both assessed in an urinary tract infection model with *E. coli*. Terminal plasma (a) and urine (b) levels of both CHD and CDCHD are displayed. N=24 for plasma and n=3 for urine.

# Supplemental tables

**Table S1.** MIC determination of epi-CHD, CHD and CDCHD against a variety of ESKAPE pathogens.

| | MIC [µg/ml] | | |
|---|---|---|---|
| **pathogen** | epi-CHD | CHD | CDCHD |
| ***E. faecium* DSM-20477** | 64 | 4 | 2 |
| ***S. aureus* DSM-346** | 16 | 4 | 2 |
| ***K. pneumoniae* DSM-30104** | 16 | 2-4 | 1 |
| ***A. baumannii* DSM-30008** | 16 | 16 | 8 |
| ***P. aeruginosa* DSM-11128** | > 64 | > 64 | 16 |
| ***E. aerogenes* DSM-30053** | 64 | 32 | 8 |

**Table S2.** MIC of CHD, CDCHD, gentamicin, levofloxacin and ciprofloxacin against strains used for *in vivo* testing.

| | MIC [µg/ml] | | | | |
|---|---|---|---|---|---|
| **pathogen** | CHD | CDCHD | levofloxacin | ciprofloxacin | gentamicin |
| ***K. pneumoniae* ATCC 43816** | 5 | 1.25 | 0.08 | - | - |
| ***E. coli* ATCC 25922** | 2 | 2 | - | 0.008 | - |
| ***E. coli* C175-94** | 2 | 2-4 | - | - | 0.75 |

**Table S3.** Mass transitions for quantification of CDCHD and CHD using caffeine as internal standard.

| | Q1 mass | Q3 mass | DP [volts] | CE [volts] | CXP [volts] |
|---|---|---|---|---|---|
| **Caffeine** | 195.024 | 138.0 | 80.0 | 27.0 | 10.0 |
| | | 110.0 | 80.0 | 31.0 | 6.0 |
| **CDCHD** | 413.069 | 396.2 | 80.0 | 21.0 | 26.0 |
| | | 378.1 | 80.0 | 21.0 | 28.0 |
| **CHD** | 412.056 | 271.1 | 80.0 | 21.0 | 14.0 |
| | | 253.1 | 80.0 | 33.0 | 16.0 |

DP: declustering potential; CE: collision energy; CXP: cell exit potential

**Table S4.** Doses and dosage regimens in the neutropenic thigh infection model with *K. pneumoniae* (low dose CHD/CDCHD).

| compound | dose [mg/kg] | route | time points [h] |
|---|---|---|---|
| **vehicle** | - | SC | 1, 3, 5, 7 |
| **levofloxacin** | 3.3 | IP | 2, 6, 10 |
| **CHD** | 10 | SC | 2, 10 |
| **CHD** | 10 | SC | 6 |
| **CDCHD** | 10 | SC | 2, 10 |
| **CDCHD** | 10 | SC | 6 |

**Table S5.** Doses and dosage regimens in the neutropenic thigh infection model with *K. pneumoniae* (high dose CHD).

| compound | Dose [mg/kg] | Route | time points [h] |
|---|---|---|---|
| **vehicle** | - | SC | 1, 3, 5, 7 |
| **levofloxacin** | 3.3 | IP | 2, 6, 10 |
| **CHD** | 10 | SC | 1, 3, 5, 7 |
| **CHD** | 15 | SC | 1, 4, 7 |
| **CHD** | 50 | SC | 1 |

**Table S6.** Doses and dosage regimens in the neutropenic thigh infection model with *K. pneumoniae* (high dose CDCHD).

| compound | Dose [mg/kg] | Route | time points [h] |
|---|---|---|---|
| **Vehicle** | - | SC | 1, 3, 5, 7 |
| **levofloxacin** | 3.3 | IP | 2, 6, 10 |
| **CDCHD** | 10 | SC | 1, 3, 5, 7 |
| **CDCHD** | 15 | SC | 1, 4, 7 |
| **CDCHD** | 50 | SC | 1 |

**Table S7.** Doses and dosage regimens in the neutropenic thigh infection model with *E. coli*.

| compound | Dose [mg/kg] | Route | time points [h] |
|---|---|---|---|
| **Vehicle** | - | IV | 1, 6 |
| **ciprofloxacin** | 20 | IV | 1, 9 |
| **CHD** | 15 | IV | 1, 6 |
| **CDCHD** | 15 | IV | 1, 6 |
| **CHD** | 50 | IV | 1 |
| **CDCHD** | 50 | IV | 1 |