Validation data for BMS-986256

https://www.invivogen.com/tlr-7-8-inhibitor-bms986256-afimetoran

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BMS-986256 (also known as Afimetoran) is a small molecule that functions as a dual and selective inhibitor of TLR7 and TLR8. The ability of BMS-986256 to inhibit TLR7 and TLR8 signaling was validated using a panel of InvivoGen's cell lines featuring an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. BMS-986256 efficiently inhibits human (h)TLR7, mouse (m)TLR7, hTLR8, but not mTLR8, in HEK-Blue™-derived cell lines overexpressing TLR7 or TLR8 **(Figure 1)**. The inhibition potency of BMS-986256 for both NF-κB and IRF signaling pathways downstream of hTLR7 and hTLR8 was also confirmed using THP1-Dual™-derived cell lines expressing NF-κB-inducible SEAP and IRF-inducible Lucia luciferase reporter genes **(Figure 2)**. The specific inhibition of TLR7 and TLR8 signaling by BMS-986256 has been verified **(Figure 3)**.

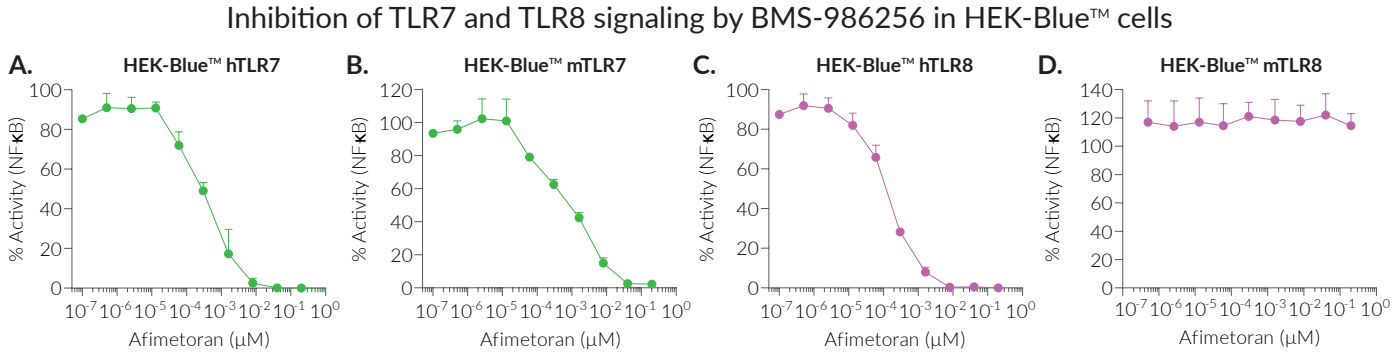

Figure 1: BMS-986256 is a potent inhibitor of human TLR7, mouse TLR7, and human TLR8, but not mouse TLR8 signaling pathways in HEK cells overexpressing TLR7 or TLR8. HEK-Blue™ cells overexpressing hTLR7 **(A)**, mTLR7 **(B)**, hTLR8 **(C)**, or mTLR8 **(D)**, were cultured with increasing concentrations of BMS-986256. After 3 hours of incubation, the following ligands were added: 100 ng/ml **(A)** or 300 ng/ml **(B)** R848, a TLR7/8 agonist, or 300 ng/ml **(C)** or 1 μg/ml **(D)** TL8-506, a TLR8 agonist. After overnight incubation, the neutralizing activity of BMS-986256 was determined by measuring the reduction of SEAP production in the supernatant using the QUANTI-Blue™ detection reagent. Data are shown as a percentage (%) of maximal TLR activation with each agonist (without inhibitor; mean +SEM).

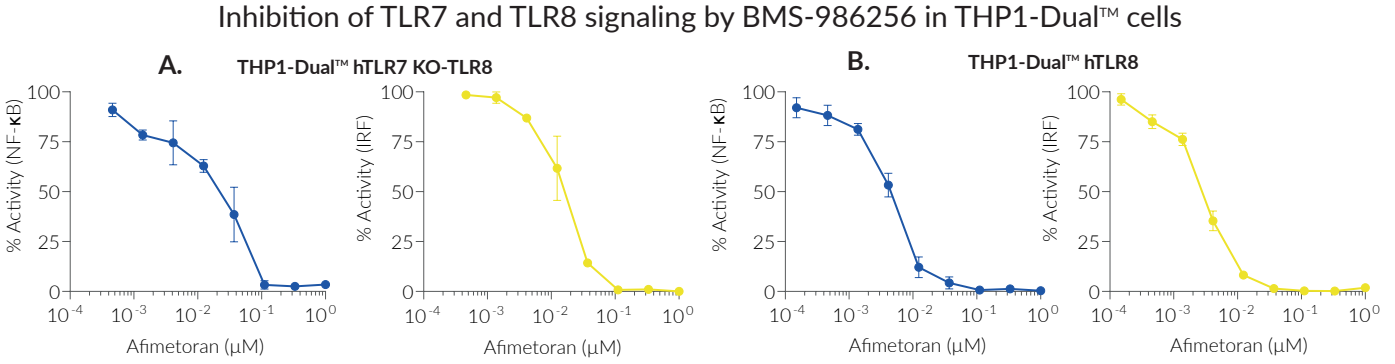

Figure 2: BMS-986256 is a potent inhibitor of NF-κB and IRF signaling pathways downstream of human TLR7 and TLR8. THP1-Dual™ hTLR7 KO-TLR8 **(A)** and THP1-Dual™ hTLR8 **(B)** cells were cultured in the presence of increasing concentrations of BMS-986256. After 3 hours of incubation, the following ligands were added: 1 μg/ml R848 (TLR7/8 agonist) (**A**), or 1 μg/ml TL8-506 (TLR8 agonist) (**B**). After overnight incubation, the neutralizing activity of BMS-986256 was determined by measuring the reduction of SEAP and Lucia luciferase production in the supernatant using the QUANTI-Blue™ and QUANTI-Luc™ 4 detection reagents, respectively. Data are shown as a percentage (%) of maximal TLR activation with each agonist (without inhibitor; mean ±SEM)

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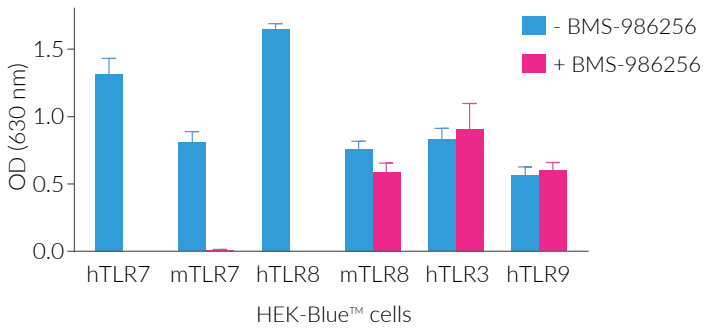

Abbreviations: hTLR7 = HEK-Blue™ hTLR7, mTLR7 = HEK-Blue™ mTLR7, hTLR8 = HEK-Blue™ hTLR8, mTLR8 = HEK-Blue™ mTLR8, hTLR3 = HEK-Blue™ hTLR3, hTLR9 = HEK-Blue™ hTLR9 cells.

Figure 3: BMS-986256 is a specific dual inhibitor of TLR7 and TLR8. HEK-Blue™ cells overexpressing hTLR7, mTLR7, hTLR8, mTLR8, hTLR3, or hTLR9 were incubated with BMS-986256 (1 µM). After 3 hours of incubation, the following ligands were added: R848 30 ng/ml (hTLR7) or 300 ng/ml (mTLR7), TL8-506 100 ng/ml (hTLR8) or 3 µg/ml (mTLR8), Poly(I:C) HMW 50 ng/ml (hTLR3), and ODN 2006 500 ng/ml (hTLR9). After overnight incubation, the neutralizing activity of BMS-986256 was determined by measuring the reduction of SEAP production in the supernatant using QUANTI-Blue™ detection reagent. Data are shown as optical density (OD) at 630 nm (mean +SEM).