

**[0186]** Biapenem MICs against KPC-producing strains including those expressing multiple classes of enzymes ranged between 8 and 64  $\mu\text{g/ml}$ . The addition of Compound I was associated with marked potentiation in characterized strains of Enterobacteriaceae with serine carbapenemases; Biapenem MICs were  $\leq 0.25 \mu\text{g/ml}$  in the presence of 0.3 to 5  $\mu\text{g/ml}$  of Compound I. Time-kill studies in KPC-producing strains (Biapenem MICs of 16-32  $\mu\text{g/ml}$ ) demonstrated significant bactericidal synergism by the addition of Compound I. In the multi-drug resistant isolate panel of Enterobacteriaceae (MIC<sub>90</sub>>32 for fluoroquinolones, aminoglycosides, cephalosporins, aztreonam and piperacillin/tazobactam), the MIC<sub>90</sub> of Biapenem (with 4  $\mu\text{g/ml}$  Compound I) was 0.5  $\mu\text{g/ml}$ . Biapenem showed potency similar to meropenem against *P. aeruginosa*, with no enhancement with the addition of Compound I. The results suggest that Compound I combined with the carbapenem Biapenem is a highly active against Gram-negative pathogens, particularly against carbapenem-resistant Enterobacteriaceae and serine carbapenemases such as KPC.

#### Example 5

**[0187]** Example 5 provides the single-dose pharmacokinetic studies of Compound I in preclinical species, including intraperitoneal administration to Swiss-Webster mice and intravenous administration to Sprague-Dawley rats, Beagle dogs, and Cynomolgus monkeys.

**[0188]** Intravenous doses of Compound I were administered as 30-minute infusions. The pharmacokinetics of Compound I were also studied with coadministration of the carbapenem, Biapenem. Compound I concentrations were determined using an LC-MS detection method.

**[0189]** The mean pharmacokinetic parameters for Compound I are shown in Table 23 below. Compound I showed linear and favorable pharmacokinetic properties across all species, with clearance and volume of distribution comparable to those reported for beta-lactam antibiotics in these species. Clearance and volume of distribution were independent of dose. No PK drug interactions between Compound I and Biapenem were observed.

TABLE 23

| Pharmacokinetics Studies of Preclinical Species |                   |                                   |  |                        |                                  |                  |
|---|-------------------|-----------------------------------|--|------------------------|----------------------------------|------------------|
| Species   | Dose              | $C_{max}$<br>( $\mu\text{g/ml}$ ) | AUC<br>( $\mu\text{g} \cdot \text{h/ml}$ ) | CL or CL/F<br>(L/kg/h) | $V_{ss}$ or $V_{ss}/F$<br>(L/kg) | $T_{1/2}$<br>(h) |
|   | (mg/kg)/<br>Route |                                   |  |                        |                                  |                  |
| Mouse   | 5 IP              | 8.26                              | 3.1  | 1.61                   | 0.38                             | 0.16             |
| Mouse   | 15 IP             | 19.50                             | 8.30                                       | 1.80                   | 0.50                             | 0.20             |
| Mouse   | 50 IP             | 67.09                             | 31.35                                      | 1.60                   | 0.60                             | 0.25             |
| Rat   | 20 IV             | 19.8 $\pm$ 0.84                   | 12.2 $\pm$ 0.43                            | 1.7 $\pm$ 0.06         | 0.79 $\pm$ 0.07                  | 1.6 $\pm$ 0.17   |
| Rat   | 50 IV             | 45.9 $\pm$ 2.00                   | 28.2 $\pm$ 2.0                             | 1.8 $\pm$ 0.13         | 1.31 $\pm$ 0.37                  | 4.5 $\pm$ 1.34   |
| Dog   | 2 IV              | 6.49 $\pm$ 0.31                   | 6.13 $\pm$ 0.65                            | 0.33 $\pm$ 0.03        | 0.25 $\pm$ 0.02                  | 0.66 $\pm$ 0.06  |
| Dog   | 6 IV              | 19.56 $\pm$ 1.42                  | 18.39 $\pm$ 2.36                           | 0.33 $\pm$ 0.04        | 0.26 $\pm$ 0.01                  | 0.71 $\pm$ 0.09  |
| Dog   | 20 IV             | 66.79 $\pm$ 6.16                  | 73.64 $\pm$ 5.38                           | 0.27 $\pm$ 0.02        | 0.27 $\pm$ 0.03                  | 1.53 $\pm$ 0.73  |
| Monkey  | 2 IV              | 5.35 $\pm$ 0.28                   | 3.96 $\pm$ 0.51                            | 0.51 $\pm$ 0.07        | 0.26 $\pm$ 0.01                  | 0.57 $\pm$ 0.03  |
| Monkey  | 6 IV              | 17.04 $\pm$ 1.68                  | 12.19 $\pm$ 1.89                           | 0.50 $\pm$ 0.08        | 0.24 $\pm$ 0.01                  | 0.55 $\pm$ 0.12  |
| Monkey  | 20 IV             | 54.89 $\pm$ 3.32                  | 41.84 $\pm$ 2.53                           | 0.48 $\pm$ 0.03        | 0.26 $\pm$ 0.03                  | 1.89 $\pm$ 1.73  |

#### Example 6

**[0190]** Example 6 provides the in vivo efficacy study of the carbapenem Biapenem in combination with Compound I in mouse models of pulmonary and thigh infection due to the carbapenem-resistant, KPC-producing *K. pneumoniae*.

**[0191]** Four *K. pneumoniae* strains with Biapenem MICs ranging between 0.25-64 mg/L were used. Neutropenic mice were infected with  $\sim 10^5$  CFU/lung or  $\sim 10^6$  CFU/thigh. Intraperitoneal treatments with 50 mpk Biapenem +/- 50 mpk Compound I were initiated 2 hours post-infection as a single dose or continued every two hours for 24 hours. Mice were sacrificed on designated time points and colony counts in tissue determined.

**[0192]** In KPC-producing strains, treatment with Biapenem/Compound I produced significantly lower bacterial counts in tissues compared to Biapenem alone groups in both infection models. In lung infection, the extent of bacterial killing after an hour of a single dose was up to 1.3 log CFU greater than that in the control (Biapenem alone) group. The thigh infection model data is shown in Table 24 below. Treatment with Biapenem/Compound I produced significant bacterial killing in both the murine thigh and lung infection models using strains resistant to Biapenem treatment. These data show this combination to be a promising therapeutic option for the treatment of infections caused by KPC producing strains.

TABLE 24

| In vivo Efficacy of Biapenem/Compound I Combination |                                   |   |                                      |   |
|---|-----------------------------------|---|--------------------------------------|---|
| Strain  | Biapenem MIC ( $\mu\text{g/ml}$ ) |   |                                      | Change in Log<br>CFU/thigh $\pm$<br>SD @ 24 h |
|   | Alone                             | with<br>Compound I<br>(5 $\mu\text{g/ml}$ ) | Compound                             |   |
| ATCC 43816  | 0.25                              | 0.25  | Biapenem<br>Biapenem +<br>Compound I | -1.09 $\pm$ 0.30<br>-1.33 $\pm$ 0.41          |