**BIOLOGICAL ACTIVITY**

**Description**
Spebrutinib besylate (AVL-292 benzenesulfonate; CC-292 besylate) is a potent inhibitor of Btk kinase activity ($IC_{50}$ <0.5 nM, $K_{inact}/K_i=7.69 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) in biochemical assays.

**$IC_{50}$ & Target**
$IC_{50}$: <0.5 nM (Btk)$^{[1]}$

**In Vitro**
Spebrutinib (CC-292) is a covalent, highly selective, orally active inhibitor of Btk with $IC_{50}$ value of 0.5 nM. Spebrutinib also less potently inhibits Yes, c-Src, Brk, Lyn, and Fyn with $IC_{50}$s of 723 nM, 1.729 $\mu$M, 2.43 $\mu$M, 4.4 $\mu$M, and 7.15 $\mu$M, respectively. Extensive analysis has revealed that the $EC_{50}$ of Btk occupancy from a Spebrutinib dose-response in Ramos cells ($EC_{50}=6 \text{ nM}$) correlated directly with the cellular $EC_{50}$ of Btk kinase inhibition with Spebrutinib ($EC_{50}=8 \text{ nM}$). 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$^{[1]}$.

**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 $\mu$M. Ramos cells are incubated with 0.1 nM-3 $\mu$M 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 $\mu$L of serum-free RPMI and BCR is stimulated with addition of 5 $\mu$g/mL α-human IgM. Samples are centrifuged, washed in phosphate-buffered saline (PBS), and lysed in 100 $\mu$L 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.

**CUSTOMER VALIDATION**

REFERENCES


Caution: Product has not been fully validated for medical applications. For research use only.
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