Validation data for CU-CPT9a

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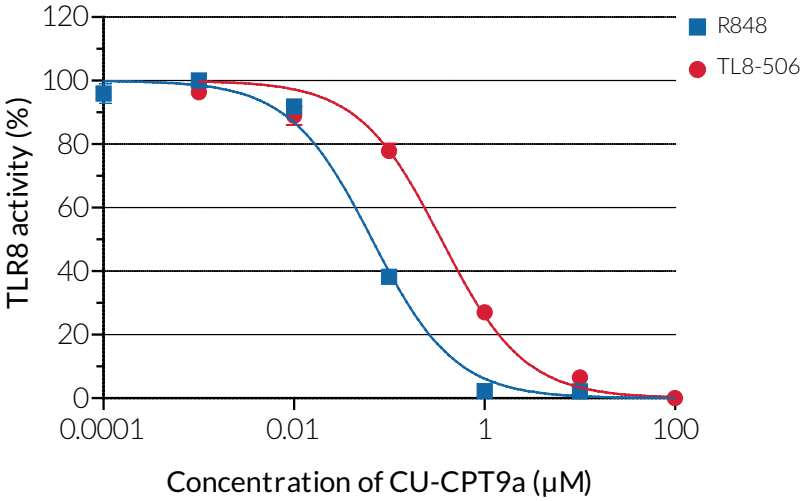
Version 19J25-ED

CU-CPT9a is a potent and selective inhibitor of human Toll-like receptor 8 (hTLR8) (**Figure 1 and 2**). CU-CPT9a stabilizes the TLR8 dimer in its resting state and not only prevents TLR8 activation but also antagonizes any binding of TLR8 ligands.

Evaluation of hTLR8 inhibition by CU-CPT9a

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. Please note that at high concentrations (i.e. 100 mM) crystal-like structures may be observed in the resuspended CU-CPT9a product.



CU-CPT9a IC_{50} (µM) ± Std Error	
R848	0.066 ± 0.004
TL8-506	0.352 ± 0.09

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, either TL8-506 (10 µg/ml), a specific TLR8 agonist, or R848 (10 µg/ml), 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 as a percentage (%) of TLR8 activity (no inhibitor).

Specificity of CU-CPT9a

Treatment of HEK-Blue™ hTLR8, hTLR7, and murine (m)TLR7 cells with CU-CPT9a results in the inhibition of the inducible NF-κB response in the hTLR8 cells only upon incubation with R848, a TLR7/TLR8 agonist. There was minimal to no inhibition noted for both human and murine TLR7, highlighting the highly specific nature of CU-CPT9a for TLR8.

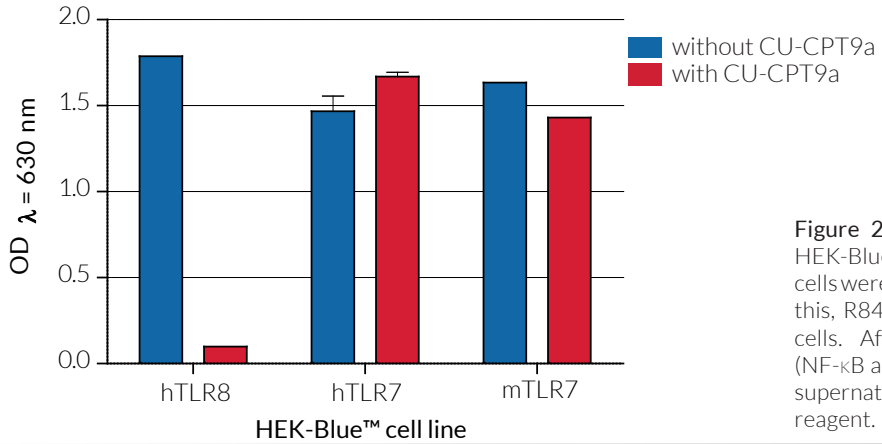


Figure 2: Specific inhibition of human TLR8 by Cu-CPT9a. HEK-Blue™ hTLR8, HEK-Blue™ hTLR7, and HEK-Blue™ mTLR7 cells were incubated with Cu-CPT9a (1 µM) for 3 hours. Following this, R848 (10 µg/ml), a TLR7/TLR8 agonist, was added to the cells. After overnight incubation, activation of TLR8 or TLR7 (NF-κB 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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