**Spebrutinib besylate**

**Cat. No.:** HY-18012A  
**CAS No.:** 1360053-81-1  
**Molecular Formula:** C₂₈H₂₈FN₅O₆S  
**Molecular Weight:** 581.62  
**Target:** Btk  
**Pathway:** Protein Tyrosine Kinase/RTK  
**Storage:** Please store the product under the recommended conditions in the COA.

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**BIOLOGICAL ACTIVITY**

**Description**
Spebrutinib besylate (AVL-292 benzenesulfonate; CC-292 besylate) is a potent inhibitor of Btk kinase activity (IC₅₀ <0.5 nM, Kᵢ = 7.69×10⁴ M⁻¹s⁻¹) in biochemical assays.

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<th>IC₅₀ &amp; Target</th>
<th>IC₅₀: &lt;0.5 nM (Btk) [¹]</th>
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**In Vitro**
Spebrutinib (CC-292) is a covalent, highly selective, orally active inhibitor of Btk with IC₅₀ value of 0.5 nM. Spebrutinib also less potently inhibits Yes, c-Src, Brk, Lyn, and Fyn with IC₅₀s of 723 nM, 1.729 μM, 2.43 μM, 4.4 μM, and 7.15 μM, respectively. Extensive analysis has revealed that the EC₅₀ of Btk occupancy from a Spebrutinib dose-response in Ramos cells (EC₅₀=6 nM) correlated directly with the cellular EC₅₀ of Btk kinase inhibition with Spebrutinib (EC₅₀=8 nM). Furthermore, the concentration at which Spebrutinib inhibits 90% of Btk activity in Ramos cells is 35 nM while the concentration of Spebrutinib required for 90% occupancy of Btk is 39 nM [¹].

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**PROTOCOL**

**Cell Assay [¹]**
Cells are incubated in serum-free RPMI media for 1-1.5 hours. Isolated human B cells are incubated with Spebrutinib at a final concentration of 0.001, 0.01, 0.1 and 1 μM. Ramos cells are incubated with 0.1 nM-3 μM Spebrutinib. Cells are then incubated in the presence of compound for 1 hour at 37°C. Following incubation, cells are centrifuged and resuspended in 100 μL of serum-free RPMI and BCR is stimulated with addition of 5 μg/mL α-human IgM. Samples are centrifuged, washed in phosphate-buffered saline (PBS), and lysed in 100 μL of Cell Extraction Buffer plus 1:10 (v/v) PhosSTOP Phosphatase Inhibitor and 1:10 (v/v) Complete Protease Inhibitor. Antibodies used for immunoblot analysis include P-PLCγ2, PLCγ2 (3871; CST), Syk (2712; CST), P-Syk (2710; CST), Btk, P-Btk, and Tubulin. Membranes are scanned on a Li-Cor Odyssey scanner using infrared fluorescence detection [¹].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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**CUSTOMER VALIDATION**

RESOURCES


REFERENCES