

IDENTIFICATION OF AND INITIAL MECHANISTIC STUDIES ON A NOVEL ANTILEISHMANIAL AGENT THROUGH *IN SILICO* PHARMACOPHORE DEVELOPMENT AND DATABASE SEARCHING

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Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania*. Currently, 12 million people are infected and one to two million new cases are reported annually. Without effective treatment, some forms of the disease are fatal. Current treatments may be toxic, ineffective due to resistance, expensive, and/or impractical to administer. Thus, novel therapeutics are urgently needed to combat this devastating disease.

We previously demonstrated the antileishmanial activity of several dinitroaniline sulfonamide (DNAS) compounds based on the herbicide oryzalin. These compounds inhibit purified parasite tubulin polymerization and arrest parasite growth in mitosis. They are also more active against *L. donovani* parasites than mammalian cells and are also selective for parasite tubulin over purified porcine brain tubulin. In this study, we used these compounds as a template to discover novel antileishmanial agents.

A three-dimensional pharmacophore was generated to describe the antileishmanial activity of the DNAs using the computer program CATALYST. This pharmacophore, containing an aliphatic hydrophobic group, an aromatic hydrophobic group, an aromatic functionality and a hydrogen-bond acceptor in specific regions of space, was then used to search databases of drug-like compounds. From the 55,000-structure Maybridge Organics database, several compounds that were able to fit into the pharmacophore were reported as hits. Nineteen of the most promising compounds were then tested for activity against the parasites.

Five of the compounds were found to be moderately active in the axenic-amastigote antileishmanial assay (IC₅₀ values between 21 and 39 μM). Two of the nineteen compounds were highly active with IC₅₀ values under 2.5 μM. The most active compound was BTB 06237. Its IC₅₀ value at 0.5 μM makes it five times more active than the most active DNAS compound. Unlike the DNAs however, this compound did not show antimitotic activity mediated through inhibition of tubulin polymerization. Transmission electron microscopy has shown that the single parasite mitochondrion becomes dialated when the parasites are treated with BTB 06237. Fluorescence staining of the mitochondria of compound-treated cells show disintegration of this organelle in a concentration-dependent manner. The exact mechanism leading to mitochondrial disintegration is currently unknown but is under investigation. We have shown, however, that the mitochondrial effect is not mediated through an apoptotic response.

Future studies include assaying the effect of BTB 06237 on specific enzymes of isolated parasite mitochondria and synthesizing analogs of the compound to develop a structure-activity relationship.