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# SYNERGISTIC STRIKES: A PROMISING RATIONAL DRUG COMBINATION AGAINST *Leishmania amazonensis* AMASTIGOTES

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Drug combinations offer a strategic approach to cutaneous leishmaniasis treatment by targeting multiple pathways to enhance efficacy and selectivity while reducing toxicity and resistance. Here, we evaluated the *in vitro* activity of POD-1 combined with MB, NMB, and its derivates, NMB-B and NMB-P (under patent secrecy) against *ex vivo* amastigotes of *Leishmania amazonensis* (LTB0016 strain, $10^6$ parasites/mL) searching for synergistic effects. Assays began from the $IC_{25,50,75}$ values of isolated compounds, followed by 24-hour exposures to fixed-ratio combinations (4:1; 3:2, 2:3, and 1:4) to determine the ICs within the combinations. FIC at 25%, 50%, and 75% were calculated, along with their mean sums ($\bar{x}\Sigma FIC_{25,\ 50,\ and\ 75}$), to classify interactions as synergistic (≤ 0.5), additive (> 0.5 and ≤ 4.0), or antagonistic (> 4.0). All combinations exhibited dose-dependent efficacy, with 81% inducing > 80% mortality and 50% reaching complete inhibition at maximum concentrations. ICs values decreased up to 26-fold compared to isolated compounds, with 90% ≤ 5 µM, 71% submicromolar, and 13% nanomolar. POD-1:NAM-P showed ICs of 0.02 µM for POD-1 (1:4 ratio) and 0.04 µM for NMB-P (4:1 ratio). Combinations significantly reduced POD-1's $IC_{75}$ ($p < 0.05$). FIC analysis indicated 60% synergy, 40% additive effect, and no antagonistic, with POD-1:NMB-P exhibiting 92% synergy, including FIC values < 0.1 µM, a pattern also observed for POD-1:MB and POD-1:NMB. The $\bar{x}\Sigma FIC$ revealed 58% synergistic combinations, 42% additive, and no antagonism, highlighting POD-1:NMB-P (67% synergy), similar to POD-1:NMB. Results underscore the potential of synergistic combinations, especially POD-1:NMB-P, to enhance efficacy at nanomolar concentrations against clinically relevant *Leishmania* form, supporting further investigations.

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