Validation data for Bay11-7082

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Bay11-7082 is a potent inhibitor of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and the NLRP3 (NOD-like receptor (NLR) pyrin domain-containing protein 3) inflammasome. NF-κB regulates multiple aspects of innate and adaptive immune functions and serves as a pivotal mediator of inflammatory responses. While the NLRP3 inflammasome is an innate immune sensor that is activated by a two-step process; a first signal (‘priming’) is provided by microbial molecules such as lipopolysaccharide (LPS), and the second signal is provided by a wide array of stimuli including endogenous molecules or crystalline substances such as monosodium urate (MSU) crystals. The ability of Bay11-7082 to inhibit the NLRP3 inflammasome was validated using InvivoGen’s THP-1/HEK-Blue™ IL-1β assay. This assay uses the secretion of IL-1β by THP1-Null2 cells as an indicator of NLRP3 inflammasome induction. The IL-1β production by these cells is measured using HEK-Blue™ IL-1β cells. Treatment with Bay11-7082 inhibited IL-1β secretion in a dose-dependent manner (Figure 1).

Figure 1: Bay11-7082 inhibits the NLRP3 inflammasome response in a dose-dependent manner.

ThP1-Null2 cells were primed with LPS-EK (1 μg/ml) for 3 h and then stimulated with MSU (150 μg/ml) and increasing concentrations of Bay11-7082. After overnight incubation, IL-1β secretion was analyzed by adding 50 μl of supernatant from treated THP1-Null2 cells to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. Data are shown as percentage (% inhibition).