Allosteric Disruption of Trypanosome Bioenergetics: Structural Discovery of a First-in-Class Alternative Oxidase Inhibitor

Drug discovery for African trypanosomiasis is severely limited by toxicity, resistance, and a lack of mechanistically innovative targets. Bloodstream-form trypanosomes rely on the trypanosome alternative oxidase (TAO), a mitochondrial terminal oxidase absent from mammalian hosts, making it an exceptional drug target. Here, we report the discovery of a first-in-class, allosteric TAO inhibitor and define its molecular mechanism through integrated structural biology, enzymology, and parasitology.

Structure–activity–guided medicinal chemistry identified a lipophilic phosphonium-linked compound that inhibits recombinant TAO with nanomolar potency (IC_{50} = 1.3 nM; K_i = 3.4 nM) and displays broad-spectrum sub-micromolar trypanocidal activity against Trypanosome species including drug-resistant strains (EC_{50} value = 1.0 nM). Enzyme kinetic analyses demonstrated a non-competitive inhibition profile, indicating a binding mode distinct from classical active-site inhibitors.

Crucially, high-resolution X-ray crystallography (2.3 Å) revealed the inhibitor bound to a previously unrecognised allosteric pocket located ~14 Å from the catalytic di-iron centre. Structural analysis showed stabilisation of an inactive TAO conformation through hydrophobic and electrostatic interactions mediated by the phosphonium moiety, providing the first direct structural evidence for allosteric regulation of TAO. These findings establish a novel, druggable site on this essential parasite enzyme.

Phenotypically, TAO inhibition caused rapid and irreversible parasite killing, with growth arrest within hours and pronounced disruption of cell-cycle progression. Selectivity profiling confirmed minimal toxicity toward human cells, yielding a high selectivity index (2300).

Together, this work defines a structurally validated, mechanism-driven strategy for anti-trypanosomal drug discovery and positions allosteric TAO inhibition as a transformative approach for developing next-generation therapeutics targeting parasite bioenergetics.