Validation data for CU-CPT9a

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Version 24A04-AK

CU-CPT9a is a potent and selective inhibitor of human Toll-like receptor 8 (hTLR8). CU-CPT9a has been used to discover the important function of TLR8 as a dominant sensor of different bacterial strains. Treatment of HEK-Blue™ hTLR8 cells with CU-CPT9a results in the inhibition of the inducible NF-κB response in a dose-dependent manner upon incubation with either R848, a TLR7/TLR8 agonist or TL8-506, a specific TLR8 agonist **(Figure 1)**. The specific inhibition of TLR7 and TLR8 signaling by CU-CPT9a has been verified **(Figure 2)**. There is minimal to no inhibition noted for TLR7, highlighting the highly specific nature of CU-CPT9a for TLR8.

Inhibition of TLR8 signaling by CU-CPT9a in HEK-Blue™ hTLR8 cells

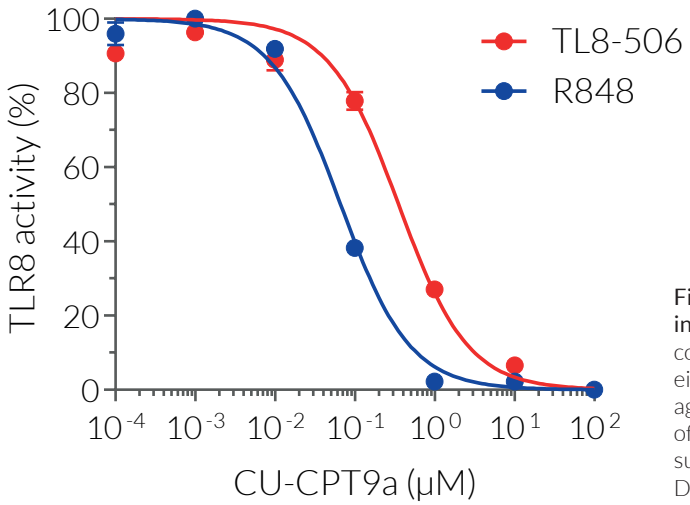

Figure 1. CU-CPT9a inhibits hTLR8 in a dose-dependent response in HEK-Blue™ hTLR8 cells. The cells were incubated with increasing concentrations of CU-CPT9a for 3 hours. Following this, 10 µg/ml of either TL8-506 (red), a specific TLR8 agonist, or R848 (blue), a TLR7/TLR8 agonist, were added to the cells. After overnight incubation, activation of TLR8 (NF-κB activity) was assessed by measuring SEAP activity in the supernatant, using QUANTI-Blue™ Solution, a SEAP detection reagent. Data are shown in percentage (%) of TLR8 activity.

Specific inhibition of TLR7 and TLR8 signaling by CU-CPT9a

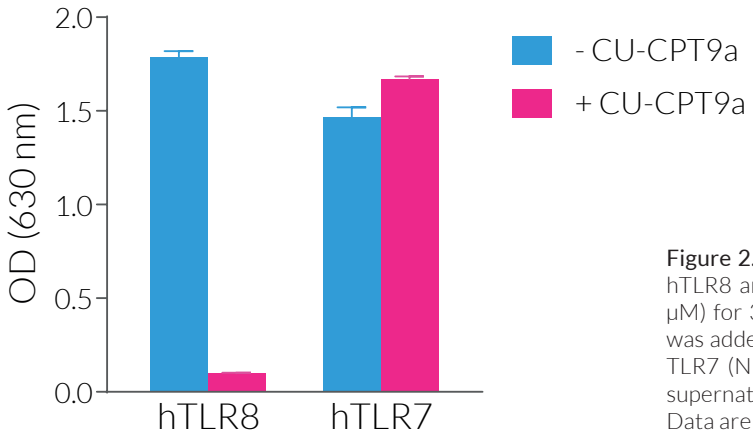

Figure 2. Specific inhibition of human TLR8 by CU-CPT9a. HEK-Blue™ hTLR8 and HEK-Blue™ hTLR7 cells were incubated with CU-CPT9a (1 µM) for 3 hours. Following this, R848 (10 µg/ml), a TLR7/TLR8 agonist, was added to the cells. After overnight incubation, activation of TLR8 or TLR7 (NF-κB activity) was assessed by measuring SEAP activity in the supernatant, using QUANTI-Blue™ Solution, a SEAP detection reagent. Data are shown as optical density (OD) at 630nm.

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