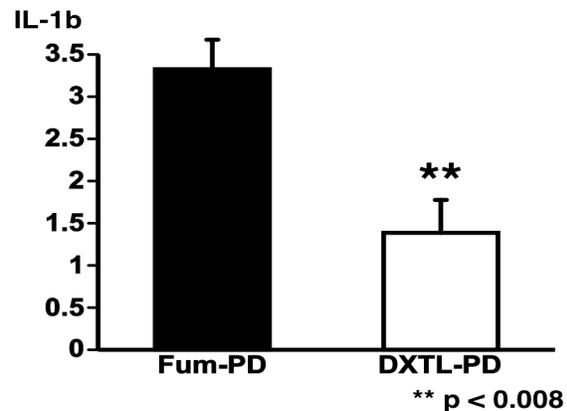
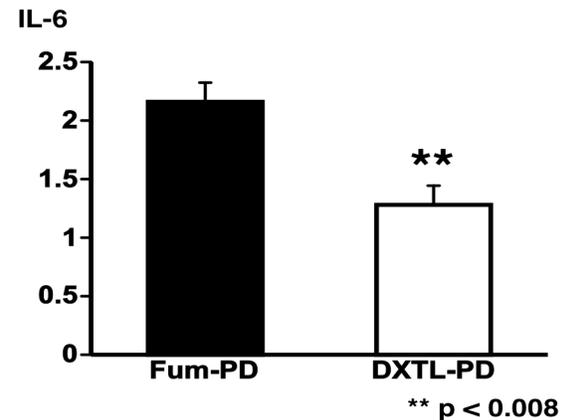


SUPPLEMENTAL FIGURE 1.

The Effect of Prodrugs on Macrophage Inflammatory Cytokine Release

Macrophages and Pro-drug treatment:

Three ml of 4% thioglycollate broth were injected intraperitoneally into wild type mice (C57BL). Four days later, mice were sacrificed and macrophages were isolated from the peritoneal cavity and seeded into 6 well plates at a density of 0.65×10^5 /ml per well. The plates were incubated overnight then stimulated with 100 ng/ml LPS for 2 h, followed by treatment with 2.4 μ M Fum-PD and Dxtl-PD in DMSO for 2h.



Quantitative reverse-transcription PCR:

RNA was extracted using PureLink RNA Mini Kit (Life Technology, Catalog number: 12183018A) and cDNA generated using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Catalog number: 11754050) SYBR Green quantitative reverse-transcription PCR was performed and the qPCR data were adjusted for a housekeeping gene.

Primer sequences:

36B4 Forward: 5' ATC CCT GAC GCA CCG CCG TGA

36B4 Reverse: 5' TGC ATC TGC TTG GAG CCC ACG TT

IL1B Forward: 5' TGT GAA ATG CCA CCT TTT GA

IL1B Reverse: 5' GGT CAA AGG TTT GGA AGC AG

IL6 Forward: 5' TGA TGC ACT TGC AGA AAA CA

IL6 Reverse: 5' ACC AGA GGA AAT TTT CAA TAG GC

The PCR results shown above corroborated the reports of others that neither fumagillin nor its analogs, including Fum-PD, are effective in macrophages. In contradistinction, the docetaxel prodrug did have bioactivity in macrophages as would be anticipated. In the present experiment, the inflammatory glycolate stimulated cells offer proof of concept that Fum-PD reaching pulmonary macrophages was highly unlikely to have bioactivity whereas the Dxtl-PD can elicit effects in the same cells.