Title: Macrolide derivatives reduce pro-inflammatory macrophage activation and macrophage-mediated neurotoxicity.

Short Running Title: Macrolides reduce macrophage neurotoxicity

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**Figure S1.** Mass spectrum of AZM1. MS (ESI) m/z: [M + H]^+ calcd for C\textsubscript{30}H\textsubscript{59}N\textsubscript{2}O\textsubscript{9} 591.4; found 591.4.
Figure S2. Mass spectrum of AZM4. MS (ESI) $m/z$: $[M + H]^+$ calcd for $C_{30}H_{57}N_2O_9$ 589.4; found 589.4.
Figure S3. $^1$H NMR spectrum (D$_2$O, 400 MHz) and $^{13}$C NMR spectrum (D$_2$O, 101 MHz) of AZM4.
Figure S4. Mass spectrum of AZM5. MS (ESI) m/z: [M + H]$^+$ calcd for $C_{42}H_{77}N_{14}O_{14}$ 834.5, found 834.5.
Figure S5. $^1$H NMR spectrum (D$_2$O, 400 MHz) and $^{13}$C NMR spectrum (D$_2$O, 101 MHz) of AZM5.
Figure S6. Mass spectrum of AZM7. MS (ESI) m/z: [M + H]$^+$ calcd for C$_{34}$H$_{63}$N$_2$O$_{11}$ 675.4, found 675.4.
Figure S7. $^1$H NMR spectrum (D$_2$O, 400 MHz) and $^{13}$C NMR spectrum (D$_2$O, 101 MHz) of AZM7.
Figure S8: Altering the antibiotic properties of AZM does not decrease macrophage viability at the time of protein and RNA isolation. Bone marrow-derived macrophages (BMDMs) were isolated from adult mice and were treated with AZM, AZM1, AZM4, AZM5, and AZM7 at concentrations of 1, 5, 25, and 125 µM for 6 hrs (timepoint at which samples are collected for protein, RNA, and neurotoxicity assays). Cell viability was measured by using MTT assay. AZM or AZM derivatives exhibited no cytotoxicity at any tested concentration as compared to unstimulated, non-treated BMDM control (dotted line). Moreover, AZM and AZM derivatives 1, and 4 at 125 µM significantly increased proliferation of BMDMs as compared to unstimulated controls at *p<0.05, **p < 0.01, ***p < 0.001, however this effect was less robust than the 24hr drug toxicity timepoint in Fig. 2. Data is mean ± SD and representative of three independent biological replicate experiments.

Methods: Performed as in figure 2, utilizing a 6hr timepoint.
Figure S9: AZM and AZM derivatives decrease pro-inflammatory macrophage activation. The pro-inflammatory cytokine, IL-1-beta, was down regulated by AZM, AZM 4, and AZM 7. Similarly the pro-inflammatory cytokine, IL-6 was, down regulated by AZM and AZM 7. Shifts in the anti-inflammatory cytokine TGF-Beta and the pro-inflammatory cytokine TNF-alpha were not statistically significant. Results indicative of 2 independent biological replications. *p<0.05, **p < 0.01, ***p < 0.001

Methods: BMDMs were polarized to be M1 macrophages by stimulating with LPS + IFN-gamma. AZM, AZM1, AZM4, AZM5, and AZM7 were co-applied to M1 cells at concentrations of 1, 5, 25, and 125 µM for 6 hr. 300µL TRIzol LS reagent (Life Technologies) was added to each well (500,000 cells) to isolate RNA. Total RNA was isolated based on the manufacturer’s protocol, with an additional phase separation using BCP, precipitation with isopropanol (Sigma-Aldrich, St. Louis, MO), and wash of the isolated RNA in 70 % ethanol. Then, 1 µg RNA was reverse-transcribed using the high-capacity complementary (cDNA) reverse transcription kit (Life Technologies). Real-time PCR amplification was performed on the mixture of 100 ng cDNA sample, Taqman Universal PCR Master Mix, and Taqman Probes (Life Technologies) using the Applied Biosystems Step One Plus Real-Time PCR System. Expression of genes was normalized to 18S mRNA for each sample, and reported values were calculated as 2-ΔΔCT.