Validation data for M5049

https://www.invivogen.com/m5049

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Version 23D27-NJ

M5049 (also known as Enpatoran) is a small molecule that functions as a dual and selective inhibitor of TLR7 and TLR8. The ability of M5049 to inhibit TLR7 and TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. M5049 efficiently inhibits human (h)TLR7, mouse (m)TLR7, hTLR8, but not mTLR8, as assessed by the expression of an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter in HEK-Blue™-derived cell lines **(Figure 1).** The inhibition potency of M5049 for both NF-κB and IRF signaling pathways downstream of human TLR7 and TLR8 was also confirmed using THP1-Dual™-derived cell lines expressing inducible SEAP and Lucia luciferase reporter genes **(Figure 2)**. The specific inhibition of TLR7 and TLR8 signaling by M5049 has been verified **(Figure 3)**.

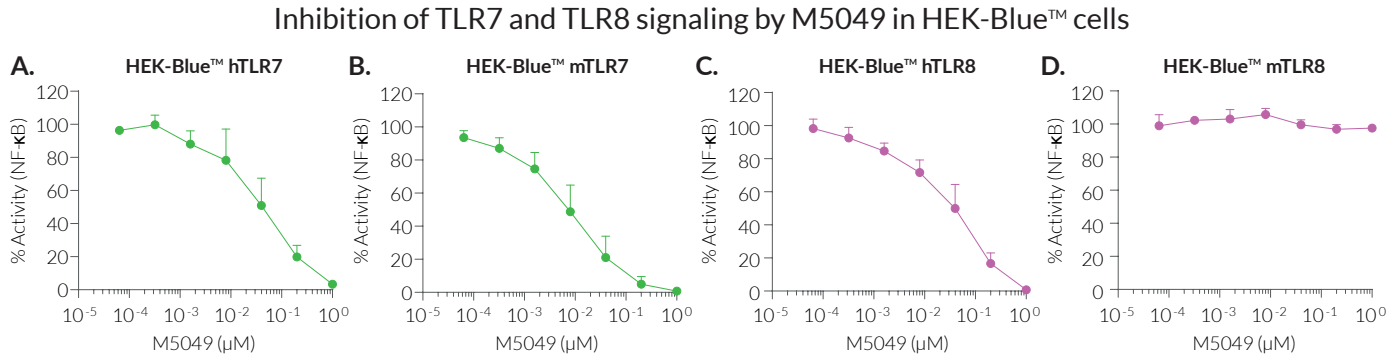

Figure 1: M5049 is a potent inhibitor of human TLR7, mouse TLR7, human TLR8, but not mouse TLR8 signaling pathways. HEK-Blue™ cells expressing hTLR7 **(A)**, mTLR7 **(B)**, hTLR8 **(C)**, or mTLR8 **(D)**, were cultured with increasing concentrations of M5049. After 3 hours of incubation, the following ligands were added: 30 ng/ml R848, a TLR7/8 agonist **(A and B)**, or 30 ng/ml **(C)** or 300 ng/ml **(D)** TL8-506, a TLR8 agonist. After overnight incubation, the neutralizing activity of M5049 was determined by measuring the reduction of SEAP production in the supernatant using the HEK-Blue™ detection reagent. Data are shown as a percentage (%) of maximal TLR activation with each agonist (without inhibitor; mean +SEM).

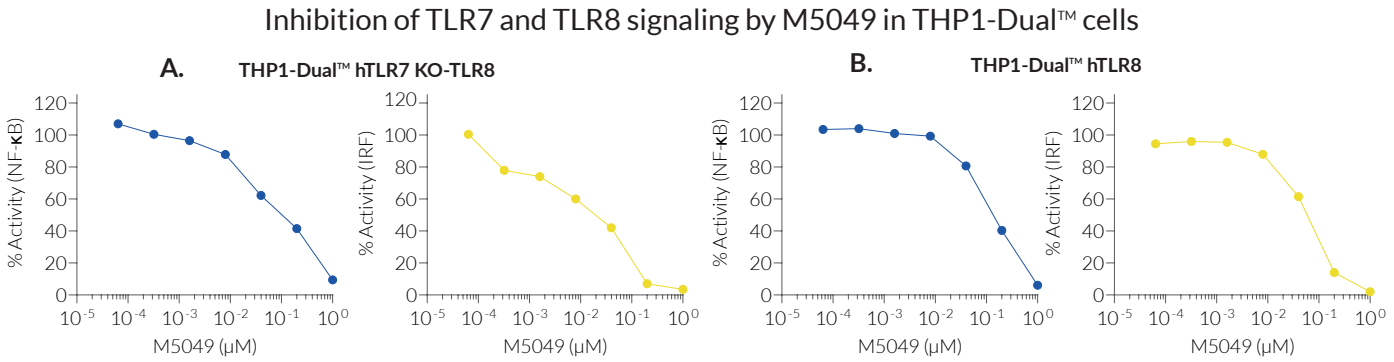

Figure 2: M5049 is a potent inhibitor of NF-κB and IRF signaling pathways downstream of human TLR7 and TLR8. THP1-Dual™ hTLR7 KO-TLR8 **(A)** and THP1-Dual™ hTLR8 **(B)** cells were cultured in the presence of increasing concentrations of M5049. After 3 hours of incubation, the following ligands were 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 M5049 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).

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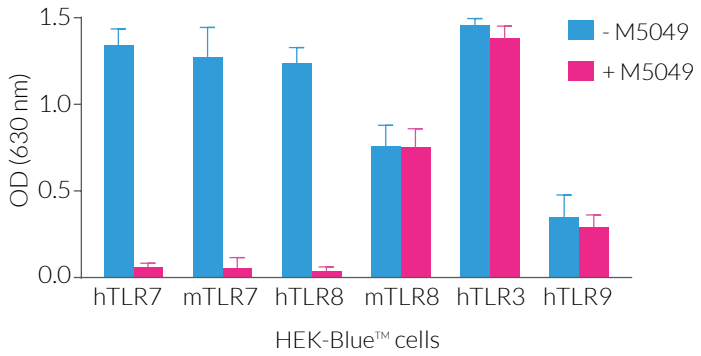

Abbreviations: hTLR7 = HEK-Blue™ hTLR7, mTLR7 = HEK-Blue™ mTLR7, hTLR8 = HEK-Blue™ hTLR8, mTLR8 = HEK-Blue™ mTLR8, hTLR3 = HEK-Blue™ hTLR3, hTLR9 = HEK-Blue™ hTLR9 cells.

Figure 3: M5049 is a specific dual inhibitor of TLR7 and TLR8. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, mTLR8, hTLR3, or hTLR9 were incubated with M5049 (1 µM). After 3 hours of incubation, the following ligands were added: R848 30 ng/ml (hTLR7) or 100 ng/ml (mTLR7), TL8-506 30 ng/ml (hTLR8) or 300 ng/ml (mTLR8), Poly(I:C) HMW 50 ng/ml (hTLR3), LPS-EK 1 ng/ml (hTLR4), and ODN 2006 500 ng/ml (hTLR9). After overnight incubation, the neutralizing activity of M5049 was determined by measuring the reduction of SEAP production in the supernatant using the HEK-Blue™ detection reagent. Data are shown as optical density (OD) at 630 nm (mean +SEM).