Antileishmanial aminopyrazoles: deconvolution of the mode of action by chemical mutagenesis

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Substantial advancements have been made in the discovery of novel antileishmanial leads and clinical candidates by phenotypic evaluation on intramacrophage amastigotes of the visceral *Leishmania* species. Aminopyrazoles have emerged as a promising series and hit-to-lead optimization by the Drugs for Neglected Diseases *initiative* (DND*i*) resulted in compounds with highly potent activity in animal models of leishmaniasis.

Molecular target deconvolution for the most potent aminopyrazoles has proven to be a major challenge because successive drug exposure failed to select for stably resistant phenotypes. Chemical mutagenesis with either ethyl methanesulfonate (EMS) or N-ethyl-N-nitrosourea (ENU) combined with drug selective pressure and whole genome sequencing was used as an alternative approach. From the obtained panel of 28 resistant lines an association between >10-fold resistance and multiple independent heterozygous mutations adjacent to the Zn^{2+} binding site of the zinc finger containing protein LINF_180011100 was discovered. Overexpression of the mutated gene increased resistance up to 10-fold, whereas susceptibility could be restored in mutant lines by transfection of a wildtype copy. Gene editing by CRISPR-Cas9 independently confirmed the contribution of the EMS and ENU mutations, resulting in H594Y and H594P substitutions respectively, to 10-32-fold resistance exhibited both at the extracellular promastigote and intracellular amastigote stage. Prediction of the molecular function of LINF_180011100 suggests a role in nucleocytoplasmic transport through the nuclear pore complex and cell cycle control.

Collectively, our data provide a sequential validation of LINF_180011100 as a drug target or resistance determinant for several aminopyrazole leads.