

Supplemental:

Experimental details of HUVEC proliferation, migration and tube-formation experiments performed by Southern Research Institute (Birmingham, Alabama).

Proliferation

Proliferation was determined using CellTiter-Glo Luminescent Cell Viability Assay. Briefly, Endothelial cells were seeded in 96-well plates in appropriate growth medium. After 24 h, various doses of CX-4945 were added, 6 replicates for each dose. After 72 hr further incubation, ATP-based luminescence signals were measured in relative luminescent units (RLU). Data were analyzed for the mean and standard deviation for each treatment condition. IC₅₀ values are determined based on the dose response curves.

Migration

HUVEC migration was determined using a trans-well plate system. Briefly, endothelial cells along with various doses of CX-4945 were seeded in the insert chambers of trans-well plates in duplicate with growth medium as the chemo-attractant in the bottom chamber. After 24 h, migrated cells were fixed, stained with Hoechst 33342 and quantified by using the Image Pro Plus software. Data were analyzed for the mean and standard deviation for each treatment condition. IC₅₀ values are determined based on the dose response curves.

Tube-formation

Briefly, HUVEC pre-stained with Calcein AM were seeded in a Matrigel coated 96-well plate in appropriate growth medium along with various doses of CX-4945. At 18 h after treatment, endothelial tubule length were quantitatively analyzed using Image Pro Plus software. Data were analyzed for the mean and standard deviation for each treatment condition. IC_{50} values are determined based on the dose response curves.